THE PHYTOCHEMISTRY OF SEVERAL SOUTH AFRICAN ALOE SPECIES.

A dissertation presented for the degree

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by

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CHAPTER 6.

CHAPTER 7.

CHAPTER 9.

CHAPTER 10.

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I N T R O D U C T I O N.

Despite the tremendous advances made with regard to synthetic organic medicinals within the last two decades, heavy reliance is still placed on plant products. This is especially true of the anthracene derivatives used medicinally as purgatives, and which are derived principally from senna, cascara, rhubarb, frangula and aloes.

While particular attention has been paid to the chemistry of the former group in recent years, aloes has been largely neglected, possibly due to the fact that the Aloe species are confined largely to areas where extensive research facilities are lacking, such as Africa, India and the West Indies. Thus research in Europe has been confined largely to the lump aloes of commerce, derived from relatively few species.

In 1953 a comprehensive report by Hodge (103) appeared on "The Drug Aloes of Commerce, With Special Reference to the Cape Species". Hodge observed that South Africa abounds in species just as abundant as A.ferox, (which is the prime source of Cape aloes), and advised that a systematic chemical survey might show certain of these to be not only higher yielders of bitter aloetic juice but also sources of a superior drug product.

Consequently an investigation along these lines is presented here, and it is observed that several species apart from A.ferox not only contain aloin, but also yield a large volume of aloetic juice. Only pharmacologic studies can reveal if the juice of these species is as safe as that of A.ferox, but without doubt they could be used for the extraction of crystalline aloin. Concurrently, the distribution of the Aloe resins, said by some to be purgative themselves, has been studied.

The investigation has revealed that the structurally similar compound homonataloin enjoys an equally wide distribution as aloin. However, almost invariably it is confined to small species yielding little aloetic juice, apart from which nothing is known regarding its pharmacologic properties. It is interesting to note that the resin distribution in the homonataloin containing species is very similar to that of the aloin-containing species, but differs widely from that of the species containing neither of these principles.

Apart from aloin and homonataloin, aloinoside and chrysophanol also occur in Aloe species, and together with the resins, these indicate that when all the South African Aloe species have been investigated, they may well be of

chemotaxonomic value.

Within the comparatively short space of the last decade some work has been performed on aspects of the metabolism of such anthracene-containing species as Rheum, Rhamnus and Rumex. These investigations have shown that the anthracene derivatives are not merely waste products, but perform definite metabolic functions. The latter portion of this work has been devoted to this relatively neglected aspect of the Aloe species.

C H A P T E R I.

ANTHRACENE DERIVATIVES.

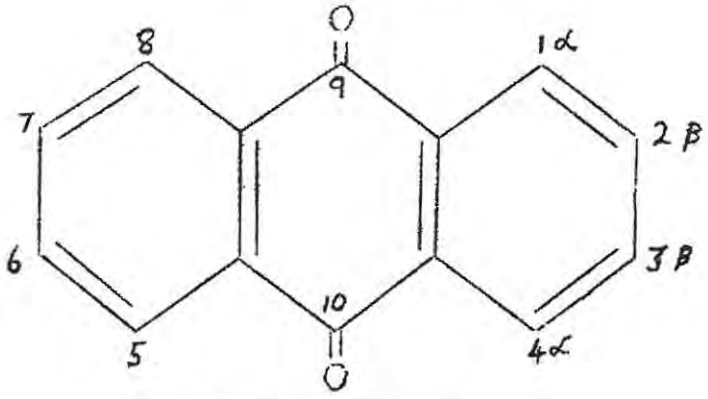
1.1.1 INTRODUCTION.

The anthraquinones (oxidised forms of anthracene) form the widest group of naturally occurring quinone compounds (THOMSON (217)). Several of these are of medicinal importance, mainly as purgatives, and in consequence much research has been directed towards the anthracene derivatives of medicinal plants.

1.1.2 OCCURRENCE.

The anthracene derivatives are widely distributed throughout the plant kingdom - in the Monocotyledons they occur chiefly in the Liliaceae, and in the Dicotyledons chiefly in the Polygonaceae, Rhamnaceae, Rubiaceae and in the genus Cassia. Some are also found in Hypericaceae, Scrophulariaceae (e.g. Digitalis), Euphorbiaceae etc. In the Gymnosperms, Pteridophytes and Bryophytes they seldom occur although some have been found in the fertile shoots of Equisetum arvense (HEGNAUER (101)). On the other hand they are comparatively common in certain fungi, e.g. ergot (FRANK & RESHKE (79)) and aspergillus (BIRKINSHAW & GOURLAY (25)), as also in lichens (FAIRBAIRN (63)). In the animal kingdom an example of their occurrence is carminic

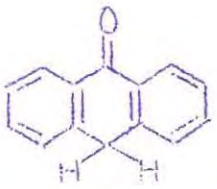
figure 1(i).



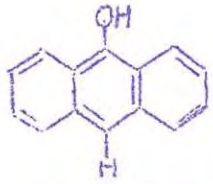
ANTHRAQUINONE.

Position	1	2	3	4&5	6	7	8	9	10
anthranol	H	H	H	H	H	H	H	OH	H
anthrone	H	H	H	H	H	H	H	O	H2
rhein	OH	H	COOH	H	H	H	OH	O	O
emodin	OH	H	CH3	H	OH	H	OH	O	O
aloe-emodin	OH	H	CH_2OH	H	H	H	OH	O	O
chrysophanol	OH	H	CH_3	H	H	H	OH	O	O
chrysazin	OH	H	H	H	H	H	OH	O	O
physcion	OH	H	CH_3	H	OCH_3	H	OH	O	O
mono-o-methyl-nataloe-emodin	OH	H	CH_3	H	H	OH	OCH_3	O	O
aloin	OH	H	CH_2OH	H	H	H	OH	O	$H\text{-}C_6H_{11}O_5$
homonataloin	OCH_3	OH	H	H	CH_3	H	OH	O	$H\text{-}C_6H_{11}O_5$
cascara-oxan-throne-gluco-side	OH	H	CH_3	H	OH	H	OH	HOC_6H_{11}	O

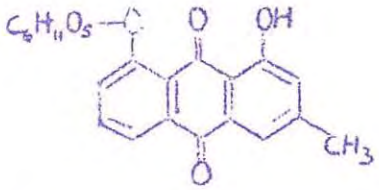
figure 1 (ii)



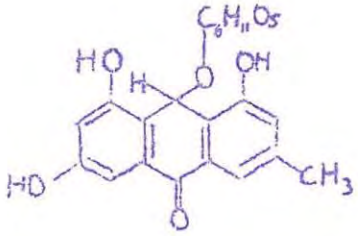
anthrone



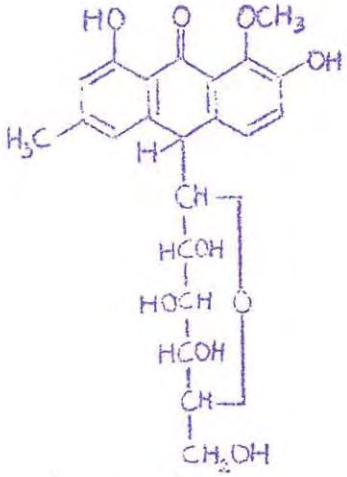
anthranol.



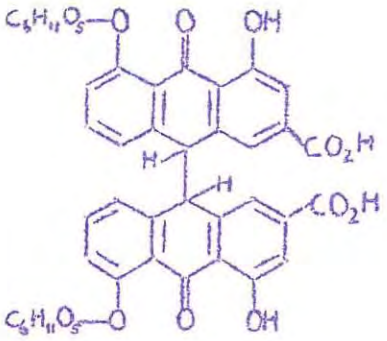
chrysophanol-8-glucoside.



emodin-oxanthrone glucoside.



homonatalein.



sennoside.

1.1.5 PHYSICAL PROPERTIES OF ANTHRACENE DERIVATIVES.

The spectrophotometric peaks of several anthracene derivatives appear in table 3(ii), while in tables 1(i) &1(ii) appear figures for melting points (218) and for hydrolysis rates of some hydroxyanthraquinone glycosides (FERGUSON & GARDNER (75).

Table 1(i) MELTING POINTS.

Name.	Position of OH group (s).	Melting point (^{o}C)
emodin	1,8	256
aloe-emodin	1,8	244
rhein	1,8	321-322
physcion	1,8	207
chrysophanol	1,8	196
homonataloin	1,8	202-4
aloin	1,8	147
erythroxyanthraquinone	1	193
β-hydroxy anthraquinone	2	302
alizarin	1,2	289
quinazarin	1,4	194
anthrarufin	1,5	280
chrysazin	1,8	193
anthraflavin	2,6	>330
anthragallol	1,2,3	314
hydroxychrysazin	1,2,8	239
anthrapurpurin	1,2,7	360
flavopurpurin	1,2,6	360
rufigallic acid	1,2,3,5,6,7	282

Table 1(ii) HYDROLYSIS RATES OF HYDROXYANTHRAQUINONE GLYCOSIDES.

Aglycone-anthraquinone	Sugar.	Time required for 50% hydrolysis HCl 0.05ON minutes	KOH minutes.
1-hydroxy	β-d-glucose	36	12
1-hydroxy	β-d-arabinose	5	4
1-hydroxy	α-d-cellobiose	98	13
1-hydroxy	β-d-maltose	30	15
2-hydroxy	β-d-glucose	360	15
1,5-hydroxy	β-d-glucose	30	9
1,8-dihydroxy	β-d-glucose	26	4
1,8-dihydroxy	α-d-cellobiose	270	40
1,8-dihydroxy	β-d-maltose	32	40
1,8-dihydroxy-3 methyl	β-d-glucose	40	16
1,9-dihydroxy-3 methyl	α-d-cellobiose	50	55
1,8-dihydroxy-3 methyl	β-d-maltose	45	85

It should be noted that whereas O-glycosides hydrolyse with dilute mineral acids (or alkalies), the C-glycosides require stronger acids and the addition of, for example, ferric chloride over a greater period of time. With regard to the spectrophotometric properties of anthraquinone, much work on this has been done by MORTON & EARLAM (158), who list spectrophotometric recordings of several hydroxyanthraquinone and

methoxy-anthraquinone derivatives in several solvents, and show how it is possible to characterise compounds in this manner. BRIGGS, NICHOLLS & PATERSON (39) have worked on anthragallol and its derivatives, and state that these show an absorption band in the region 240-245 mμ, due to the chromophore - C_6H_4COR, one of the fundamental bands of anthraquinone itself. Absorption in the region of 276-288.5 mμ is characteristic of the quinonoid nucleus of anthraquinone. Wavelengths of 356-362.5 mμ show no free hydroxyl groups, while one free hydroxyl group is shown by wavelengths of 407.5 - 413.5 mμ and two free hydroxyl groups by wavelengths of 427.5 - 432.5 mμ.

1.2.1 DISTRIBUTION OF ANTHRACENE DERIVATIVES.

As was mentioned before, anthracene derivatives are widely distributed in nature. Those of medicinal importance are found in the *Cassia*, *Frangula*, *Rheum* and *Aloe* species, and these will be dealt with later.

A complex variety of anthraquinone derivatives have been found by BOWIE & co-workers and by BRIGGS & co-workers. The appearance of several derivatives in one species is the rule rather than the exception, and doubtless this will be the case with the *Aloe* species when this field has been thoroughly explored. Only recently has HAYNES (personal communication) isolated aloesin, a C-glycosyl derivative, from aloes. This substance is dealt with in Chapter 8.

BOWIE, COOKE & WILKEN (33) have in recent years isolated several anthraquinones from two Coelospermum species (C.reticulatum and C.paniculatum), of the family Rubiaceae, tribe Morindeae. The anthraquinones in C.reticulatum occur mostly in the free state, the principal constituent being a new natural anthraquinone coelulatin, which has been identified as 1,3,8-trihydroxy-2-hydroxymethylanthraquinone. Small amounts of rubiadin and lucidin have also been isolated. In C.paniculatum the anthraquinones occur mostly as glycosides which have been hydrolysed to a mixture of rubiadin, rubiadin-1-methyl ether and lucidin. The name "medicine bush" given to the plant suggests that it mayat one time have been used as a purgative.

It has long been noted that a 1,8-arrangement of hydroxyl groups is usual in anthraquinones isolated from plants of the family Rhamnaceae, and from fungi and lichens. This arrangement could arise naturally by the polyacetate route of biogenesis. It has been customary to say that this pattern of hydroxyl groups never occurs in the anthraquinones of the Rubiaceae, but coelulatin provides the exception to this rule.

BOWIE & COOKE (32) have obtained anthraquinones from the whole roots of Morinda citrifolia, namely nordamnacanthal (which appeared in the Coelospermum species), morindone, rubiadin, and rubiadin-1-methyl ether. (These latter two have also been isolated from the root bark of Morinda jasminoides.)

Another minor component isolated was soranjidiol (1,6-dihydroxy-2-methyl-anthraquinone). From the root bark of Ventilago viminalis three new colouring matters have been isolated by COOKE & JOHNSON (52) namely ventimalin, viminalin and ventilagone, as also the known anthraquinones chrysophanol and islandicin. Ventimalin is 1,3,4,5,6-pentahydroxy-2-methylanthraquinone, while viminalin is 4,5-dimethyl ether of ventimalin.

Several of these compounds also occur in the Coprosma species. Thus BRIGGS & DACRE (35) isolated the following anthraquinone derivatives from Coprosma australis:-

a) morindin, a water soluble glycoside of morindone.

b) morindone, 1,5,6-trihydroxy-2-methylanthraquinone.

c) rubiadin-1 methyl ether (3-hydroxy-1-methoxy-2-methyl-anthraquinone.)

These were found both in the bark (17%) and in the root.

In Coprosma areolata BRIGGS, CRAW & DACRE (36) obtained a high yield of 23% for the two compounds, rubiadin-1 methyl ether and areolatin, which is probably 1,5,6,7-tetrahydroxy-2-methyl-anthraquinone. From Coprosma lucida BRIGGS & NICHOLLS (37) found a number of anthraquinones both free and as glycosides. No fewer than seven free anthraquinones occur, namely anthragallol its 2-methyl and 1,2-dimethyl ether, rubiadin, 1,6-dihydroxy-2-methyl-anthraquinone, 3-hydroxy-2-methyl anthraquinone, and a new trihydroxy-2-methyl anthraquinone for which the name lucidin is proposed.

From Coprosma acerosa, BRIGGS & THOMAS (38) have isolated the following by chromatography, namely lucidin, anthragallol 2-methyl ether, anthragallol 1,2-dimethyl ether, rubiadin 1-methyl ether and 1,6-dihydroxy-2-methylanthraquinone, some of which were also combined as glycosides.

Later work by BRIGGS, NICHOLLS & PATERSON (39) accounted for soranjidiol, rubiadin and copareolatin in Coprosma australis, which has been mentioned earlier. Finally, to complete the extensive picture, BRIGGS & TAYLOR (40) have obtained rubiadin 1-methyl ether and anthragallol 1,2-dimethyl ether from the bark of Coprosma rhamnoides. Physcion, rhein, aloe-emodin, frangula emodin and chrysophanol have been obtained from Oreoherzogia fallax Boiss by POETHKE et alia (171).

1.3.1 BIOSYNTHESIS OF ANTHRAQUINONES.

Hand-in-hand with the detection and isolation of the vast number of anthraquinone or anthraquinone derivatives existing today has gone an intense interest in the biosynthetic pathways by which these compounds have arisen, as also an interest as to whether these compounds can be used as a means of establishing a chemical taxonomy of the species producing them.

Several theories have been offered to account for the production of anthraquinones. Thus GILSON (92) as long ago as 1902 suggested that the anthraquinone nucleus could arise from condensation of phenolic compounds, for example, two molecules

of gallic acid condensing to form rufigallic acid (1,2,3,5,6,7-hydroxy anthraquinone). MORITZ (ex 81) suggested the possibility of condensation of phenylmethane bodies (e.g. vanillin, salicylaldehyde, salicylic acid, etc.) Supporting this theory is the presence of resinous bodies of the phenylpropane series in Aloe, and the content of salicylic acid in senna leaves. Conspicuous, also, is the presence in rhubarb of both anthraglycosides and gallotannic acid materials.

More popular conceptions of the biosynthesis favour acetic acid (as acetate unites), shikimik acid, or orsellinic acid as the basis of biosynthesis. However, HEGNAUER (101) states that comparison of some of the compounds accompanying anthraquinones in certain plants indicates that there may be several metabolic pathways to anthraquinones in higher plants. Quoting several species which include, amongst others, Polygonaceae, Rubiaceae, Rubiaceae-Coffeoideae (e.g. Anthospermae, Morindeae, and Galieae), Hegnauer is of the opinion that the acetate theory is not likely to tell the whole story of anthraquinone synthesis in higher plants. In several cases relations between anthraquinones and flavanoid compounds, phenylpropane derivatives and still other phenolic compounds seem probable. In other instances the anthraquinones show great similarity to naphthoquinones accumulated in the same or systematically related species. On the other hand, the naphthalene derivative of Rhamnus japonica could arise by

oxidative degeneration of anthranols. From a taxonomic viewpoint, however, HEGNAUER is convinced that an accurate study of the anthraquinones present in the several species may be an aid to the arrangement of this large number of species in taxa.

1.3.1.1 SHIKIMIC ACID.

According to RICKARDS (177), the recognition of the shikimic acid route, (stated to be one of the two major synthetic routes), is due to DAVIS and his associates (sensu RICKARDS). This method gives rise to amino acids and similar aromatic compounds, and DAVIS based his findings on work performed on mutants of micro-organisms (mainly E.coli and A.aerogenes) which were deficient in the ability to produce certain essential metabolites, including phenylalanine, tyrosine, tryptophane and p-aminobenzoic acid. Phenylalanine formation passes from shikimic acid (1,carboxy-3,4-hydroxybenzene) through three intermediary stages.

1.3.1.2 ORSELLINIC ACID.

According to BIRKINSHAW (24), SESHADRI favoured orsellinic acid units as components of anthraquinones, since orsellinic acid is the invariable component of lichen acids and depsidones. The orsellinic acid was considered to arise by aldol condensation of a hexose and a biose. Since the lichen products are probably produced by the fungal symbiont of the lichen, it was an obvious further step to extend the hypothesis to mould metabolites.

Some hydroxylated anthraquinones are formed in very high yield from glucose by the action of moulds (RAISTRICK (ex 192)). A noteworthy feature of these mould metabolic components is that they bear a carbon constituent in the 3-position (a methyl group or an oxidation product thereof.) Thus Penicillium cyclopium gives rise to the trihydroxy anthraquinone compounds emodic acid and ω-hydroxyemodin, while tetrahydroxy compounds are produced by Helminthosporium species.

1.3.1.3 ACETIC ACID.

As long ago as 1907 COLLIE speculated on "acetate units" as being the route whereby higher aromatic compounds were synthesised. This work has been reviewed by BIRKINSHAW (24) who states that the acetate hypothesis, developed by ROBINSON & BIRCH following COLLIE'S original speculation, has achieved considerable success in offering a plausible route for the biosynthesis of fungal metabolites, particularly the quinones. The method of linkage of acetate units is perhaps most readily envisaged in the case of certain anthraquinones. ROBINSON (ex 24) drew attention to the folding of the C16 chain formed from 8 acetate units so as to give the skeleton of endocrocin.

The acetate hypothesis has been further examined and extended by BIRCH & DONOVAN (23) as a result of inductive reasoning from structural comparisons of a large number of natural products,

chiefly phenols. In some cases they have, by this hypothesis, predicted the correct structure of fungal quinones when the chemical evidence offered alternatives. Thus when ASTILL & ROBERTS (sensu BIRKINSHAW (24)) had shown that flaveolin was either 2,5,7-trihydroxy-1,4-naphthoquinone or the 3,5,7-compound BIRCH & DONOVAN (21) predicted the former structure on the basis of the acetate theory of biogenesis. HAYNES et alia (99) have used this method also for the structure of homonataloin.

Evidence in favour of the acetate hypothesis has been obtained by incorporation in the substrate of acetate labelled in the carboxyl group with C^{14} to produce labelled quinones. This method has been used by BIRCH et alia for helminthosporin from Helminthosporium gramineum, and by GATENBECK (84) for emodin derived by dithionite fission of skyrin produced by P.islandicum.

1.3.1.4 CHEMICAL CONSIDERATIONS OF ACETATE BIOSYNTHESIS. (AFTER RICKARDS (177)).

Phenolic biosynthesis from acetic acid can conveniently be considered at two levels: firstly, generation of the main carbon-oxygen skeleton from acetate units, and secondly, subsequent structural modifications of this skeleton, including the introduction and removal of certain substituents.

1.3.1.4(1) GENERATION OF THE CARBON-OXYGEN SKELETON.

Hypothetically, acetic acid units (biologically activated for acylation as their thiolesters with co-enzyme A) are joined by formol elimination of water in head-to-tail linkage, with each other or with naturally occurring carboxylic acids to form polyketomethylene acids. Ring closure by aldol condensation or C-acylation would then produce phenols of the orcinol or acylphloroglucinol type respectively. The depsides from lichens are typical orcinol-types.

1.3.1.4(2) MODIFICATION OF THE CARBON-OXYGEN SKELETON.

Modification is frequently caused by introduction or removal of oxygen. Oxidation of phenols at their ortho- and para-positions is chemically facile, and similar biological introduction of oxygen is indicated notably by the frequent co-occurrence of anthrones with the related anthraquinones. Oxidation may also occur at methyl groups, and stages of oxidation of physcion (emodin-6-methylether, $R=CH_3$) produces fallacinol ($R=CH_2OH$) and fallacinal ($R=CHO$).

Removal of oxygen probably involves reduction of a carbonyl group to a hydroxyl group in a non-aromatic intermediate, followed by dehydration. In some cases intermediate stages in this oxygen removal process are found: both flavoskyrin and its dehydration product, chyrysophanic acid, have been

isolated from Penicillium islandicum, and may be compared with emodin and helminthosporin, in which both removal and introduction of oxygen have occurred.

1.4.1 CLASSIFICATION OF ANTHRAQUINONES.

According to BIRKINSHAW (24) the largest group of fungal quinones occurs in the anthraquinone series. Nearly all of them have a methyl group or an oxidised form of it in the β-position. They may be classified according to the number and position of the nuclear hydroxyl groups as follows:-

Group 1

Those possessing only α-hydroxyl group e.g. chrysophanol and islandicin.

Group 2

Those possessing both α- and β-hydroxyl groups. These may be further subdivided into group2A (the emodin type) having two α-and one β-hydroxyl or potential hydroxyl group, and group 2B, having three α- and one β-hydroxyl (or potential) group. For example, Group 2A - frangula emodin, physcion, 6-hydroxyemodin, emodic acid and roseo-purpurin all conform closely to emodin, the type substance, while endocrocin, nalgiovensin and nalgiolaxin show less conformity. Group 2B - catenarin, erythroglaucin and tritisporin conform to the emodin type with the insertion of an additional nuclear hydroxyl group.

1.5.1 TAXONOMY.

Whenever groups of plants are studied having similar chemical constituents, it is customary to see if these compounds can be used as a basis for a chemical taxonomic classification. According to FLÜCK (78) we may distinguish the so-called primary plant products, for example starch and proteins, and the secondary products such as mucilages and gums, glycosides, tannins, alkaloids, essential oils, pectins, etc. These groups fortunately also represent the most useful substances for chemical taxonomy. Whenever chemicals in plants are studied for taxonomic purposes it must be borne in mind that the absence or presence of constituents in related plants may vary due to geographical and climatic conditions, and seasonal and even diurnal variation cannot be overlooked.

1.5.2 METHOD OF CLASSIFICATION.

Chemical contributions to the classification of plants are based on their chemical constituents, that is, on their "molecular characteristics" (ERDTMAN (61)). These characteristics are genetically controlled, and have the advantage over morphological ones, that they can be very exactly described in terms of definite structural and configurational formulae. The elucidation of the structures and configurations of naturally occurring organic compounds paves the way to an understanding of their biosynthesis, which is a matter of fundamental systemic

importance. The method of chemical taxonomy is thus simple in principle, and consists of the investigation of the distribution of chemical compounds, or groups of biosynthetically related compounds, in series of related, or supposedly related plants. Possibly in future the enzymes will be found to be more important for this purpose than the low molecular weight secondary compounds. However, with our growing knowledge of the structure of natural products and their occurrence in plants the potentialities of "chemotaxonomy" are now becoming increasingly obvious.

Many substances such as amino acids, fatty acids and sugars occur in almost all plants and are therefore of little or no taxonomic interest (ERDTMAN (61)). Similarly, compounds found only in a single species are also taxonomically useless if not biosynthetically related to plant constituents of intermediate distribution. It is among the latter substances that we may expect to find compounds of the highest taxonomic value.

Chemotaxonomic studies should include studies of all the various parts of plants such as bark, wood, leaves, roots, cuticles and seeds. In Chapter 9 this has been done for the flowers, seeds and fruits of the species A.arborescens, A.ferox, A.speciosa and A.africana, but the glycanthrones have invariably been found in the leaf sap and occasionally only in the leaf (e.g. A.striatula var caesia.)

Finally ERDTMAN (61) says that it is always dangerous to draw taxonomic conclusions from the occurrence or non-occurrence of a single compound from a single part of a plant. Consequently in this work attention has been paid not only to the glycanthrones, but also to the resinous bodies occurring in the various Aloe species. However, the glycosides (in which group can be included the C-glycosyl compounds, or glycanthrones) have taxonomic importance. According to HEGNAUER (100) from the taxonomic point of view glycosides appear to be interesting at the level of the species or variety, and one can distinguish "chemical" races in this manner. In this work taxonomy of the species of Aloes is under consideration.

1.5.3 TAXONOMY OF ALOE SPECIES.

An interesting aspect of chemical taxonomy is to see if correlation exists between this method and classification according to botanical features.

In his classification REYNOLDS (176) says of the section Pachydendron Haw. that of the species comprising it, "two species, A.globuligemma and A.angelica fit far from comfortably in this section."

Of the thirteen species that comprise this section, all have been examined (VAN OUDTSHOORN (227); McCARTHY & PRICE (147); BRUCE (44) and only two do not contain aloin, namely A.globuligemma which contains no glycanthrones, and A.angelica

which contains homonataloin. The finding is in agreement with REYNOLDS' observation (176).

In this work it has been possible to study several Aloe species which fall into subdivisions named by REYNOLDS. Perhaps the most striking of these is the Series Latebracteatae, of the Section Eualoe Subsection Grandes. This comprises A.cryptopoda, A.wickensii, A.wickensii var lutea, and A.lutescens. Not only do these four contain homonataloin (McCARTHY & PRICE (148)) but it will be shown in this work that they have almost identical resin distribution and have a compound (resin) yielding a rose-pink colour with nitrous acid, which reaction is not given by the other species. It is interesting to note that A.speciosa, which differs in botanical appearance, but is also of the section Eualoe, has almost identical resin distribution and reaction, and contains homonatalaoin. As it forms the only member of the Series Principales, Subsection Magnae, it is wondered if, on a basis of chemical taxonomy, it should not be classified with the former group.

Another group which on chemical grounds agree with REYNOLDS' classification are the three species comprising the Series Paniculatae of the Section Eualoe, Subsection Humiles, these being A.striata, A.karasbergensis, and A.reynoldsii. These three, although containing neither aloin nor homonataloin, have similar chromatographic appearance, and a yellow spot between Rf 0.8 - 0.9 in chloroform: ethanol (3:1).

In similar manner VAN OUDTSHOORN (227) has described aloin in the four species comprising the Series Arborescentes, Section Eualoe, Subsection Magnae. In the work presented here aloin has been found in A.arborescens and A.vanbalenii of that series, but not in A.pluridens, while in A.mutabilis, homonataloin and not aloin was found. With regard to the latter, VAN OUDTSHOORN (227) has shown clearly that two distinct chemical races exist with regard to A.marlothii, one containing aloin and the other homonataloin. In this work, in both A.peglerae and A.mutabilis from the Eastern Cape homonataloin has been found, whereas working on Transvaal plants, VAN OUDTSHOORN (227) found aloin in these species. It thus seems probable that chemical races exist for several species, possibly depending upon locale, as was the finding with A.marlothii.

Another group complying with REYNOLDS' classification is the Section Anguialoe, four of the five species of which could be obtained, and contained aloin, namely A.vryheidensis, A.sessiliflora, A.dolomitica, and A.castanea. A further striking example of more than one component contributing to the taxonomic picture is found with the Series Mitriformes, of the Section Eualoe, Subsection Grandes. Of the four species comprising this Series, A.arenicola could not be obtained, but the remainder (A.mitriformis, A.distans and A.comptonii) not only contain homonataloin, but also a resin spot adjoining the homonataloin, which gave rise to the peaks appearing in

figure 5(iv). This resin spot colours ochre with nitrous acid.

Insufficient species have been obtained to classify further groups, but where a few species from a Series have been obtained, it has been seen that no close chemical relationship exists between groups having some botanical similarities. Thus for the Series Prolongatae of the Section Eualoe, Subsection Grandes, examination of four of the seven species obtained revealed aloin in one, (A.striatula var caesia), homonataloin in one (A.pearsonii) and neither glycanthrone in the other two (A.tenuior and A.gracilis). Despite these, chemical taxonomy can be usefully applied to the Aloe species.

CHAPTER 2.

ANTHRACENE DRUGS OF MEDICINAL IMPORTANCE.

2.1 INTRODUCTION.

The main drugs of importance as medicinal purgatives are cascara (Rhamnus purshiana), rhubarb (Rheum species), senna (Cassia species), and aloes (Aloe species), although frangula bark (cortex Rhamnus frangula) is also used. On account of the large volume of work that has been done on these groups, it is proposed to deal with each one in somewhat further detail.

2.2 CASCARA (Rhamnus purshiana).

Work on cascara has been on comparatively recent origin, namely, within the last two decades. In 1948 SCHINDLER & SEEBECK (187) isolated emodin oxanthrone glycoside from cascara bark, while in 1951 LEE & BERGER (126) isolated an active substance named 'casanthranol'. In 1958 BETTS, FAIRBAIRN & MITAL (18) used paper chromatography to show the presence of three anthraquinone glycosides which they called Compounds A, B and C. Since Compound A required hydrolysis with ferric chloride this compound was shown to be a C-glycosyl derivative, while Compounds B & C were split by normal acid hydrolysis and were thus O-glycosyl compounds. In 1959 BAUMGARTNER & LEUPIN (9) showed by means of paper chromatography that aloin was present

both in Rhamnus purchiana and Rhamnus frangula. The yellow fluorescence due to the aloin had been erroneously reported by STEINER (208) to be the flavanol glycoside xanthorhamnin. Later, in 1961, BAUMGARTNER & LEUPIN (10) were able to isolate from cascara bark 11-deoxy-aloin (10-glucosyl-1,8-dihydroxy-methyl anthrone).

In the interim FAIRBAIRN & MITAL (69) isolated an aloin-like substance from cascara bark. This compound had similar spectral peaks to aloin and was a C-glycosyl compound, but differed from aloin in being both sweet and more water soluble than aloin. Furthermore, ferric chloride oxidation led to the production of both aloe-emodin and chrysophanol.

The aloin-like substance from cascara bark was studied further, and by 1960 FAIRBAIRN & SIMIC (71) had resolved this compound into four allied anthraquinone derivatives, using counter-current extraction and paper chromatography. Initially only two of these compounds could be isolated and purified, and these were named Cascaroside A & Cascaroside B. At that stage the remaining two compounds were found to be derivatives of chrysophanol and not of aloe-emodin as is aloin, and the name chrysaloin was suggested for these compounds.

To date FAIRBAIRN & co-workers have obtained evidence of some twelve glycosidic anthracene derivatives. At least six are aloin-like (or C-glycosides) while the remainder are

O-glycosides. Two of the C-glycosides are barbaloin and deoxybarbaloin (which they have named chrysaloin because of its close relationship to chrysophanol). The renaining four C-glycosides are based on these compounds and the following is a tentative scheme showing their interrelationships:-

Cascaroside A is based on (+)-barbaloin and

Cascaroside B on (-)-barbaloin with the optical isomerism based on C_{10}; otherwise they are identical (FAIRBAIRN, FRIEDMAN & SIMIC (72).

Each contains one glucose molecule attached by a normal glycosidic link and a second component whose molecular weight is less than that of glucose, but whose structure and point of attachment have not yet been ascertained. Their work also suggests that Cascarosides C & D have a similar basic structure. It is interesting to note that these Cascarosides, being primary glycosides of barbaloin and chrysaloin, are both O- and C-glycosides. The remaining four to six compounds are normal O-glycosides, based mainly on emodin.

Thus cascara contains two types of anthracene compounds, (i) normal glycosides (based mainly on emodin), representing 10-20% of the total anthracene glycosides, and (ii) aloin-like compounds representing the remaining 80-90% of the total.

2.3 RHAMNUS FRANGULA.

This drug has not been as thoroughly examined as has cascara, and most of the work has been confined to the bark. However, BEZANGER-BEAUQUESNE (20) has examined the leaves of Rhamnus frangula and found that reduced anthraquinone forms are practically absent from the leaves, mainly free aglycones and anthraglycosides being found, but to a considerably lower extent than in the bark.

In 1963 SIEPER, LONGO & KORT (198) obtained the following as a methanolic extract of frangula bark, namely glucofrangulin, frangulin, frangulin-monoside, emodin, physcion and chrysophanol. MÜHLEMANN & SCHMID (160) have isolated the reduced form of glucofrangulin, namely glucofrangulin anthranol and its dianthranol, glucofrangulin dehydroanthranol. FAIRBAIRN & LOU (66) showed only free emodin, chrysophanol and small amounts of emodin methyl ether, (partly present as glycosides) to be in the bark, and FAIRBAIRN (66) has found no C-glycosyl derivatives in frangula, whereas BAUMGARTNER & LEUPIN (9) have found aloin in Rhamnus frangula.

2.4 RHUBARB.

The study of Rheum palmatum and associated species has been complicated both by the large variety of anthracene derivatives present in the plant, as also other constitutents

such as tannin, which are known to interfere with the bio-assay (FAIRBAIRN & LOU (66)).

The following basic anthraquinones have been demonstrated by FAIRBAIRN & LOU (66), namely, chrysophanol, aloe-emodin, rhein, emodin and emodin monomethyl ether. Their early work suggested that the main active fractions were glycosides of rhein anthrones. BELLAART (12) later isolated three quinone glycosides from Chinese rhubarb, while HÖRHAMMER et alia (112) isolated a large number of compounds from Rheum palmatum by the useful device of column chromatography on a polyamide (Perlon) column, which has the property of binding tannins, which normally interfere with crystallisation. The following were obtained, namely rhein-monoglycoside, aloe-emodin-diglycoside, chrysophanol-diglycoside, rhein, aloe-emodin monoglycoside, a mixture of chrysophanol glycoside and rheum-emodin glycoside, and finally a mixture of chrysophanol, rheum-emodin, aloe-emodin and physcion.

This serves to illustrate the diversity of anthracene derivatives occurring in rhubarb, but is by no means the complete picture. In 1963 WAGNER, HÖRHAMMER AND FARKASS (235) isolated genuine anthraquinone glycosides from the root of Rheum palmatum var tangut. In addition to known glycosides the following diglucosides were identified: chrysophanol-diglucoside, aloe-emodin-diglucoside and rhein-diglucoside.

Much work on rhubarb has been done by LEMLI & co-workers. Thus LEMLI, DEQUEKER & CUVEELE (128) utilised paper chromatography to isolate four dianthrones of rhubarb, at the same time describing an interesting colour reaction with formamide, (which is described further in section 3.6.4.3.). They used paper saturated with 2% formamide in acetone as stationary phase and found on spraying with alcoholic KOH and heating for 5 minutes at 100°C as aftertreatment that purple colours were given by anthrones and dianthrones and a red colour by anthraquinones. They used this reaction of formamide with alkali as a basis for determining the nature of several anthracene derivatives.

They later (129) isolated a dianthrone of rhein by preparative paper chromatography, the ultra-violet spectrum of which was identical to that of sennidine A & B. Oxidation with ferric chloride produced rhein.

Further to this (130) they isolated a new dianthrone which they called rheidine A, which is a heterodianthrone composed of one molecule of emodin anthrone and one molecule of rhein anthrone. The dianthrone rheidine A was prepared by heating equimolecular mixtures of emodin anthrone and rhein anthrone with twice the amount of ferric chloride in glacial acetic acid. The synthesised compound had an ultra-violet spectrum almost identical with that of the isolated compound. Soon after (134), they demonstrated the presence of rheidine A in senna leaves.

Later (135), they were successful in isolating two further heterodianthrones from the fresh roots of Rheum palmatum, namely rheidine B and rheidine C. The former consists of rhein anthrone and chrysophanol anthrone, and the latter of rhein anthrone and physcion anthrone. Thus together with rheidine A and sennidine C, four heterodianthrones have been obtained from rhubarb by these workers. A compound not frequently mentioned is raponticine, a new assay method for which has been devised by HENNEBERG & HORAK (102). Raponticine is separated by paper chromatography, eluted with alkali and a colour complex formed with potassium ferricyanide which can be measured against a standard.

Japanese workers have also contributed to the work on rhubarb. They (147) isolated rhein from Rheum coreanum Nokai, and it is interesting to note that they dispute certain findings by HÖRHAMMER (105), who thought that rhein was di-rhein. HÖRHAMMER came to this conclusion because a methanolic solution of rhein treated with hydrochloric acid was transformed to a compound which, with alkali and air, reformed rhein. From this HÖRHAMMER concluded that the substance was di-rhein, and that the substance obtained by reaction of acid was mono-rhein. On repeating the process, the Japanese workers obtained two compounds on acidification, which according to HÖRHAMMER should have been mono-rhein and its anthranol, both of which are soluble in

bi-carbonate solution due to the presence of a carboxyl group in each. However, only one compound could be dissolved by the Japanese, who showed the insoluble substance to be rhein methylester. The formation of the latter is due to the fact that methylation occurs readily with hydrochloric acid and methanol.

2.5 SENNA.

With senna, both the leaf and pod of Cassia acutifolia and C.angustifolia, in the main, contain important anthracene derivatives. Since TUTIN (223) first isolated aloe-emodin and rhein from senna, much progress has been made. STOLL, KUSMAUL & BECKER (209) made a very important advance when they isolated two active glycosides from senna, namely sennosides A & B, which are optical isomers, and which are excellent non-griping purgative principles. They found that both these compounds had a composition $C_{21}H_{20}O_{10}$ with one molecule of glucose combined with the anthracene grouping. On oxidation the aglycone gave rhein. Seven years later they were able to isolate these compounds in far purer condition by extracting the drug with chloroform-ethanol to remove impurities, followed by methanol containing oxalic acid. STOLL, BECKER & HELFENSTEIN (210) in 1950 showed that sennosides A & B had the molecular formula $C_{42}H_{38}O_{20}$.

In 1951 FAIRBAIRN & SALEH (61) showed the presence of a third active glycoside in senna, which when present in sufficient quantity has a marked synergistic effect on the activity of the sennosides A & B. Since the leaf contains more of this constituent than the pod, it explains why the leaf, though containing a smaller proportion of sennosides A & B than the pod, nevertheless has a greater biological activity.

In 1958 FAIRBAIRN, FRIEDMAN & RYAN (68) isolated a primary glycoside which on mild hydrolysis broke down to one molecule of sennoside and two molecules of glucose. In 1961 CRELLIN et alia (53) isolated five anthracene glycosides from senna. One of these rhein-9-glucoside, isolated from the pod, appeared to be similar to the compound described earlier that year by VICKERS (207). According to the latter, the compound, which could account for 10-20% of the sennoside content, was non-purgative. The other glycosides isolated by CRELLIN et alia (53) were rhein-8-diglucoside, rhein-anthrone-8-glucoside, and a primary glycoside of unknown structure, but of molecular weight 2000. This compound is more active than either the sennosides or the unknown compound of molecular weight 1164 isolated in 1958 by FAIRBAIRN et alia (68).

Reverting to rhein-anthrone-8-glucoside, STOLL, BECKER & HELFENSTEIN (210) were able to break this compound down to the sennosides, and finally to rhein.

The work discussed has mainly been on senna pod. From senna leaf, CRELLIN et alia (53) isolated aloe emodin glycoside and report that there is good evidence for the presence of an aloe-emodin anthranol glycoside. LEMLI (131) in 1962 isolated a new compound, the aglycone sennidine C, from both the leaf and pod of senna. This compound was shown to be the dianthrone of aloe-emodin and rhein (LEMLI (131)). In 1964 LEMLI, DEQUEKER & CUVEELE (133) isolated the following compounds from senna leaves, namely emodin, aloe-emodin, aloe-emodin dianthrone, rheidine A, sennidine C, rhein and sennidine A & B.

Adding to the complexity of compounds found in senna, KHORANA & SANGHAVI (120) have isolated two new glycosides from C.angustifolia pods, these being glucosides of rhein and chrysophanol. These have a synergistic action on the sennosides, a mixture of both being biologically more active than either group. The possibility of traces of aloe-emodin and emodin glucoside are reported.

From a plant related to senna, namely Adiperi (Cassia) jaknii, found in the Andes of Venezuela, SEELKOPF & TERAN (190) report the finding of glycosides which are not sennosides. The total anthraquinone content of the leaf, flowers, pods and seeds was 0.9, 0.94, 0.87 and 2.06% respectively, showing the wide distribution in the plant.

2.6 ALOES.

The anthracene derivatives of aloes appear to be far fewer and far less complex in nature than those of the preceding drugs. In fact, although much literature has appeared on aloes, it has been confined mainly to aloin, and to non-anthraquinones like p-coumaric acid and the resins. It is only in recent years that homonataloin and aloinosides have been discovered, and have opened up a new field of research.

The literature on the structure of aloin has been vast and confused. It is not the intention to discuss the structure of aloin further here, as several reviews have appeared on this subject. The later and more accurate work on the structure of aloin is reviewed in Chapter 4.

The anthracene derivatives found in aloes consist of aloin, isobarbaloin, aloinosides, homonataloin, chrysophanol, aloe-emodin and beta-barbaloin. With the exception of the latter which is absent from unboiled juices, the remainder have been dealt with in the ensuing chapters, together with the literature available on each compound, and to avoid repetition, these will not be treated here.

Work on aloes has been confined almost exclusively to commercial aloes (lump aloes) from the aloe-producing parts of the world such as the Cape, Natal, East Africa, India and the West Indies. In South Africa we are fortunate in having

some one hundred and thirty Aloe species (REYNOLDS (176)), and work in recent years has been rather on the distribution of aloin-like compounds than on discovering new compounds. (VAN OUDTSHOORN & GERRITSMA (226), McCARTHY & PRICE (147). It is hoped that the work presented here will contribute to the expansion of knowledge regarding both anthracene and non-anthracene derivatives in Aloe species.

Despite the relative paucity of work that has been performed on the anthracene derivatives of aloes, a surprising amount of work has been done on the biological and certain phytochemical aspects of Aloe species. Consequently, that work is reviewed here.

2.6.1 BIOLOGICAL WORK.

For years the native tribes of Southern Africa have used aloes or extracts thereof for a variety of purposes ranging from abortifacients, disinfectents, eye disinfectants, to purgatives, etc. (WATT & BREYER-BRANDWIJK (237)). Much of this usage has been based (not without accuracy) on folklore, but the work now described is the result of scientific research.

In a study of the ecbolic properties of Indian medicinal plants SAHA & co-workers (1961 (185)) found that the leaf extract of A.barbadensis had oxytocic properties, 0.5 mgm of leaf extract being equivalent to 0.003 International Unit of

oxytocin. Hypotensive activity is reported by BENBASSAT & co-workers (1959 (13)), who found that aqueous and saliva extracts of Lignum aloes lowered blood pressure in cats for 40-80 seconds. The effect was mainly on the blood vessels, although possibly a central action was also concerned. Tachyphylaxis and adrenaline reversal were not observed, and neither pulse rate nor respiration was affected.

Previous to this, in 1952 GONNARD & co-workers (93) had discovered that aloe extracts caused a drop in arterial pressure both in cats and dogs. They reported that this drop was not caused by histamine.

The Russian workers KARAEV et alia (118) in 1958 reported that extracts of five plants, included amongst which was A.arborescens, markedly improved the utilisation of glucose in rabbits, as shown by glucose tolerance tests. They recommended that these preparations be tested in humans having diabetes mellitus.

FILATOV & BIBER (76) found that aloe leaves act as biogenic stimulators (i.e. causing yeast activity) due to cinnamic and hydroxycinnamic acids. It is postulated that these may act as hydrogen acceptors, and stimulate redox processes on introduction into animals and man, and become hydroxycinnamic or hydroxycoumarin derivatives. They suggest that these acids occur as a result of hydrolysis of glycosides, and increase in

concentration when stored in the dark. (No such glycosides are known in aloes). SUKHORUKOV & BOLSHAKOVA (214) used the method of yeast cell growth to measure the concentration of plant hormone in selected plants. They found that decrease in temperature from 18° to 4°C caused both free - and bound-hormone to increase in potted wheat, rye, and Aloe species. When brought to -25° free hormone always increased, but the bound hormone behaved irregularly. They found no relation between free hormone and amine nitrogen.

Using FILATOV'S technique, FREYTAG (80) found that aloe tissues, stored in the dark for 2-4 days at 2-4°C caused wound hormone reaction in the bean hull, that is, caused cellular hyperplasia, new cell formation etc. By using large amounts of the tissues he was able to arrest the growth of mature cells of the bean mericarp. It is not yet established that the compound known as "traumatic acid" is responsible for these reactions (although it seems likely for aloes), but clearly cofactors, such as glutamic acid, accelerate these reactions.

It has been known for some time that the leaves of A.vera and A.arborescens are efficacious in X-irradiation burns (REYNOLDS (176)). It comes as no surprise then that FLAGG (77) reports the usefulness of the gel from A.vera leaves in suntan preparations. The Russians ROSTOTSKII & ALESHKINA (180) have made an aloe emulsion consisting mainly of bio-stimulated aloe

leaves, castor oil eucalyptus and emulgent for the treatment of skin diseases, for example, radiation injuries, kraurosis, dermatitis, eczema, neurodermatitis, psoriasis, and herpes.

Radiation is an important factor these days, and SHAMATOV & ZAPISKI (193) have shown that subcutaneous injections of aloe extracts cause a sharp rise in the absorption of Ca^{45} & P^{32} at fractured sites and throughout healthy bone, but no regularity in absorption trends could be observed.

Workers of the National Cancer Institute (BELKIN & FITZGERALD (11)) examined 32 cathartic plants for their capacity to damage "sarcoma 37". Amongst the 15 having effect were Rhamnus cathartica, Rheum officinale and Rumex crispus, while lesser damage was caused by Aloe perryi and Cassia alata.

Aloes have also been of importance in the field of microbiology. LORENZETTI et alia (142) have found that the freeze-dried juice of A.vera, heated at 80°C for 15 minutes, inhibited the following bacteria, namely S.aureus 209, S.pyogenes, C.xerose and S.paratyphi. Since the juice of aloe is known to contain anthraquinone-type compounds, aloe emodin, emodin and chrysophanol were tested for inhibition of S.aureus 209, but the results were negative. They found that none of the leaf parts, but only the juice, was a positive inhibitor. DOFF (60) however, reports that the juice of aloe leaves, or alcoholic or

aqueous extracts of the leaf, of aloin and aloe-emodin all show tuberculostatic activity. The most active compound was p-coumaric acid, which was effective in dilutions down to 1-100,000 in Sauton medium. Antibiotic compounds in aloes and in Rheum officinale (but not R.rhaponticum) have been reported by D'AMICO (54). In an effort to find adjuvants to enhance the production of 7-chlorotetracycline from certain strains of Streptomyces aureofaciens, McCORMACK (152) found that out of 22 compounds which were effective for the task, (barb)aloin was 17th on the list in descending order.

2.6.2 PHYTOCHEMICAL WORK.

BRUNO (45) reports that the leaves of A.plicatilis Mill. are rich in lipoids, but that lipoids are scarce in the external layers of leaves of A.cassia Salm Dyck., but slightly more abundant in the internal layers. The starch content of the latter plant is reported to be scarce in spring and very low in winter.

In Madras aloes DAS & MITRA (58) report that the stiffness is due to the high xylan (15%) and polyuronide (6%) content, since its lignin content is only 6%, and is lower than that of white jute (C.capsularis). However its α-cellulose content is higher (65%) as is its fat content (2%).

In the Cuban aloe KAUFMAN & LEIVA (119) report only small

amounts of (barb)aloin and isobarbaloin, but the residue on ignition yielded 19.2% calcium, 12.2% aluminium, 40% silicon oxide and a trace of iron.

The fibre content of aloe plants has been reported by LINCOLN (139), who states that the fibre content of leaves of Aloe species grown under shade and in full sunlight at four different localities showed no change.

Saponin has been reported in A.vera leaves by WASICKY & HOEHNE (238). The saponin (2.91%) was crude, and had a very low haemolytic index. (In the four species studied in Chapter 9 only A.arborescens extract caused no frothing when shaken with water, whereas A.ferox and A.africana frothed considerably, suggesting saponin).

CAMPBELL & COOPER (50) have found a relatively high content of 3,4-benzopyrene in certain Aloe snuff (prepared by heat treatment of Aloe stems.) They deduce that this is probably the cause of the high rate of nasal cancer (73%) in Bantu of the Transvaal.

DANILOVIC & PECI-POPOVIC (55) have identified four sugars in the hydrolysate of Aloe, these being rhamnose, xylose, arabinose, and galactose. These, plus glucose and fructose, appeared also in senna, frangula, cascara and rhubarb. (The presence of glucose in several Aloe species is reported in Chapter 6.)

A study of the flowers of several Aloe species by BUSCH & RESENDE (48) has revealed that as many as twelve flavonoids and four carotenoids could be found in some flower extracts, using column chromatography. Among normal plants of A.striatula var caesia, LINSKENS (140) observed two spontaneous mutants having flowerless inflorescences. Paper chromatography showed no difference in aqueous extracts of the normal and mutant plants, but methanolic fractions showed fluorescent fractions which differed from those in the normal plants. Differences in absorption spectra indicated that in the mutants the formation of a specific protein was blocked, resulting in an accumulation of nucleic acids.

Some work has also been performed on the chromosomes of Aloe species. CONAGIN (51) treated plant tissues, including Aloe species, with p-dichlorobenzene before fixation, and found that although this caused no abnormality in the chromosomes, some shrinkage was caused, which facilitated counting and separation of the chromosomes.

Thus it will be seen that apart from the anthracene derivatives in Aloe species, several interesting constituents are contained, including some having medical and pharmaceutical importance.

2.7 STRUCTURE - ACTIVITY RELATIONSHIPS.

Since all the vegetable purgatives named contain anthracene derivatives, it is natural to assume that these are the active principles. However, attempts to correlate the quantity of anthracene derivatives present - as determined colorimetrically by the Börntrager reaction (31) or one of its modifications (62) - with the purgative activity were nearly always unsuccessful (3) (65) (212). Furthermore, pure anthraquinones were found to be practically inert in quantities corresponding to those present in normal doses of the drugs (63) (221). An interesting attempt to explain this latter fact was made by LIDDEL et alia (138), who showed that mixtures of pure anthraquinones were synergistic. As a result of a comprehensive investigation FAIRBAIRN (63) showed that the anthracene derivatives were highly active as anthrone (i.e. partially reduced) glycosides; less active as free anthrones, and much less active as free anthraquinones. This accounted for the difference between results by biological and chemical methods, since by the latter method all forms were measured in terms of anthraquinone content.

For maximum activity at least two factors are important, firstly the presence of sugar groups. Their function is to act as a transporter enabling the aglycone to reach the site of action in the large intestine intact. In the absence of sugars

the majority of the aglycones disappear during metabolism and only a small amount reaches the large intestine (211). According to Okada (165) the glycosides are hydrolysed by enzymes on reaching the large intestine and the liberated aglycones then exert their action.

The second factor is the partial reduction of the meso-carbonyl groups: thus aloe-emodin anthrone is more effective than aloe-emodin.

A third possible factor is the presence and position of the phenolic groups. Work by FAIRBAIRN (64) on synthetic and naturally occurring anthraquinones suggests (i) that those with one phenolic group are inactive, (ii) those with two are active and (iii) those with three are active provided all are not α-phenolic groups. Removal or acylation of phenolic groups of known cathartics leads to loss of activity.

The route of administration is important, as IPPEN (114) has shown. Thus when 1,8-dihydroxy-anthranol is absorbed cutaneously it is oxidised in the tissues to 1,8-dihydroxy-anthraquinone, the latter being found in the urine, but not the former. If, however, 1,8-dihydroxyanthraquinone is administered orally, the quinone is partially reduced in the large gut to the corresponding anthranol. FAIRBAIRN (74) has shown that mice respond to oral dosages of sennosides, but not to parenteral dosages of the same quantity.

2.8 DETERMINATION OF ANTHRACENE DERIVATIVES.

In 1959 KRAUS (125) reviewed the methods used for estimation of the anthracene derivatives in the plants mentioned before, citing 124 references. It is not proposed to repeat KRAUS'S findings, but his subdivision of methods is as follows:-

a) gravimetric analysis, volumetric, colorimetric and photometric,
b) methods involving prior purification by extraction,
c) methods involving prior chromatographic purification,
d) fluorimetric,
e) polarographic,
f) chromatographic (paper, thin-layer- dye-reagents, column chromatography and paper electrophoresis).

One omission from KRAUS'S work is the method of bio-assay. This has been used extensively by FAIRBAIRN'S co-workers, and LOU (143) devised an assay using white mice which has been used successfully for senna for several years. FAIRBAIRN has commented on the close correlation found between this method and chemical methods. This correlation, based on mice, has been shown by clinical trials to hold true for man also. These biological methods have demonstrated the important synergistic effects of anthracene compounds, a fact which could never have been found by chemical assay. Commenting on the efficacy of the mouse assay method, BRITTAIN & co-workers (42) state that

although the method is successful for senna preparations, it is not so well suited to the evaluation of other anthraquinone purgatives, e.g. cascara and rhubarb, and does not demonstrate the purgative activity of aloin (D'ARCY et alia (57)).

LISTER & PRIDE (141) have used the rat faecal pellet method for the bio-assay of barbaloin, aloin and amorphous aloin, in which the activity of the first two over the last-named is clearly shown.

Only comparatively few new assay methods have been described since KRAUS'S review in 1951. Thus JANIAK & BOEHMERT (115) have described an assay method for aloin in which the samples are passed through a Perlon column to remove impurities, then heated with 4% borax in methanol on a water-bath. The yellow green fluorescence which results obeys Beer's law and is stable for about an hour. The colour is measured at 450 mμ with an error of $\pm$ 0.05%. The method required about 30 minutes to perform. This method has recently been criticised by BÖHME & KREUTZIG (28a)(1966) as not being specific for aloin, other constituents giving false results. GERRITSMA & VAN OUDTSHOORN (89) have described a prior purification using thin-layer chromatography, and the cutting off of the aloin spot and its elution with methanol, which is read at 355 mμ. This method also takes some 30 minutes. A similar method for paper chromatography is described by

BÖHME & KREUTZIG (28b). Potentiometric titration in non-aqueous milieu is described for hydroxy-anthraquinones (partially free, partially as glycosides) by RUGGIERI (181). Pyridine is used as the base and the titrant is 0.1N sodium methoxide standardised with pure 1,8-dihydroxyanthraquinone and expressed as emodin, while MÖHRLE (156) has used a colorimetric method involving sodium periodate and ammonia, the resulting red coloured solution being examined at 505 mμ. Finally, the Chinese workers WANG et alia (236) have described a method of determining anthraquinones in Rheum palmatum, Cassia tora and Polygonum multiflorum, involving soxhlet extraction, purification with solvents and the reading at 490 mμ of the alkali-coloured solution, which is compared to a standard graph obtained with 1,8-dihydroxyanthraquinone. For total anthraquinones a prior hydrolysis with 5N sulphuric acid is used.

C H A P T E R 3.

EXPERIMENTAL METHODS.

3.1.1 INTRODUCTION.

In this work the main analytical methods involved have been chromatographic, spectrophotometric and volumetric.

For Qualitative work, use has been made of the first two methods, aided, in the case of chromatography, by ancillary identifications of a chemical nature, particularly spray reagents.

For Quantitative work, use has been made of all three analytical methods, although chromatography has been used only for separation of constituents prior to quantitative estimation of glycanthrones.

The volumetric method of analysis has been of the simple acid/base nature and requires no further elucidation, but it is proposed to deal more fully with chromatographic and spectrophotometric methods, as also the use of spray reagents and chemical tests performed on Aloe juices.

3.1.2 CHROMATOGRAPHIC METHODS.

Paper chromatography.

Having been discovered several years prior to thin-layer chromatography, this analytical tool was widely used for earlier

research work, including research on plants containing anthraquinone derivatives. Its greatest asset is the fact that, provided experimental methods are identical, exactly the same Rf values will be obtained by a series of workers. However, the identical analytical criteria are critical and include:-

(i) identical chromatographic paper
(ii) identical (controlled) temperatures
(iii) identical solvents
(iv) identical saturation of paper.

Despite this, variation in results can still occur due either to use of excess starting constituents, or due to chemical attraction (bonding forces) between a number of constituents. Thus frequently the Rf of a component run singly differs from the Rf of that substance run as a mixture with other components.

The disadvantages are important, namely, that paper chromatography is time-consuming in relation to the newer thin-layer method; that the spots are not as discreet as in thin-layer chromatography, and tend to merge; that these spots are not as readily eluted as those from thin-layer chromatograms, and finally, but most important, paper chromatograms are not resistant to corrosive chemicals like sulphuric acid, bromine etc., and consequently identification in situ cannot readily be performed.

3.1.3 THIN-LAYER CHROMATOGRAPHY (T.L.C.)

Several of the advantages of thin-layer chromatography have been mentioned in the preceding section. The speed and relative robustness of the method have made it an extremely popular and useful analytical tool, and although thin-layer chromatograms cannot, like paper chromatograms, be kept as a permanent record, this fault has been largely overcome by the use of photographic methods for record purposes, as also tracing of revealed constituents. Recently a spray has been introduced which seals the chromatogram, which may then be kept indefinitely.

The colour photography of fluorescent zones on chromatograms has been described in a paper by ROSILLO (179). The actual colour produced by components is often hard to describe by different workers, in particular, colour shades such as mauves, violets, greys etc. Colour photography is the solution to this problem (Fluorescent colour photography has been utilised in Chapter 5). To obtain the Rf values, ROSILLO projected his colour transparency onto a screen divided by 11 equidistant horizontal black lines. The projector was adjusted so that the origin and solvent front were superimposed on the first and last lines respectively, whereupon the Rf value of each spot could then be read directly.

3.1.4 REPRODUCIBILITY OF Rf VALUES.

One of the major disadvantages of thin-layer chromatography is that Rf values of substances are not reproducible. Furthermore, different samples of the same compound sometimes show slightly different behaviour even on the same layer. These difficulties are more common in adsorption thin-layer chromatography and are probably traceable to the complexity of surface adsorption or to the fact that no two laboratories prepare layers in exactly the same manner. An excellent review on the subject has been given by BOBBIT (26).

3.1.5 FACTORS INFLUENCING Rf VALUES.

The main factors influencing the reproducibility of Rf values are:

(i) Nature of the adsorbent.
(ii) Developing system.
(iii) Nature and amount of applied sample.
(iv) Saturation of the chamber.
(v) Temperature.

3.1.5.1 Nature of the adsorbent.

BRENNER et alia (34) have stated that amongst the principal factors involved in the movement of solutes are the quality of the adsorbent, the degree of activity of the layer, and the

thickness of the layer. These workers have noted an appreciable difference between batches of Silica gel G. Furthermore, it should be remembered that an activated layer becomes less active after exposure to a moist atmosphere for appreciable times. The use of standard, commercial adsorbents with a layer thickness over 0.15 mm and consistent drying times will help to reduce variations, however. STAHL (204) claims that there is little difference in Rf value if the layer is spread at a thickness between 200 μ and 300 μ (0.2-0.3 mm), and 250 μ is recommended for analytical work. However, in order to produce a layer of wet thickness 250 μ, the operator has to avoid having the slurry too thick or too thin, and must not move the applicator too quickly or too slowly, since all these factors can produce variation in layer thickness. MEINHARD AND HALL (195) go further and state that variations can be caused by:-

(i) time taken to prepare the slurry
(ii) time taken for drying and activating plate
(iii) the temperature used for (ii)
(iv) storage conditions of prepared plates
(v) age of plates at time of use.

3.1.5.2 Developing systems.

The nature of the developing system is a prime factor, and use must be made of reagent grade solvents, which should be made fresh to avoid esterification or other possible interaction.

This is especially true when very volatile components such as ether or pentane are being used, while the mere opening of a chamber containing such a volatile solvent in a mixture can change the concentration appreciably. Solvent "demixing" is also an important factor, producing several "fronts" (SHELLARD (195)).

3.1.5.3 Nature and amount of applied sample.

The nature of the sample, that is, the properties of its components, is the third major factor in the determination of Rf values, and is not controllable. The amount of substance applied at the starting point will affect both the shape and position of the spot, the actual difference depending upon the shape of the adsorption isotherm, because the amount of substance absorbed per mg of adsorbent will vary with the concentration of the substance according to the nature of the substance itself. With substances having a convex adsorption isotherm the front of the spot will move faster than the back, so that the spot will be more elongated as concentration increases, with corresponding changes in Rf value. Furthermore the reversal of the relative position of two substances has been observed when the load has been substantially increased.

Furthermore, the Rf of a given substance may not be exactly the same in a mixture as it is in the pure state

(PASTUKA (168)). According to SHELLARD (195) interaction could occur between two substances, either in the solvent or on the surface of the adsorbent; if in the solvent the adsorption would be less, with increased Rf value, while if on the surface the opposite effect would occur.

3.1.5.4 Saturation of the chamber.

Care should be taken to saturate the chamber before use, and this is normally accomplished by partially lining the walls with filter paper which dips into the solvent, and when possible, by shaking the chamber before introduction of the chromatogram. This saturation (called oversaturation by STAHL) will speed up development by one-third, will give lower, more consistent Rf values, and will produce straighter solvent fronts and rounder spots. In this manner "edge effects" can also be eliminated, since these are a source of large errors.

3.1.5.5 Temperature.

Although MULLER, HONERLAGEN & HARTHORN (see (195)) consider the Rf value to be strongly dependent on temperature, this is not a widely held view. SHELLARD (195) has found slight variations with temperature, especially with mixed solvent systems.

3.2.1 RECENT ADVANCES IN T.L.C.

A most important advance in the T.L.C. field is the use of this method for quantitative separation of constituents, by what is known as preparative chromatography, and this method is slowly superseding column chromatography for that function. HALPAAP (95) states that the use of a two-dimensional surface to effect a segregation offers considerable advantages over the three-dimensional system of column chromatography as a manufacturing method. Initial amounts of up to 100 g of mixtures can be effectively and almost quantitatively separated to afford isolation of substances showing high purity after only one recrystallisation.

STAHL (204), developing this technique, investigated the optimum layer thickness for this process, using layer-thicknesses from 20-2,000 μ. The best separations were achieved with small samples and layer-thicknesses in the range 150-500 μ. Above 750 μ the separations deteriorated with increasing sample size. Layer thicknesses of 1,500 μ and more may be used only for substances which show a marked difference in their Rf values on normal chromatograms. The means of application of sample was in the form of a spray, although traversing pipettes are also popular for quantitative work.

Further advances by STAHL (205) include an apparatus for providing gradient layers of T.L.C. In this manner it is

to obtain gradient mixtures of, for example, basic to acidic alumina, pH gradient layers for specific profile study, and for development in the direction of the gradient layers in mixtures of kieselguhr and silica gel.

Low-temperature T.L.C. using refrigerant gases (e.g. monofluorodichloromethane) has also been devised by STAHL and co-workers (205). In several instances he and his colleagues have succeeded in achieving a clean separation of complex mixtures by using low temperatures. This method could be applied with advantage to the separation of very unstable substances.

Furthermore, in co-operation with Zeiss, an instrument for obtaining directly the ultra-violet absorption spectrum of chromatographed material by reflectance technique has been developed.

KABARA et alia (116) have used finely etched (sandblasted) plates in preference to clear-glass plates for T.L.C. They claim that the surface is more adhesive, a binder need not be included with the adsorbent, and that Rf values are slightly increased.

3.2.2 UTILITY OF THIN-LAYER CHROMATOGRAPHY.

The wide-spread usefulness of thin-layer chromatography has been shown in recent reviews by TEIJGELER (215) & (216),

who enumerates over fifty analytical fields from alkaloids to vitamins, citing 375 references.

3.2.3 GAS CHROMATOGRAPHY.

It is interesting to record that FURAYA (82) has recently separated emodin glucoside and several glycosides by gas chromatography. Further to this, FURAYA et alia (83) (1966) have succeeded in separating 26 anthraquinones, either free or as trimethylsilyl ethers, and including the anthraquinones of rhubarb.

3.3.1 OWN WORK.

For thin-layer chromatography of Aloe species the following adsorbent bases were tried, namely, kieselguhr, alumina, cellulose and silica gel G. The latter was by far the most satisfactory, although for high molecular weight substances (antibiotics) BRODASKY (43) says that the greatest reproducibility is found with paper and cellulose.

In view of what has been said concerning reproducibility of Rf values in the foregoing pages, Rf values are rarely quoted in this work. Where a reference substance has been available, the Rf values of substances relative to this have been quoted, e.g. as for the resinous bodies. Where no known substance has appeared on the chromatogram, then Rf values are

tentatively reported as an indication of the zone where the unknown compound exists.

It has further been found that another factor influencing Rf values has been the time taken to spot many samples on to a freshly activated plate as compared to few samples on a smaller plate. Rf values are only quoted for machine-spread plates, since PATAKI & KELEMEN (169) have shown that the layer thickness of hand-spread plates, (and consequently Rf values) is unreliable.

3.3.2 PREPARATION OF PLATES.

Silica gel G (Merck) (3 parts) was mixed with distilled water (6 parts) and spread on plates 20 cm x 20 cm, a layer thickness of 250 μ being employed. Use was made of either a DeSaga or Pleuger spreader. Also, plates 12½cm x 9cm were used for routine scanning of samples, for testing spray reagents, etc. These were made by spreading with a simple spreading device having raised sides.

All plates were activated before use by heating at 105°C for 30 minutes, and were used immediately after cooling, wherever possible. SHELLARD (195) states that Rf variations can occur due to direction of thin-layer chromatography, namely ascending, horizontal or descending, and consequently advises that the direction of chromatography be reported along with findings. Ascending thin-layer chromatography was used for all thin-layer chromatograms in this work.

3.3.3 SOLVENTS USED FOR CHROMATOGRAPHY.

A large number of solvent systems have been used for chromatography of anthraquinone derivatives by both paper and thin-layer chromatography. Some of these solvent systems are described in table 3(i).

Table 3(i). CHROMATOGRAPHIC SOLVENTS.

	Solvent composition.		Method.	Used for.	Authors.
1.	benzene	3	T.L.C.	anthraquinones in	POETHKE,
	ethyl acetate	1		Oreoherzogia fallax (Boiss)	BEHRENDT & MATSCHKE (171)
2.	dichloromethane	10	T.L.C.	anthraquinones in	SIEPER, LONGO & KORT (198)
	methanol	3		frangula bark	
3.	dichloromethane	10	T.L.C.	ditto	ditto
	methanol	10.5			
4.	benzene	1	T.L.C.	ditto	ditto
	carbon tetrachloride	1			
5.	n-propanol	4	T.L.C.	rheinglucoside and	HÖRHAMMER, WAGNER &
	ethyl acetate 4	4		sennosides	BITTNER (110)
	water	3			
6.	ethyl acetate	100	T.L.C.	universal solvent for Aloe	ditto
	methanol	16.5		Rhamnus and Rheum species	
	water	13.5			

	n-butanol	4	paper	sennosides A & B	KAPADIA & KHORANA (117)
7.	glacial acetic acid	1			
	water	5			
8.	ditto		paper	**Aloe**	AWE, AUTERHOFF & WACHSMUTHMELM (5)
9.	ditto		paper	Aloe	AWE & KUMMEL (6)
10.	ditto		paper	Aloe	" BÖHME & BERTRAM (27)
10b.	ditto		paper	Aloe	KRAUS (124)
	n-butanol	40	paper	sennosides A & B	KAPADIA & KHORANA (117)
11.	acetic acid (1.93N)	10			
	water	5			
	ethyl acetate	4	paper	commercial aloes	" BÖHME & KREUTZIG (28)
12.	glacial acetic acid	1	(& T.L.C.)		
	water	5			
	toluene	60	paper	Rheum palmatum	LEMLI, DEQUEKER &
	butanol	10	(treated with		
	propanol	30	20% formamide		CUVEELE (128)
13.	tetrahydrofuran	10	in acetone)		
	formamide	30			
	formic acid	0.05			
	toluene	6	paper	sennidine in Senna	LEMLI (131)
14.	butanol	1			
	propanol	3			
	formamide	2			

	petrol ether (40°)	4	paper	anthraquinones	" HORHAMMER, WAGNER & LEUE (107)
15.	toluene	1			
	xylene	1			
	methanol	2			
16.	toluene		paper	anthraquinones	BETTS, FAIRBAIRN & MITAL (18)
16b.	ditto		paper	Senna	KHORANA & SANGHAVI (120)
	cyclohexane	90	paper (treated with dimethyl formamide/formamide 80:20)	anthraquinones in Rhamnus purshiana	KINGET (122)
17.	octanol	9			
	pentanol	1			
	benzene (45-70°)		paper	anthraquinone	SHIBATA, TAKITO &
18.	saturated with 97% methanol			pigments	TANAKA (196)
19.	butanol, water satured		paper	Aloe	AUTERHOFF & BALL (4)
	chloroform	3	T.L.C.	Aloe	GERRITSMA & VAN OUDTS-
20.	ethanol (95%)	1			HOORN (89)
	n-propanol	6	paper	anthraquinones &	MERCK (153)
21.	ethyl acetate	1	& T.L.C.	glycanthrones	
	water	3			
	petrol ether (65-110°)		paper	aloes and	MARY, CHRISTENSEN &
22.	saturated with 97% methanol			cascara sagrada	BEAL (145)
	amyl alcohol	4	paper	Rheum	SCHULTZ & MAYER (189)
23.	glacial acetic acid	1			
	water	5			
	amyl alcohol	2	paper	Rheum	SCHULTZ & MAYER (189)
24.	glacial acetic acid	1			
	water	2			

Table 3(ib) SOLVENTS USED IN THIS WORK.

Solvent Designation.	Composition.
Z	Toluene
Y	Benzene:glacialacetic acid (2:1)
X	Isopropyl ether
W	Toluene/petrol ether
V	Chloroform: ethanol 95% (3:1)
U	Ethyl acetate: n-propanol: water: acetone (50: 15: 15: 30)
T	Acetic acid: formic acid: ethyl acetate: water (3:1:18:4)
S	Butanol: ethanol 95%: water (40:11:9)
R	N-propanol: ethyl acetate: water (4:4:3)
Q	Benzene: carbon tetrachloride (1:1)
P	Ethyl acetate: methanol: water (100:16.5:13.5)
O	Butanol: chloroform (5:2)
N	Butanol: glacial acetic acid:water (4:1:5)
M	Petrol ether (40°): toluene: xylene: methanol (4:1:1:2)
L	Ethyl acetate: glacial acetic acid: water (4:1:5)

3.4.1 SPECTROPHOTOMETRIC ANALYSIS.

Valuable evidence with regard to the identification of compounds by their spectra, as also to the orientation of substituent groups on the molecule can be afforded by spectrophotometric methods. Use has been made of this method to identify here both aloin and homonataloin in Aloe species, since both these compounds give clearly defined peaks in the ultra-violet region of the spectrum. The spectra of both aloin and homonataloin as appearing in several Aloe species are shown in figures 4(i) & 5(iii).

BRIGGS, NICHOLLS & PATERSON (39) have used this method to study the molecules of anthragallol and anthraquinone. Thus anthragallol and all its derivatives show an absorption band in the region 240-245.5 mμ, due to the chromophore - C_6H_4.COR, one of the fundamental bands of anthraquinone itself. Absorption in the region of 276-288.5 mμ is characteristic of the quinonoid nucleus of anthraquinone. Wavelengths of 356-362.5 mμ showed no free α-hydroxyl group; wavelengths of 407.5 - 413.5 mμ showed one free α-hydroxyl group while wavelengths of 427.5 - 432.5 show two free α-hydroxyl groups.

VAN OUDTSHOORN (227) has examined several hydroxy-anthraquinone substances with reference to the work of BRIGGS and coworkers. He found that nataloe-emodin (1,7,8-trihydroxyanthraquinone), a compound with two free α-hydroxy groups, has a maximum at 434 mμ, which is 1.5 mμ wider than the range given by

BRIGGS et alia (39). He contends therefore that the limits for two free hydroxy groups can be widened. Several of the maxima quoted by VAN OUDTSHOORN (227) for hydroxyanthraquinone derivatives appear in table 3(ii). Recognition of subsituent groups in anthraquinone is given by MORTON & EARLAM (158) who list spectrophotometric recordings of several hydroxyanthraquinones and their derivatives in several solvents showing how it is possible to characterise compounds in this manner.

Table 3(ii) U.V. Spectra of anthraquinone derivatives.

Anthraquinone derivative.	Wavelength of peaks.					
chrysophanol	225	255	277	287	-	430
chrysophanol anthrone	220	255	-	295	355	-
aloe-emodin	225	254	276	286	-	430
aloe-emodin anthrone	220	255	-	285	355	-
rhein	230	257	265	-	-	430
rhein anthrone	220	-	265	295	365	-
emodin	225	252	265	289	-	437
physcion	222	252	265	287	-	435
nataloe-emodin	232	260	-	290	-	434
nataloe-emodin-8-methyl ether	228	-	275	295	-	400
nataloe-emodin-dimethyl ether	228	-	272	293	-	396
chrysazin	228	255	-	285	-	430
chrysazin anthrone	222	255	-	285	355	-
aloin	222	260	-	295	359	-
homonataloin	222	250	273	294	347	-
aloe-emodin monoglucoside	223	255	-	-	-	410
chrysophanol monoglucoside	220	257	-	284	-	423

3.4.2 SPECTROPHOTOMETRIC APPARATUS USED.

In this work exclusive use was made of two instruments, namely, the Beckman D.B. recording spectrophotometer, and the Unicam S.P. 600. The former was used to obtain ultra-violet spectra of compounds, and for the quantitative estimation of homonataloin (Chapter 9), while the Unicam S.P. 600 was used for quantitative estimations of aloin (Chapter 9). In all cases 1 cm matched quartz cells were used, with methanol as solvent.

3.5.1 CHEMICAL TESTS ON ALOE JUICES.

Wherever possible, chemical tests have been performed on the Aloe juices examined in this work. These include the use of concentrated sulphuric acid, followed by fumes of nitric acid (Histedt's reaction), concentrated nitric acid, nitrous acid, and potassium periodate solution. It has been found that Histedt's reaction is not specific for homonataloin (McCARTHY & PRICE (148)), and the non-homonataloin containing Aloes react with nitrous acid, and with periodate, giving similar colours to those given by homonataloin. The component(s) causing this colour change are not known, but are situated among the resins and might well be resinous derivatives. Since with few exceptions the juices react with alkali to produce yellowy or brownish-green solutions, alkali reactions will be reported only for exceptional reactions.

For conciseness, Aloes giving similar reactions are grouped together in table 3(iii), and only the specific name for each species is given. Methanolic solutions of juice were used, these being tested on a white spotting tile.

Table 3(iii). REACTIONS WITH CONCENTRATED ACIDS.

Species.	Sulphuric.	Sulphuric & nitric fumes.	Nitric.
A.ferox & Group (i)(follows)	orange brown	brown	yellowy brown
A.africana	orange brown	brown with red edge	ditto
A.cryptopoda & Group (ii)	yellow brown	green	red changing to orange red
A.munchii	greenish brown	deep green & blue	red changing to orange red
A.simii	greenish yellow	deeper green with blue edge	evanescent cherry turning yellow
A.comptonii	yellowy brown	rapidly deep red with green & blue edge	deep red
A.globuligemma	brown	unchanged	light brown
A.succotrina	yellowy brown	green, blue & purple	red going orange red
A.broomii	reddish brown	purplish red with faint green edge	red going orange
A.dyeri	brown	unchanged	weak yellow
A.fosterii	greenish brown	green & blue	red going orange
A.reynoldsii	greenish yellow	green & blue	pale green
A.karasbergensis	olive	bottle green	pale yellow going mauve after 5 min.
A.lineata	orange	orange with purple edge	brown

A.distans	yellowy brown	green, blue & pink	red going orange red
A.melanacantha	yellowy brown	green, blue & pink	red going orange red
A.chabaudii	light green	deep green & finally purple	red going orange red
A.hereroensis	greenish brown	deep green & finally brown	red going orange red
A.arborescens	yellow	yellow	brown
A.angelica	orange yellow	green, blue & purple	yellow
A.aculeata	reddish brown	deeper reddish brown	slowly green
A.claviflora	cherry pink	cherry pink	-
A.petrophylla	brownish green	greener	evanescent red going orange
A.ramosissima	yellowy brown	dark yellowy brown	yellowy brown
A.plicatilis	grey green	no change	pale orange
A.grandidentata	yellowy green	blue	yellowy brown
A.longibracteata	yellowy green	blue	yellowy brown
A.branddraaiensis	lime green goes olive	deep green & blue	evanescent red going orange
A.suprafoliata	mustard	slowly green	orange red slowly deepening
A.candelabrum	red brown	deep red with green edge	brown going emerald green

Group (i).

A.castanea, A.reitzii, A.vanbalenii, A.dolomitica, A.cameronii, A.marlothii, A.sessiliflora, A.vryheidensis, A.excelsa.

Group (ii)

A.mutabilis, A.wickensii var lutea, A.lutescens, A.mitriformis, A.pachygaster.

3.5.2 REACTION OF NITROUS ACID WITH ALOE JUICES.

This is described fully in section 5.3.4, where the reaction of nitrous acid on homonataloin is also discussed.

3.6.1 SPRAY REAGENTS.

Under section 3.1.4 the reproducibility of Rf values was discussed. SHELLARD (195) has this observation to make: "Although they should never be regarded as definite proof of identity, Rf values give a reliable indication of the identity of the substance, especially in association with the colours produced by specific spray reagents".

Spray reagents have been much used in this work, and have aided in the identification of glycanthrones, anthraquinones, glycosides, sugars and resins, and have been used for the detection of these on chromatograms.

The following spray reagents have in the main appeared in the literature, and many of them have been used in this work, and will appear under the relevant chapters.

These are:

2-6, dibromocinchoninechlorimide
Fast Blue B (4,4'-Bi(2-methoxybenzoldiazonium chloride)
nitrous acid
copper sulphate and hydrogen peroxide
antimony trichloride
vanillin in absolute alcohol
piperidine 50% in benzene
silver nitrate and ammonia
alum
baryta water
borax
ferric chloride
potassium permanganate
potassium permanganate and soda
potassium hydroxide and hydrogen peroxide
magnesium acetate
magnesium acetate and ammonia
ammonia vapours
phosphoric acid 25%
antimony pentachloride
stannous chloride
sodium hydroxide
sulphanilic acid and sodium nitrite
zinc acetate
lead tetra-acetate 1% in benzene
periodate-starch
para-anisidine
formamide

The previously named spray reagents are used for three main classes of compounds, namely resins, sugars or glycosides, and anthraquinone derivatives.

3.6.2 REAGENTS FOR RESINS.

These are alcoholic alkali (2-5%)

Fast Blue B 0.5% aqueous,

antimony trichloride 4% in chloroform,

2,6-dibromocinchonine chlorimide 0.4% methanolic.

The use of these has been described fully in Chapter 8, sections 8.5.2 to 8.5.5, and will not be repeated here.

3.6.3 REAGENTS FOR SUGARS AND/OR GLYCOSIDES.

(i) 35% borax containing 0.8% KI, 0.9% boric acid, and 3% sol. starch - for glycosides and cis-hydroxy compounds (METZENBERG & MITCHELL (154)).

(ii) p-anisidine HCl 2% in water-saturated butanol:- for cis-hydroxyl compounds.

(iii) 4 parts sodium metaperiodate (2% aq)

1 part 1% aq. $KMNO_4$ in 2% $Na_2 CO_3$ aq.

This reagent converts carbohydrates to detectable aldehydes.

(iv) Lead tetra-acetate 1% in benzene - (BUCHANAN et alia(47))
Used for the detections of 1-2 glycols, for glycosides and non-reducing carbohydrates.

Reagents (i) and (ii) have been used in Chapter 6, sections 6.1.9 to 6.1.12.

3.6.4 REAGENTS FOR ANTHRAQUINONE DERIVATIVES.

The following reagents were used for homonataloin, and full details therefore appear in Chapter 5, sections 5.1.3 to 5.2.1.

(i) potassium periodate aqueous (saturated solution)
(ii) magnesium acetate 0.5% methanolic solution
(iii) potassium hydroxide 10% aqueous & hydrogen peroxide 3%
(iv) sodium nitrate 5% aq., followed by glacial acetic acid
(v) $CuSO_4$ 0.5% aq., followed by hydrogen peroxide 3%
(vi) potassium hydroxide 5% alcoholic
(vii) vanillin 3% in absolute alcohol containing ½% H_2SO_4 conc.

The following were used for aloin and full details thereof appear in Chapter 4, section 4.1.5.

(i) potassium hydroxide 10% + hydrogen peroxide 3% (1:1)
(ii) magnesium acetate 1% methanolic
(iii) KOH 5% alcoholic.

The following reagent has been used by RAMAUT (174) in studying the products of metabolism of Aspergillus versicolor, namely, piperidine 50% in benzene. With this reagent anthraquinones give a violet colouration.

3.6.4.1 Differentiation using spray reagents.

A useful property of certain spray reagents is not only that they assist in identifying a general group, but that they

can also differentiate members of that group. Three reagents having this property are potassium (or sodium) hydroxide, formamide and magnesium acetate.

3.6.4.2 Alcoholic KOH reagent.

Not only does this reagent, (in concentrations from 2-5%) indicate 1,8-hydroxy anthraquinones, but it also distinguishes between anthraquinones, which colour red on heating, and anthrones and dianthrones, which colour brown or yellow. If heat is not applied, sennidine A & B give yellow spots which slowly change to violet on standing (KHORANA & SANGAVI (120)).

Furthermore, this useful reagent indicates the presence of p-coumaric acid as a clear blue spot, as also certain resins, which colour various shades of blue (u.v.).

3.6.4.3 Formamide reagent.

If formamide-saturated paper is sprayed with 5% KOH in 50% methanol, an excellent distinction between anthraquinones, anthrones and dianthrones is obtained. Thus anthraquinones, on heating at 100°C for 5 minutes, give a red colour, while anthrones and dianthrones under these conditions give purple colours (LEMLI et alia (133)).

LEMLI & co-workers utilised this procedure to produce a specific colour reaction on a semi-micro scale as follows:-

100-200 mgm of anthraquinone is heated in a boiling waterbath for ten minutes with 2 ml of 10% KOH in formamide. After cooling, the solution is diluted to 10 ml with formamide. On exposure to sunlight or ultra-violet light (max. 365 mμ) the colour changes from red (max. 510 mμ) to blue (max. 610 mμ) for chrysophanol, aloe-emodin and rhein. The 2,7-substituted anthraquinones emodin and physcion remain red (max. 510-540 mμ) (N.B. This method offers a differentiation of two chromatographically similar substances, namely, physcion and chrysophanol). The dianthrones e.g. sennidine A & B go from red to purple.

It is interesting to note that once the anthraquinone compound has been warmed with formamide - KOH, it forms a complex from which aloe-emodin, chrysophanol and rhein cannot be recovered from acidified aqueous mixture by means of organic solvents. However, emodin and physcion do not form complexes, and can be recovered by means of ether. This allows an important quantitative differentiation of chrysophanol and physcion.

3.6.4.4 Magnesium acetate reagent.

Thanks largely to the work of SHIBATA, TAKITO & TANAKA (196) magnesium acetate 0 5% methanolic has been widely used in attempts to classify the position of hydroxyl groups on the anthraquinone nucleus. Anthraquinones with at least one of the hydroxyl groups in the α-position develop with the reagent,

when heated at 90°C for 5 minutes. Compounds which contain two in the 1,3-position e.g. emodin, chrysophanol or aloe-emodin, give an orange-red or pink colour; those with two in the 1,4-position i.e. quinizarin, produce a purple, and those with two in the 1,2-position i.e. alizarin, exhibit a violet colour. VAN OUDTSHOORN (227) using a temperature of 110°C for 10 minutes, describes the following daylight colours:-

chrysophanol, physcion and chrysazin - orange-red

rhein, emodin and aloe-emodin - red

nataloe-emodin and alizarin - violet

methylnataloe-emodin - orange.

Apart from these three reagents, SCHULTZ & MAYER (189) have described no fewer than seventeen reagents which they used to distinguish between an anthraquinone (frangula emodin), an anthranol (aloin Merck) and a dianthrone (Pursennid Sandoz). Those reagents not already described appear in table 3(iv).

Table 3(iv). COLOUR REACTIONS OF ANTHRAQUINONE DERIVATIVES.

Reagent	Anthraquinone		Anthranol		Dianthrone	
	daylight	u.v.	daylight	u.v.	daylight	u.v.
contol	yellow	shining orange	grey yellow	shining orange	weakly yellow	brown
$AgNO_3$ 1%+NH_3 1%	weak bright yellow	bright grey	intense grey	intense red brown	weakly yellow	brown
alum 5% aq	yellow	bright brown	grey yellow	shining dark yellow	weakly yellow	grey brown
baryta water 5%	intense red	dark red	intense yellow	yellow brown fluorescent	bright grey yellow	dull brown
borax 5% aq	intense red	red	intense bright brown	yellow fluor.	weakly yellow ish	dull grey brown
$FeCL_3$ 1% aq	weak bright brown	-	intense black grey	-	intense grey	-
$KMnO_4$ 1% aq	yellow	bright brown	grey-videt	intense brown	weak yellow-ish	dull brown
$KMNO_4$ + soda	intense red	purple	intense brown	intense grey yellow	clear brown	dull brown
Mg/cet - NH_3	intense red	shining red	intense bright brown	yellow fluor.	bright yellow brown	intense brown
NH_3 vapours	intense red	dark red	intense yellow	shining orange	intense yel-low	brown

H_3PO_4 25%	weak yellow	dull grey	weak yellow brown	intense brown	weak bright yellow	brown
$SbCl_5$ 1% aq	yellow	shining brick-red	clear brown	dull dark brown	clear yellow	brown
$SnCl_2$ 1% aq	weak yellow	shining bright grey	weak yellow brown	shining orange	weak bright yellow	brown
Soda 5% aq	intense red	purple	intense brown	shining yellow	weak orange	brown
Sulphanilic acid + $NaNO_2$	red	red	intense violet-brown	shining yellow	bright orange	dull grey-brown
Zn Acet 5% aq	bright orange	shining bright orange	bright brown	orange	weak yellow-ish	brown

3.7.1 HERBARIUM SPECIMENS (INTRODUCTION).

Aloe species are very prone to mould growth once picked, and a suitable method of preservation is required. Acknowledgement for the following advice is due to Miss G. Britten, Albany Museum: "The method used to dry succulents these days is to soak in paraffin or dip in boiling water. I prefer the old method - formalin 1 to 15 parts water. Immerse the plant for a day or so, then dry in the usual way. Drying Aloes is rather a long job and they must be thoroughly dry before mounting. The specimens require constant changing to avoid mould. As the flowers tend to drop off it is advisable to treat the specimens as soon as possible. When put into a press a good heavy weight is needed, or it should be strapped tightly and left in the sun to hasten drying.

If the leaves are thick they can be cut in half longitudinally and scraped out. If it is not possible to cut them, a few slits along the margins will allow the juice to ooze out when the specimens are under pressure.

When collecting Aloe species such as A.ferox or A.africana, the flowerheads should be cut in sections long enough to fit the sheets on which they will eventually be mounted. It is better to cut the thick flowering stems in half, as this makes it quicker and easier to dry them.

Aloes, like most succulents, lose their colour after

treatment, and it is advisable to make notes of the colours of flowers and leaves, whether spotted etc."

3.7.2 PREPARATION OF HERBARIUM SPECIMENS.

All large specimens of leaves were sliced and scraped to remove the pulp, and the remaining epidermis was then swabbed inside and out with formaldehyde (35% v/v). Both the upper and lower halves were then dried in the sun and stapled onto white mounting boards, on which the description of the leaf and flowering portion was recorded. Large flowering heads were cut in half, scraped internally, painted with formaldehyde and left to dry before attaching to the mounting boards. A few flowers were detached and placed in an envelope, which was fixed to the board. Small leaves were merely pricked to eliminate juice, while small flowering heads were dipped in formaldehyde, dried, and affixed to the board.

3.7.3 HERBARIUM SPECIMENS.

Table 3(v). HERBARIUM SPECIMENS (leaf and flowering top)

Species.	Origin.	Herbarium No.
A.aculeata Pole Evans	Uitenhage	M001
A.arborescens Mill.	Port Elizabeth	M002
A.cameronii (Rhodesian)	Uitenhage	M003
A.dolomitica Groenewald	Uitenhage	M004

A.reitzii Reynolds	Uitenhage	M005
A.sessiliflora Pole Evans	Uitenhage	M006
A.vanbalenii Pillans	Uitenhage	M007
A.vryheidensis Groenewald	Uitenhage	M008
A.ferox Mill.	Port Elizabeth	M009
A.africana Mill.	Port Elizabeth	M010
A.marlothii Berger	Uitenhage	M011
A.candelabrum Berger	Uitenhage	M012
A.castanea Schonl.	Uitenhage	M013
A.excelsa (Rhodesian)	Port Elizabeth	M014
A.striatula var caesia Reynolds	Grahamstown	M015
A.suprafoliata Pole Evans	Uitenhage	M016
A.cryptopoda Bak.	Uitenhage	M017
A.lutescens Groenewald	Uitenhage	M018
A.pachygaster Dinter	Uitenhage	M019
A.melanacantha Berger	Uitenhage	M020
A.hereroensis Engler	Uitenhage	M021
A.comptonii Reynolds	Port Elizabeth	M022
A.mitriformis Mill.	Uitenhage	M023
A speciosa Bak.	Port Elizabeth	M024
A.wickensii var lutea Reynolds	Uitenhage	M025
A angelica Pole Evans	Uitenhage	M026
A.mutabilis Pillans	Port Elizabeth	M027
A.munchii Christian	Uitenhage	M028
A.pearsonii Schonl.	Uitenhage	M029

A.distans Haw.	Uitenhage	M030
A.grandidentata Salm Dyck	Port Elizabeth	M031
A.simii Pole Evans	Uitenhage	M032
A.dyeri Schonl.	Uitenhage	M033
A.petrophylla Pillans	Uitenhage	M034
A.saponaria (Ait) Haw.	Port Elizabeth	M035
A.globuligemma Pole Evans	Uitenhage	M036
A.longistyla Bak.	Grahamstown	M037
A.polyphylla Schonl.	Grahamstown	M038
A.broomii Schonl.	Uitenhage	M039
A.succotrina Lam.	Uitenhage	M040
A.lineata (Ait) Haw.	Port Elizabeth	M041
A.ramosissima Pillans	Uitenhage	M042
A.pretoriensis Pole Evans	Port Elizabeth	M043
A.longibracteata Pole Evans	Uitenhage	M044
A.fosteri Pillans	Uitenhage	M045
A.ammophila Reynolds	Grahamstown	M046
A.branddraaiensis Groenewald	Port Elizabeth	M047
A.chabaudii Schonl.	Uitenhage	M048
A.karasbergensis Pillans	Uitenhage	M049
A.pratensis Bak.	Uitenhage	M050
A.reynoldsii Letty	Uitenhage	M051
A.peglerae Shonl.	Grahamstown	M052
A.dominella Reynolds	Port Elizabeth	M053
A.striata Haw.	Port Elizabeth	M054

A.plicatilis (L) Mill.	Port Elizabeth	M055
A.glauca Mill.	Grahamstown	M056
A.aristata Haw.	Grahamstown	M057
A.variegata Linn.	Port Elizabeth	M058
A.claviflora Burchell	Grahamstown	M059
A.pluridens Haw.	Port Elizabeth	M060
A.gracilis Haw.	Port Elizabeth	M061
A.humilis (L) Mill.	Port Elizabeth	M062
A.greenii Bak.	Port Elizabeth.	M063

C H A P T E R 4.

ALOIN AND RELATED COMPOUNDS.

4.1.1 INTRODUCTION.

Aloin and its isomer isobarbaloin and the aloinosides have been extracted from several Aloe species, aloin predominating in its occurrence. Furthermore, aloin has been obtained as a hydrolysis product from plants other than the aloe, but in this chapter discussion of the occurrence of the above principles is confined exclusively to Aloe species.

4.1.2 ALOIN.

Aloin was extracted in crystalline form as long ago as 1851 by T & H SMITH (201). Since the aloin was extracted from Barbados aloes, the name barbaloin was adopted, its isomer being known as isobarbaloin. However, the terms aloin and barbaloin are synonymous, and the term barbaloin will only be used in this chapter where it is directly quoted from the original literature.

4.1.3 STRUCTURE OF ALOIN.

Work on the structure of aloin commenced over 50 years ago. Workers of that and later periods were hampered by the lack of modern methods of instrumental analysis, and in consequence much of the work of people such as LÉGER (127),

HAUSER (97), ROSENTHALER (178) and CAHN AND SIMONSEN (49) is only of historic interest, although some information of the structure of the basic anthraquinone nucleus was obtained.

In 1937, the American workers GARDNER & JOSEPH (87) produced evidence that hydrolysis of both aloin and isobarbaloin gave the same anthrone nucleus (9-anthrone), thus refuting the earlier claims of HAUSER (97) that the nucleus was an aloe-emodin anthranol.

In 1952 MÜHLEMANN (159) described a method for synthesising chrysazin-9-anthrone glucoside. Shortly after, by analogy, the formula for aloin was stated to be 10 (1':5'-anhydro-glucosyl) aloe-emodin-9-anthrone. This formula, MÜHLEMANN pointed out, allowed of isobarbaloin being one or other of the optical antipodes at C(10).

Confirmation of this was given by BIRCH & DONOVAN (1955)(22) and by BARNES & HOLFELD (1955)(8), use being made of the ultra violet spectra of methyl-, acetyl-, ethyl- and bromo-derivatives of aloin. The latter workers stated that since both barbaloin and isobarbaloin had maxima at 267-268 mμ and at 359 mμ, this indicated that the sugar moiety of the aloins was joined at C(10) by a C-C bond. Final confirmation of the formula for aloin proposed by MÜHLEMANN came from HAY & HAYNES (1956)(98), using infra-red spectroscopy, X-ray crystallography and chemical methods.

In recent years, further C-C glycosyl compounds have been isolated, including carminic acid (ALI & HAYNES) (2) and homonataloin (HAYNES, HENDERSON & TYLER) (99). following recent nomenclature, these compounds are termed glycopyranosyl compounds. These C-C glycosyl compounds are not confined to anthraquinone derivatives, and recently SEIKEL & MABRY (191) have reported the isolation of a glycoflavonoid from Vitex lucens.

4.1.4 PHYSICAL CHARACTERISTICS OF ALOIN.

Aloin occurs as a yellow crystalline powder with a melting point of 146-147°C, although in the literature, temperatures reported range from 142°C to 150°C. Amorphous aloin has a darker colour, and is usually contaminated with water-soluble resins. Crystalline aloin is relatively soluble in polar solvents, and virtually insoluble in non-polar solvents. Thus Harders (96) lists 11 solvents, the greatest solubility being in methanol and dioxan (1-13.2, 1-13.3 resp.,) the least in chloroform (1-33,333). Further to this, HÖHME & BERTRAM (27) state that aloin is soluble to the extent of 57% in pyridine (18°C).

4.1.5 CHEMICAL REACTIONS OF ALOIN.

A comprehensive list of 17 reagents producing characteristic colours with aloin is given by SCHULTZ & MEYER (189). However,

it must be borne in mind that as starting point they used Aloin Merck, which has been found to be not entirely free from resins. Those giving perhaps the best differentiation appear in table 4(i).

Table 4(i). COLOUR REACTIONS OF ALOIN (MERCK).

Reagent	Daylight.	Ultra-violet.
1) $AgNO_3$ 1%+NH_4OH 10% (1:1)	Intense grey	Intense red brown
2) $FeCl_3$ 1% aqueous	Intense grey-black	-
3) KOH 10%+H_2O_2 3% (1:1)	Intense bright brown	Bright green fluorescence
4) Mg acet 1% methanolic	Intense orange	Yellow fluorescence
5) NH_3 vapours or solution	Intense yellow	Shining orange

For identifying aloin on chromatograms, preferential use was made of reagents 3), 4) and 5) in the above table, magnesium acetate indicating possible groupings of unknown compounds revealed by it.

Furthermore, to differentiate crystalline aloin from amorphous aloin without recourse to chromatography, LISTER & PRIDE (126) utilise the colour of the fluorescence of the compound in ammonia solution when placed under ultra-violet light, the crystalline aloin fluorescing bright yellow, the amorphous aloin fluorescing bright blue. (This bright blue

fluorescence is later shown to be due to aloesin which itself appears bright (light) blue on unsprayed aloetic juice separated by chromatography). This differentiating test of LISTER & PRIDE has been included in the B.P.C. 1963 (41).

4.1.6 CHEMICAL REACTIONS OF JUICES.

As a routine examination, all juices (in methanol) were tested separately on a white spotting tile with the following: concentrated sulphuric acid, concentrated nitric acid, and ammonia solution. In the case of aloin-containing juices, no great differentiation could be achieved, as this group merely gives yellow brown solutions with sulphuric acid, unchanged by the fumes of nitric acid (in contrast to homonat-aloin-containing juices). With nitric acid no immediate colour change is observed, but on leaving several minutes one species (A.aculeata) slowly turned green. According to TREASE (220), a 1% aqueous solution of dried aloes gives with nitric acid a brownish colour rapidly changing to green. This reported rapidity of action was not observed with methanolic solutions of Aloe juices, and aloin itself merely forms a pale yellow solution with nitric acid, but far deeper yellow solutions with either sulphuric acid or with ammonia solution.

4.2.1 EXPERIMENTAL.

Separation of the constituents, including aloin, of several Aloe species was achieved by using thin-layer chromatography. Methanolic solutions of the aloetic juices were spotted onto Silica gel G plates, activated by heating at 105°C for 30 minutes.

4.2.2 SOLVENT SYSTEMS

Two solvent systems are described widely in the literature, and give good separation of aloin (and if present, aloe-emodin). These two solvent systems have been described throughout this work as Solvents V and P, the Rf value for aloin being 0.42 and 0.87 respectively.

Solvent V:	chloroform: ethanol (95% v/v)	3:1
Solvent P:	ethyl acetate: methanol: water	100:16.5:13.5

4.2.3 DETECTION OF ALOIN.

Due to its characteristic colour when viewed under ultra-violet light, aloin is one of the easiest compounds of Aloe species to identify. Identification can be simplified by utilising spray reagents followed by observation of the plates in daylight and in ultra-violet light. Further confirmation is by melting point, ultra-violet spectra and infra-red spectroscopy. Only the latter was not used in this work.

4.2.4 SPRAY REAGENTS.

Although aloe-emodin was not specifically sought, its identification if present is not difficult, as it runs close to the front with both the afore-mentioned solvents, and gives characteristic colour reactions with several reagents. The reactions of aloin appear in table 4(i). Aloe-emodin gives with these three spray reagents (KOH/H_2O_2; Mg acetate: ammonia) characteristic red colourations.

4.2.5 MELTING POINT DETERMINATIONS.

It was possible to determine the melting point of aloin by removing the aloin spot from the chromatoplate, placing it in a small sintered-glass funnel, and eluting with a few drops of methanol, the filtrate being collected on a watch-glass. This was allowed to evaporate, and the yellow residue was transferred with a needle to the block of a micro-melting point apparatus, and observed through a microscope. The residue was amorphous in character, in contrast to crystalline aloin similarly treated as control. However, a defininte rounding of the yellow amorphous matter indicated the melting points, which appear in table 4(ii). (It should be noted that records of melting points and ultra violet spectra were made only of Aloe species which at the time had not been reported in the literature. Chromatographic data was used to corroborate aloin in species such as A.ferox, A.candelabrum and A.africana).

Table 4(ii). MELTING-POINTS OF ALOIN FROM ALOE SPECIES.

Species.	Melting-point (°C uncorrected).
A.aculeata	146-147
A.arborescens	146-147
A.cameronii	142-144
A dolomitica	144-146
A reitzii	146-147
A.sessiliflora	142-144
A.vanbalenii	144-145
A vryheidensis	144-145

(Phenazone (Merck) (m.pt. 111-113°C) melted at 110-111°C, while phenacetin B.P.C. (m.pt. 134-136°C) melted at 134°C on this apparatus.)

4.2.6 ULTRA VIOLET SPECTRA.

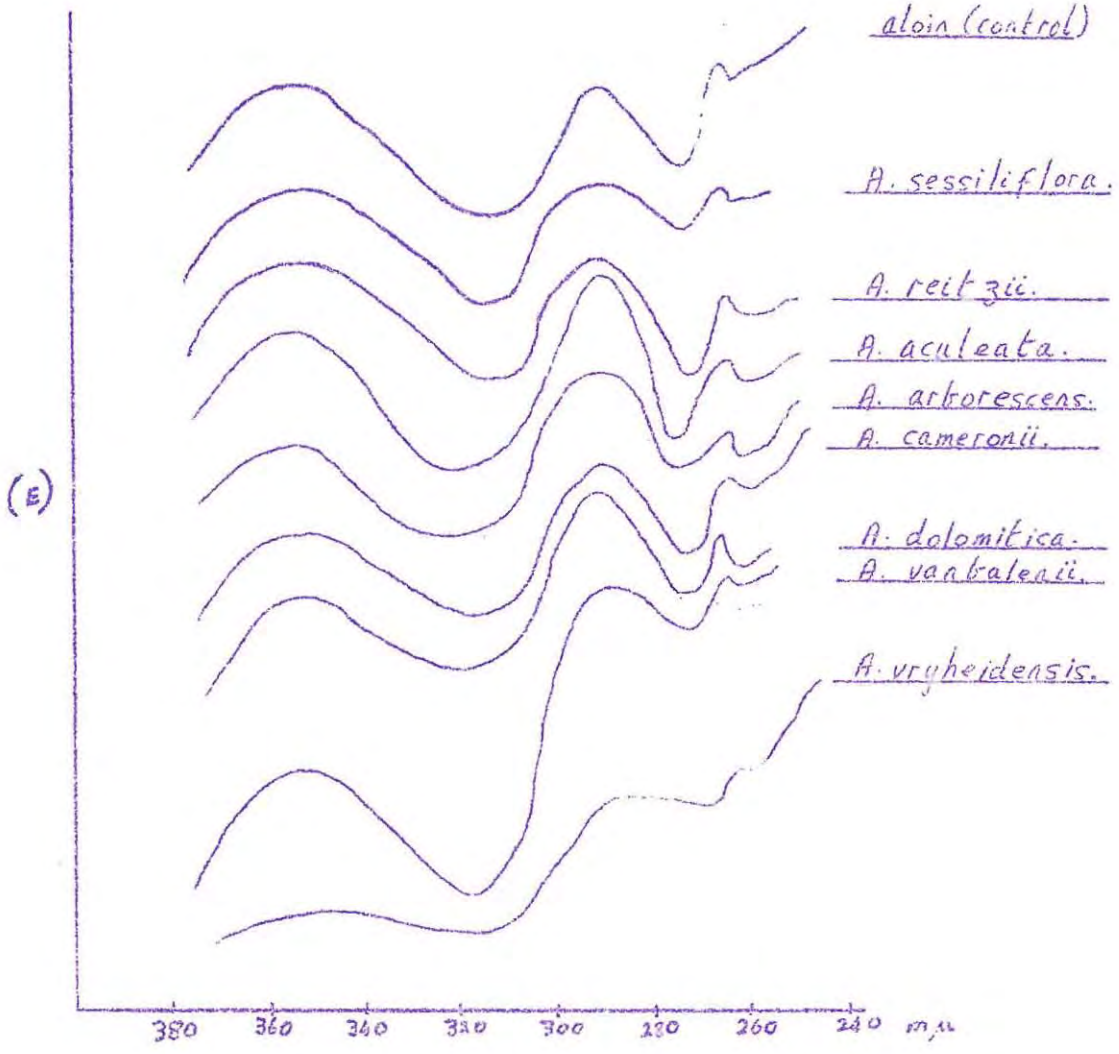
The absorption curves for eight Aloe species appear in figure 4(i). As was the case with melting point determinations, spectra were plotted only for those species which at that time had not been reported in the literature.

Use was made of a Beckman D-B automatic recording spectrophotometer, fitted with 1 cm cells. The spot was taken from the chromatoplate, dissolved in methanol and filtered free from silica gel prior to reading. The spectra are arranged in

figure 4(i)

ABSORPTION CURVES

ALOIN



descending order for clarity, and consequently extinction values are not reported (McCARTHY & PRICE (147)).

4.3.1 SPECIES CONTAINING ALOIN.

Aloin was found in the juice of 14 Aloe species, these being:

A.aculeata Pole Evans,
A.cameronii (Rhodesian),
A.reitzii Reynolds,
A.vanbalenii Pillans,
A.ferox Mill.,
A.excelsa (Rhodesian),
A.africana Mill.,
A.arborescens Mill.,
A.dolomitica Groenewald,
A.sessiliflora Pole Evans,
A.vryheidensis Groenewald,
A.candelabrum Berger,
A.marlothii Berger,
A.castanea Schonl.

Aloin was also found in the leaf of A.striatula var caesia, as reported by VAN OUDTSHOORN & GERRITSMA (226), but not in the leaf of A.pluridens Haw., as reported by VAN OUDTSHOORN, methanolic extracts of both plants being used. Leaves from several A.pluridens plants growing in Port Elizabeth were found not to contain aloin, and it is possible that the former workers obtained a hybrid of pluridens x arborescens which would be difficult to distinguish from A.pluridens. The above-named 14 species appear in figure 8(ii). It is interesting to note that with the exception of A.arborescens and A.striatula var.caesia,

all the above-named Aloe species produce sufficient aloetic juice to warrant their use commercially as a source of aloin.

4.3.2 DISCUSSION OF CERTAIN SPECIES.

Work on the resins contained in Aloe species (Chapter 8) showed how similar in chromatographic appearance the Aloe species are, especially those containing aloin. All yield thick, orange aloetic juice when the leaf is incised, except A.arborescens, where the juice is extremely watery, due possibly to the lack of resins in the juice. With regard to A.marlothii, VAN OUDTSHOORN (227) has shown that in the Transvaal this species forms two sub-groups according to its locality of origin, one sub-group containing aloin, the other homonataloin. At least three A.marlothii plants from the Port Elizabeth-Uitenhage complex have been found to contain aloin, and as yet none has contained homonataloin, as is the case in the Pietersburg district of the Transvaal.

In A.aculeata a compound above aloin turned a purplish colour on treatment with magnesium acetate reagent, followed by heating for 5 minutes at 105°C. This is strongly suggestive of a 1,2-dihydroxy anthraquinone compound (196).

Difficulty was encountered with A.castanea when an attempt was made to determine the melting point of aloin in the manner described in section 4.2.5. The compound melted

at 135°C, showing impurity. A close scrutiny of the chromatogram of A.castanea run in Solvent V showed that the aloin spot in u.v. light appeared to consist of an upper yellow rim and a light brown base. It was possible to separate this spot (using butanol/chloroform/ethanol 4:3:1) into an orange spot (yellow tipped) and immediately above this a pinky red spot. Both these spots appeared yellow in daylight. The orange spot was separated and its melting point was found to be 140°C, indicating that some of the impurity had been removed. Shortly after this VAN OUDTSHOORN (227) extracted crystalline aloin from A.castanea.

4.4.1 ISOBARBALOIN.

Although isobarbaloin, the isomer of aloin, is referred to frequently in textbooks of pharmacognosy as being a constituent of Curaçao aloes, it is surprising how little has actually been written about this compound in the literature.

4.4.2 STRUCTURE OF ISOBARBALOIN.

GARDNER & JOSEPH (87) in 1937 produced evidence that hydrolysis of both aloin and isobarbaloin gave the same anthrone nucleus, namely chrysazin-9-anthrone. MÜHLEMANN (159) described a method of synthesising the glucoside of chrysazin-9-anthrone and shortly after this showed that by analogy the

formula of aloin could be stated as 10-(1':5'-anhydroglycosyl) aloe-emodin-9-anthrone. This formula allowed of isobarbaloin being one or other of the optical antipodes at C(10).

BARNES & HOLFELD (8) showed that spectroscopic examination of both (barb)aloin and isobarbaloin indicated a basic nucleus for each of chrysazin-9-anthrone. They showed that the ultra violet spectra of synthetic anthrones of this type varied with the substituent at C(10). Naming R and R' as substituents at C(10), and R'' as substituent at C(3), they showed the following change of peaks for the different compounds: thus

a) When R,R'&R'' = H, then there is a peak at 355 mμ.

b) When R=Br or Ome, the peak above shifts to 370 mμ.

c) When R' & R'' = H, and R= any substituent other than H, a peak appears at 266-268 mμ.

Since both aloin and isobarbaloin both had peaks at 267 mμ and 359 mμ, this indicated that the sugar moiety of the aloins was joined at C(10) by a C-C bond. As further evidence they found that both aloin and isobarbaloin consumed two moles of HIO_4, indicating only stereochemical differences between the compounds.

HAYNES (personal communication) states that the infra-red spectra of barbaloin and isobarbaloin are almost, but not quite, identical and he and his co-workers believe that isobarbaloin is an optical isomer of barbaloin. They hope in

the near future to study both with N.M.R. Haynes is of the opnion that Klunge's test is the best for checking for the presence of ~~sio~~ isobarbaloin, since chromatography on paper is problematical as the two isomers are not separated by solvents which do not cause streaking.

TESTS FOR ISOBARBALOIN.

4.4.3 KLUNGE'S ISOBARBALOIN TEST (220).

To 20 ml of an aqueous 0.5% solution of aloes is added a drop of saturated copper sulphate solution, followed by 1g sodium chloride and 10 ml of alcohol 90% v/v. Isobarbaloin (Curaçao aloes) gives a wine-red colour which is hastened by warming and which persists for 12 hours. No other commercial variety of aloes gives this test. (The test is however unsuitable for chromatograms.)

4.4.4 NITROUS ACID TEST (220).

To an aqueous solution of aloes is added a few crystals of sodium nitrite followed by a little acetic acid. A rich pink to carmine (isobarbaloin) is given by Curaçao, a lesser pink by Cape, while Socotrine and Zanzibar aloes show little change in colour.

The above test is easily adapted to give a spray reagent. Thus chromatograms were sprayed with a 5% aqueous solution of

sodium nitrite, followed by glacial acetic acid, whereupon isobarbaloin gave a rich crimson colour which deepened to carmine. However, this reaction was found to be far from specific for isobarbaloin, as a similar reddish colour was observed when methanolic solutions of the juices of several Aloe species were treated on a spotting tile with nitrite solution followed by acetic acid.

4.4.5 REACTION OF ALOETIC JUICES WITH NITROUS ACID.

Using the nitrous acid reagent, Aloe species could be divided roughly into six broad colour groups, namely carmine, purple, mauve, red mixed with yellow, rose red and yellowy brown.

a) carmine

This colour was given by Curaçao aloes, and also by A.suprafoliata, A.speciosa & A.wickensii var lutea, which contain homonataloin.

b) purple

A.succotrina (instantaneously), A.branddraaiensis, A.longibracteata, A.longistyla (fading rapidly) & A.mitriformis.

c) mauve

A.lutescens (rapid), A.mutabilis (rapid) A.cryptopoda, A.petrophylla, A.ammophila, A.fosteri, A.dyeri and A.simii.

d) red and yellow

A distans, A.comptonii, A.hereroensis, A.angelica, A.melanacantha & A.saponaria (weak).

e) rose-red

A.pachygaster and A.munchii

f) yellowy brown

All the aloin-containers (except A.aculeata which was instantly carmine, fading rapidly). Also the following non-aloin-containers: A.karasbergensis, A.broomii, A.globuligemma, A.chabaudii, A.pratensis and A.polyphylla.

4.4.6 CHROMATOGRAPHIC SEPARATION.

Several of these Aloe species were run chromatographically, then sprayed with nitrous acid to see which components gave the colour reaction. All the homonataloin-containers gave a rich carmine, changing fairly rapidly to purple, as is described for homonataloin in Chapter 5. Most of these gave a purple tail near the base, and the reaction is probably between nitrous acid and resin. (It is interesting to note, however, that both dithranol and chrysarobin, which contain complex anthraquinones, turned pink with HNO_2, albeit slowly.) The purple tail described above was also given by KIO_4 solution, where in many Aloe species a tail extending up to Rf 0.2 is formed.

BÖHME & KREUTZIG (28) also describe a periodate-positive spot which appears only in Cape aloes, and with periodate gives a blue-violet colour which fades rapidly. This spot also gives Klunge's reaction, which is regarded as specific for isobarbaloin. They state that it is for this reason that previous workers erroneously came to regard Cape aloes as also containing isobarbaloin. Unfortunately they do not attempt to identify their compound.

4.4.7 CHROMATOGRAPHY OF ISOBARBALOIN.

BÖHME & KREUTZIG (28) have separated isobarbaloin by thin-layer and paper chromatography utilising a solvent consisting of ethyl acetate: glacial acetic acid: water (4:1:5). In this solvent isobarbaloin on paper has an Rf of 0.43. They describe the compound as a dark orange spot under ultra-violet light, changing to yellow-orange after treatment with KOH. In daylight it is apparent as a gradually increasing purple-violet spot. After treatment with periodate the spot increases in density and is visible as a strong violet colour which is permanent.

According to HÖRHAMMER, WAGNER & BITTNER (111) isobarbaloin has a yellowy brown colour when viewed under ultra-violet light, whereas aloin and aloinoside are yellow. After spraying with KOH the three compounds retain these colours,

while homonataloin goes yellowy brown.

After spraying with Fast Blue B followed by methanolic KOH, isobarbaloin, aloin and aloinoside all appear green in daylight, whereas homonataloin appears dark green. Isobarbaloin has an Rf value of 0.38 (Silica gel G) using the solvent system ethyl acetate: methanol: water (100:16.5:13.5).

Using the same solvent system and Silica gel G, HÖRHAMMER, WAGNER, BITTNER & GRAF (113) report isobarbaloin in West Indian aloes, but not in Cape, East African, Socotrine or Natal aloes. This is in agreement with work described under EXPERIMENTAL, where isobarbaloin was, however, found also in Zanzibar aloes.

4.4.8 ISOLATION OF ISOBARBALOIN.

One of the few methods that is described for the isolation of isobarbaloin is that of GOLSE (92): macerate aloes with twice the amount of acetone containing a little acetic acid. The aloins remain insoluble. The deposit is collected, dried in air and treated with boiling methyl alcohol, which dissolves it. The barbaloin crystallises on cooling. The isobarbaloin is more soluble and remains in the supernatant liquid. (Presumably once the barbaloin has all crystallised out, the mother liquor will deposit isobarbaloin on concentration.) The two substances are described as crystalline yellow solids, giving the same coloured solutions in water and alcohol.

4.4.9 EXPERIMENTAL.

CHROMATOGRAPHY OF COMMERCIAL ALOES.

Seven varieties of commercial aloes were obtained from the School of Pharmacy, Rhodes University. These seven varieties were A.Curaçao, A.vera, A.Barbados, A.Natal, A.Socotra, A.Zanzibar and Cape aloes. Methanolic solutions of these were run on Silica gel G plates using as solvent ethyl acetate: glacial acetic acid: water (4:1:5).
When observed in daylight, after 16 hours, the samples of Curaçao and Zanzibar aloes, and to a lesser extent of A.vera and A.Barbados all had a mauve spot under the aloin spot, and a mauve spot above aloin in the two former species. This lower spot is isobarbaloin, its Rf being 0.36 (BÖHME & KREUTZIG (28) 0.38). These spots appeared brown under ultraviolet light. On spraying with KlO_4 the isobarbaloin turned violet purple, while a spot immediately under aloin in Natal and Socotra aloes turned purple, which faded rapidly. This seems to be the "periodate-positive" compound described by BÖHME & KREUTZIG (28).

A separate plate was sprayed with HNO_2, whereupon the isobarbaloin, the spot at Rf 0.51 and one at Rf 0.12 turned crimson and later purple in the case of Curaçao aloes, while the spot at Rf 0.51 was present together with isobarbaloin in Zanzibar aloes. The isobarbaloin of both A.vera and Aloe

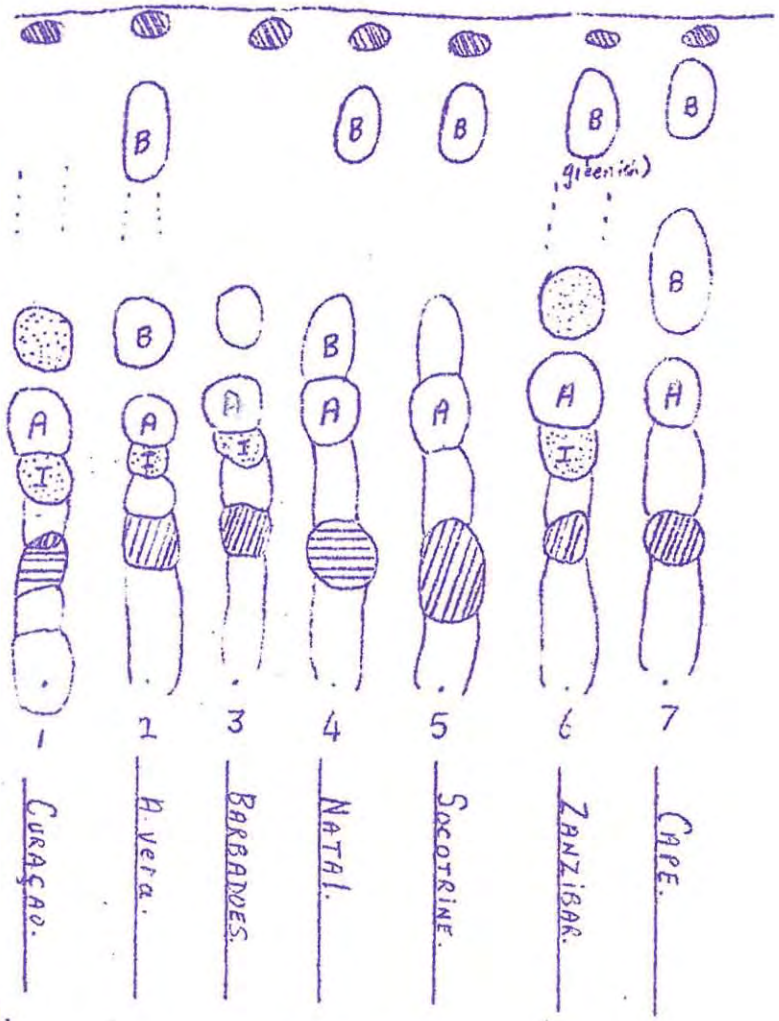
Barbados was shown as a crimson spot by the nitrous acid, but no other spots were visible, while the spots just below aloin in Natal aloes and Socotra aloes were brown (daylight colours).

Finally, a plate was sprayed with alcoholic KOH, which revealed aloe-emodin as a red spot at the front of each sample. (It was noteworthy that even the Natal aloes contained aloin, in contrast to some Natal samples which contain homonataloin. It was probably tapped from A.candelabrum or A.marlothii, or even A.thraskii).

When oversprayed with Fast Blue B solution the resins became apparent, all samples having an orange spot extending from the base to Rf 0.20. Only Cape aloes had an orange spot immediately above the aloin spot, while only Zanzibar aloes had a greenish spot (due to KOH) at Rf 0.81, whereas the others had a blue spot with KOH (resin) which turned orange with Fast Blue B reagent. Chromatograms of the above appear in figure 4(ii).

Thus, as maintained by HÖRHAMMER and co-workers (111) it is possible to differentiate the various commercial species chromatographically, especially if the sample of Natal aloes contains the more usual homonataloin, and if the Cape aloes (type A) contains aloinoside. That these species are not easily differentiated by chemical tests is shown by reference to table 4(iii).

figure 4 (ii).



Colour Code.

A = aloin (orange)

I = isobarbaloin (mauve)

= violet-purple (KIO_4)

= grey (u.v.)

= beige (u.v.)

= red (KOH)

B = blue (KOH - u.v.)

4.4.10 Table 4(iii). CHEMICAL TESTS ON METHANOLIC ALOE SOLUTIONS.

Type	H_2SO_4	followed by HNO_3 fumes	HNO_3
Curaçao	deep yellow brown	green then blue	crimson - orange red
A.vera	ditto.	ditto.	ditto.
Barbados	ditto.	ditto.	ditto.
Natal	ditto.	redder brown	faint crimson - rapidly green
Socotra	ditto.	brown	yellow brown
Zanzibar	ditto.	green then blue	crimson - orange red
Cape	ditto.	no change	yellow brown.

It will be noted that the sample of Natal aloes gives the general reaction for Cape aloes, while Histedt's reaction, which was thought previously to indicate homonataloin specifically, is given by no fewer than four samples, although the first three are merely varieties of West Indian aloes. It should further be noted that isobarbaloin was found to be absent from any of the South African Aloe species examined in this work.

4.5.1 ALOINOSIDE.

As recently as 1963, HÖRHAMMER, WAGNER & BITTNER (111) in an investigation of Cape aloes, described compounds resembling aloin and which they called aloinosides A & B. They found that

aloinoside B had a laxative effect similar to aloin at a dosage of 300 mg/Kg.

4.5.2 STRUCTURE OF ALOINOSIDE.

In the same year McCARTHY (146) noticed similar spots in A.africana, which grows profusely around Port Elizabeth in the Eastern Cape, and from this species VAN OUDTSHOORN & GERRITSMA (226) isolated aloinoside B, which they showed to be a rhamnoside. In 1964 HÖRHAMMER and co-workers (112) isolated aloinoside B from Socotra aloes, and showed it to be aloe-emodin-9-anthrone-10-D-glucosyl-11-mono-α-L-rhamnoside. This compound gave identical melting point, U.V. and I.R. spectra and micro-analyses as that isolated by VAN OUDTSHOORN & GERRITSMA (226). It is interesting to note that aloinoside B has a similar U.V. spectrum to aloin, but the I.R. spectrum differs widely.

4.5.3 CHARACTERISTICS OF ALOINOSIDE B.

A sample of aloinoside B (kindly supplied by Dr. Van Oudtshoorn) had the following characteristics: a pale white to faintly buff coloured compound, with melting point of 234°C. It gave the same reactions to H_2SO_4, HNO_3 and NH_4OH as aloin, but its solution in methanol was colourless to faint yellow, in contrast to the deep yellow colour of methanolic aloin solution. Both aloinoside and aloin (methanolic solutions) fluoresce a

bright yellow colour under ultra-violet light, and show no blue colour when ammonia solution is added (distinction from amorphous aloin).

In contrast to aloin, which is a C-glycosyl compound, aloinoside has both a C-glycosyl and an O-glyccsyl attachment. Thus it can be hydrolysed into aloin merely by heating with 5% HCl, whereas hydrolysis of aloin requires both HCl and ferric chloride solution to sunder the C-C bond.

4.5.4 CHROMATOGRAPHY OF ALOINOSIDES.

Initially it was the work of HÖRHAMMER et alia (100) which demonstrated aloinosides A & B in Cape aloes. They reported the presence of aloinoside only in samples of aloes obtained from the Mossel Bay area of the Cape, whereas commercial samples from the Eastern Cape contained only aloin. Soon after this it was shown by McCARTHY (146), who used samples of fresh aloe juice as distinct from aloe lump used by the former workers, that the presence of aloinosides was confined to Aloe species grown in the Mossel Bay - George region of the Cape, but the line of demarcation stretched further west than Port Elizabeth. Thus as far west as Steytlerville aloetic juice contained aloin alone.

Nevertheless in the Port Elizabeth area, which abounds in A.africana, several samples of juice showed the presence of

aloinoside, which is to be expected from the hybridisation of two such closely similar species. In fact, it is to be wondered if the species A.ferox found in the Mossel Bay area is not a ferox-type which originally was a hybrid form of A.africana and A.ferox, because every sample of A.africana analysed to date has contained aloinoside. Thus just as VAN OUDTSHOORN (227) has demonstrated that two classes of A.marlothii exist in the Transvaal, it may well be that the original species that contained homonataloin has, in merging with A.marlothii, kept its chemical character while losing its physical character. A similar explanation may apply to A.ferox. types.

4.5.5 QUANTITATIVE ESTIMATION OF ALOINOSIDE.

Aloinoside can either be obtained quantitatively by crystallisation from the hydrolysate of a suitable species, or estimated spectrophotometrically. Using the former method, VAN OUDTSHOORN (227) obtained a yield of 3% aloinoside from A africana from the Port Elizabeth area.

In this work, aloinoside has been estimated as aloin spectrophotometrically. This involves a slight error only, as VAN OUDTSHOORN (227) has shown the U.V. spectra of aloin and aloinoside to be nearly identical. Thus, A.africana from the Port Elizabeth area contains 4-7% aloinosides (Chapter 9).

4.5.6 ISOLATION OF ALOINOSIDE.

In this work aloinoside has been found only in Cape aloes, in A.africana and A.reitzii. Using the solvent system chloroform:ethanol (3:1), the aloinosides tend to merge as one spot, which is the reason for the higher percentage obtained above by the spectrophotometric method. The merged spot has an Rf of 0.20 - 0.25 on Silica gel G. Using this same medium, and the solvent system ethyl acetate: methanol: water (100:16.5:13.5), HÖRHAMMER et alia (111) have separated the two aloinosides into discrete spots, aloinoside A having an Rf of 0.29 and aloinoside B an Rf of 0.31. Aloinosides give the same reactions as aloin with the spray reagents use in this work.

4.5.7 DISCUSSION OF THIS CHAPTER.

Aloin has been demonstrated in 14 species of Aloe, two of which (A.africana and A.reitzii) contained, also, aloinosides. Isobarbaloin has not been found in any South African species. In addition to the aloin-containing species reported here, VAN OUDTSHOORN & GERRITSMA (226) have found aloin in the following species: A.pretoriensis, A.petricola, A.peglerae, A.rubrolutea and A.striatula var. caesia. Thus aloin is an important and widely spread entity in Aloe species.

CHAPTER 5.

HOMONATALOIN.

A sample of homonataloin was kindly supplied by Professor L.J. Haynes having the following characteristics - melting point 200-204°C, $[\alpha]_D^{27}$ -111·5° in ethanol, and showing U.V. and I.R. absorption identical with that of the material used for the structural configuration of homonataloin (HAYNES, HENDERSON & TYLER (99)). This sample was extracted from A.wickensii.

5.1.1 REACTIONS OF HOMONATALOIN.

The crystalline yellow sample when dissolved in methanol gave a faintly yellow solution which fluoresced pinky brown under ultra-violet light. Addition of 4N ammonia solution produced a bright yellow solution (daylight) which under ultra-violet light appeared a fluorescent blue (distinction from aloin but not from amorphous aloin) (LISTER & PRIDE (141)). The methanolic solution, spotted on a silica gel plate, gave with potassium periodate reagent a purple colour after some three hours.

The methanolic solution was left to evaporate spontaneously. After a few days these crystals were redissolved, and gave a mauve solution. Homonataloin is thus readily subject to methanolysis.

Crystals of homonataloin were finely powdered. A small portion was treated on a white tile with concentrated sulphuric acid and gave a deep yellow colour which, when fumes of concentrated nitric acid were blown over it, changed through green to deep blue, fading to brown (Histedt's reaction). A similar portion, when treated with concentrated nitric acid, went deep crimson, fading slowly to orange (this differs from the reaction on the sap of species containing homonataloin, inasmuch as there the change from crimson to orange is more rapid - (see section 3.5.1).

In GATHERCOAL & WIRTH (85) the observation is made that the powder of Natal aloe dissolved in concentrated nitric acid assumes a permanent crimson colour, and also gives HISTEDT'S reaction, these tests differentiating it from other aloe varieties. Jaffarabad aloe is also reported as giving a deep crimson with concentrated nitric acid, but no mention is made of HISTEDT'S reaction.

In the opinion of this writer neither test can be claimed to be diagnostic for homonataloin since it has been shown that these reactions are given by several other Aloe species (see section 3.5.1). It is significant that most of these species contain a principle which also reacts with nitrous acid to give an instantaneous crimson-purple colour, as do those containing homonataloin. This former principle tails

on thin-layer chromatograms even in dilute solutions of sap, and has a low Rf value in the chloroform/ethanol solvent system.

5.1.2 COLOUR REACTIONS OF HOMONATALOIN WITH SPRAY REAGENTS.

Spray reagents are of great assistance in the identification of compounds separated by chromatographic means. This is particularly true of homonataloin which takes some 24 hours to form its characteristic mauve-purple colour, but considerably less time when spray reagents are used.

Samples of homonataloin, and of methanolic A.speciosa, (which VAN OUDTSHOORN (224) had shown to contain homonataloin) were run on Silica gel G plates in Solvent V (chloroform/ethanol) and sprayed with the reagents listed below. The colour of the homonataloin before spraying was yellow (daylight) and fluorescent light brown (ultra-violet), which after 24 hours changed to purple and purplish brown respectively.

5.1.3 LIST OF SPRAY REAGENTS.

(i) KlO_4 aqueous (saturated solution).

On immediate spraying the homonataloin was yellowy-brown (daylight) and brown (ultra-violet), these colours changing to mauve-purple and purplish-brown respectively after a period of 4 hours.

(ii) Magnesium acetate 0.5% methanolic solution.

After spraying, the plate was heated at 105°C for 5 minutes, when homonataloin appeared bright yellow (daylight) and fluorescent lime-yellow (ultra-violet).

(iii) Potassium hydroxide 10% aqueous + hydrogen peroxide 3%.

Equal volumes mixed immediately before use produced bright yellow (daylight) and brown (ultra-violet). Excess of spray reagent produces a green tinge both in daylight and ultra violet light.

(iv) Sodium nitrite 5% aqueous, followed by glacial acetic acid.

This reagent produces an almost instantaneous purple-red (daylight) which appears as a deep plum red under ultra-violet. The former colour is far more specific.

(v) Copper sulphate 0.5% aqueous solution, followed by hydrogen peroxide 3% solution.

The plate, on warming at 105°C for a few minutes, shows homonataloin as a deep reddish purple (daylight), which under ultra-violet light is a deep red.

(vi) Potassium hydroxide 5% alcoholic.

This reagent is recommended by BÖHME & KREUTZIG (28) but has nothing to recommend it over the previously-mentioned reagents. In daylight homonataloin appears as a light brown spot after some hours, changing to purplish brown after only 24 hours. Colours under ultra-violet light are correspondingly deeper.

(vii) Vanillin 3% in absolute alcohol containing ½% H_2SO_4 conc.

The plates require heating at 105°C for 5-20 minutes, whereupon homonataloin appears as a yellowy brown tinged with green (daylight), and is greenish brown under ultra-violet. (Under ultra-violet after this treatment aloin and aloe-emodin are orange, while several blue spots turn pink).

Chromatographic investigation.

All the Aloe species listed in table 3(v) were screened for the presence of homonataloin on Silica gel G plates activated at 105°C for 30 minutes.

5.2.1 SOLVENT SYSTEMS EMPLOYED.

Solvent V chloroform/ethanol 95% (3:1)

Solvent O n-butanol/chloroform (5:2)

Solvent P ethyl acetate/methanol/water (100:16.5:13.5)

Solvent V was used initially and pure homonataloin was run as a marker. When examined under ultra-violet light a compound was observed in several species which had an Rf very similar or equal to the homonataloin control marker, and which reacted in identical manner to the control homonataloin when treated with the spray reagents (i), (ii), (iii) & (iv). This compound, which appeared to be homonataloin, was present in the following species:

A.angelica Pole Evans

A.comptonii Reynolds

A.cryptopoda Bak.

A.distans Haw.

A.hereroensis Engler.

A.lutescens Groenewald

A.melanacantha Berger

A.mitriformis Mill.

A.munchii Christian

A.mutabilis Pillans

A.pachygaster Dinter.

A.pearsonii Schonl.

A.speciosa Bak.

A.suprafoliata Pole Evans

A.wickensii var lutea Reynolds

5.2.2 CHEMICAL REACTIONS ON ALOE JUICES.

Methanolic solutions of each of the above species was examined on a spotting tile using, respectively, concentrated sulphuric acid, concentrated nitric acid, and concentrated sulphuric acid followed by fumes of nitric acid.

All the species gave the reactions described before for homonataloin, but there were two variations with the nitric acid test - A.suprafoliata instead of forming an immediate

crimson colour slowly gave an orange-red colour which deepened on standing, while with A.angelica only a slight darkening of the yellow methanolic solution resulted.

5.2.3 CHROMATOGRAPHIC ANALYSIS.

It was observed that in homonataloin-containing species especially, the fresh sap rapidly forms an amorphous deposit, particularly when obtained from the larger species. Methanolic extracts of these species were run on Silica gel G plates activated at 105°C for 30 minutes prior to use, Solvent V (chloroform/ethanol) being employed initially.

This solvent produces relatively large spots, in contrast to the compact, flattened spots produced in Solvent P (ethyl acetate/methanol/water). The different chromatographic pictures obtained are reflected in the chromatograms traced from the original plates (20cm x 20cm), and from the colour photograph of these species, run in the former case in Solvent V, and in the latter case in Solvent P.

5.3.1 PHOTOGRAPHY.

Black-and-white photographs of chromatoplates fluorescing under ultra-violet light can be taken without difficulty using an ordinary camera and a fast film. These, of course, do not produce the characteristic colours visible in Aloe species

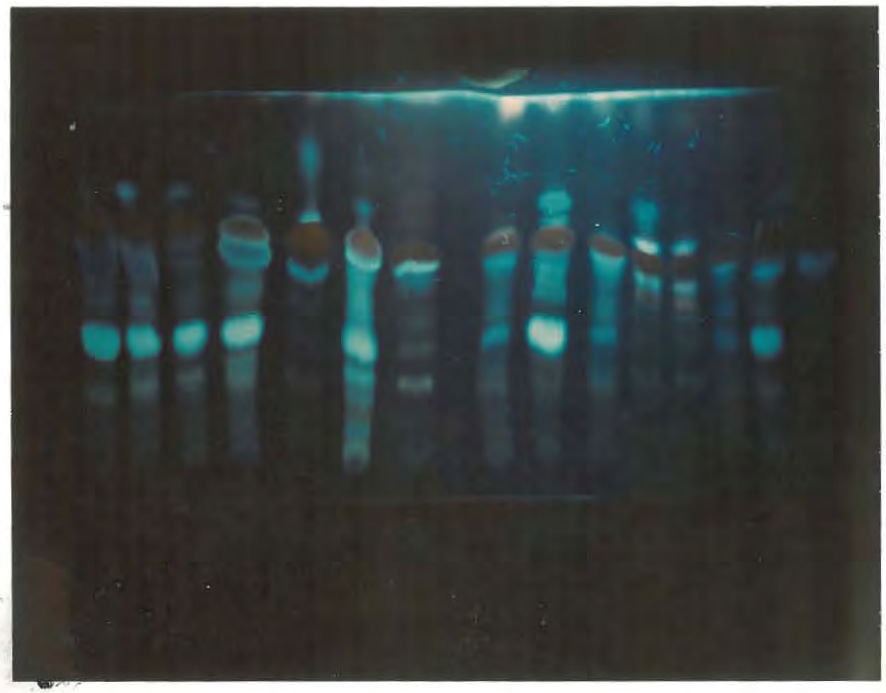
when placed under ultra-violet light, and apart from the speed with which the permanent record can be made, are in no way better than records made by tracing the outlines of each spot, which has been delineated under u.v. using a long needle. The latter method, in fact, enables individual Aloes to be traced with sufficient distance between each to enable the colour to be written next to each spot, while labelling of photographs is extremely difficult.

Colour photography obviates such a necessity, but although immensely superior to tracings which merely name the colour, the colours produced are not exact reproductions of the colours observed under ultra-violet light. The photograph of the 15 homonataloin species (using Solvent P)(figure 5(i)) were taken using Polacolor Reversal Film, f .4.5 for 85 seconds (courtesy Mr. B. Mann, A.R.P.S.). The photograph does, however, clearly show the preponderance of blue colours and the multiplicity of different components, in each Aloe species. The brown homonataloin spots are clearly shown, while the luminous whitish blue spots at Rf 0.40 are aloesin (section 8.7.12).

5.3.2 LOCALISATION OF SPOTS.

When viewed under ultra-violet light, each species was observed to have a spot fluorescing with the same characteristic

figure 5(i).



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 (obscured)

1. A. mitriformis
2. A. distans
3. A. pearsonii
4. A. comptonii
5. A. cryptopoda
6. A. angelica
7. A. suprafoliata
8. A. hereroensis
9. A. munchii
10. A. pachygaster
11. A. lutescens
12. A. wickensii (var. lutea)
13. A. melanacantha
14. A. mutabilis
15. A. speciosa

hydrolysis of the homonataloin to mono-o-methyl nataloe-emodin. VAN OUDTSHOORN (227) has described the daylight colours of methylnataloe-emodin and nataloe-emodin with mag. acetate as orange and violet respectively, hence differentiation is possible. Thus no free anthraquinones occurred in these species.

However, both with this reagent, and with the alcoholic KOH, several yellow spots were observed. According to BETTS et alia (18), combined anthraquinones give yellow to orange colour on treatment with magnesium acetate. SHIBATA et alia (196) describe the colours of several hydroxyanthraquinones as orange, only rubiadin forming orange-yellow. As stated before, yellow spots which fluoresced bright yellow under ultra-violet light have been observed in several species i.e. A.mitriformis, A.distans and A.comptonii (all at Rf 0.60 where homonataloin was 0.66), and at Rf 0.27-0.29 in the species A.munchii, A.lutescens, A.wickensii var. lutea, A.melanacantha, A.mutabilis, and A.speciosa. These are probably resins. MARY et alia (145) have reported an anthranol in Curaçao, Cape and Socotrine aloes as also in commercial aloin and cascara sagrada, which gave a yellow colour with alkalies "instead of the orange or pinkish colour characteristic of the other anthracene drugs": which further gave a yellow colour with magnesium acetate: and which exhibited no absorption

peak at any wavelength over a wide range of the ultra-violet and visible spectrum.

KHORANA AND SANGHAVI (120) observe that sennidin A & B both produce a yellow colour with alcoholic sodium hydroxide which slowly changes to violet on standing. A violet colour was observed on spraying A.mitriformis with alcoholic KOH, but it appeared fairly rapidly. The production of a mauve colour which tails even in dilute concentration has been mentioned for both the magnesium acetate and nitrous acid reagents, and also occurs with potassium periodate. It might conceivably be the case that dianthrones, which occur in Rheum, Frangula and Cassia species, also exist in Aloe species. The tailing of the compound even in low concentration is concordant with a substance of high molecular weight.

5.4.1 EXTRACTION AND SPECTROPHOTOMETRY.

The 15 species described in this chapter were chromatographed in Solvent V on small Silica gel G plates. These, when suitably developed, were visualised under ultra-violet light, and the brown homonataloin spot carefully scraped off and placed into small test tubes. The contents of each tube was dissolved in methanol, and filtered through fine filter paper.

The resulting filtrates were examined and recorded using a Beckman D.B. automatic recording spectrophotometer. To

obtain a measure of uniformity, each solution was diluted (if necessary) to give an initial transmission at 390 mμ of 80% to 90%. The peaks recorded for the 15 species appear in figures 5(iii) & 5(iv), where they have been arranged in descending order to enable a comparison to be made. Consequently, extinction values are not quoted.

Spectrophotometric Differences in Species.

It will be observed from figures 5(iii) & 5(iv) that the 15 species fall into two broad groups, which shall be termed Group X and Group Y.

5.4.2 GROUP X.

According to HAYNES et alia (99), homonataloin has the following maxima: 347, 294, 273 (infl), 250 (infl), 222 mμ. The following species have almost identical peaks, although the inflection at 250 mμ is in several cases very small:
A.angelica, A.speciosa, A.suprafoliata, A.lutescens, A.cryptopoda, A.wickensii var. lutea, A.mutabilis, A.melanacantha, A.munchii, A.pachygaster, A.hereroensis.
In the cases of A.hereroensis and A.pachygaster the inflection at 273 mμ is absent.

5.4.3 GROUP Y.

In four species, namely A.mitriformis, A.distans, A.comptonii and A.pearsonii, the spectral picture varies from

figure 5(iii).

ABSORPTION CURVES.

HOMONATALOIN.

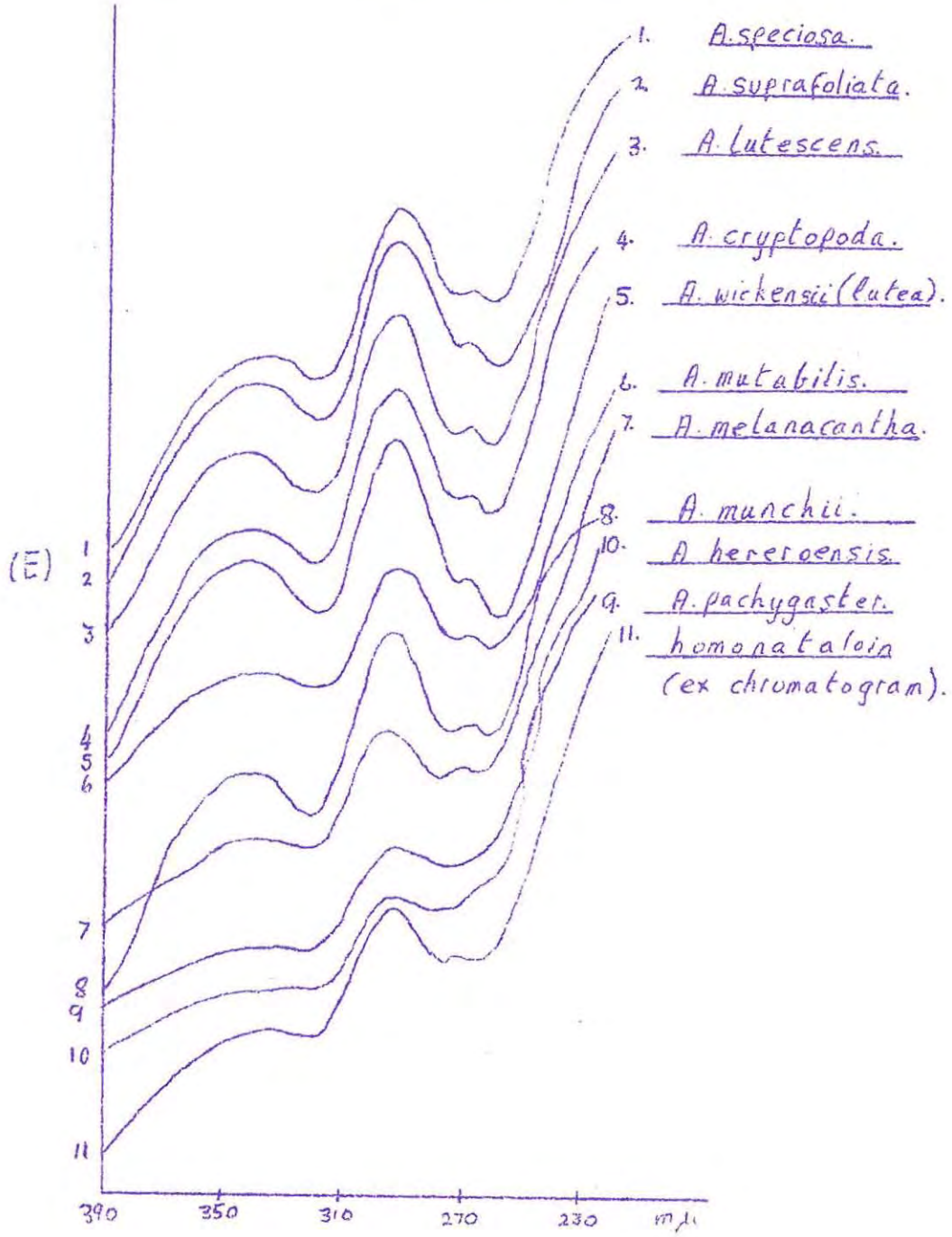
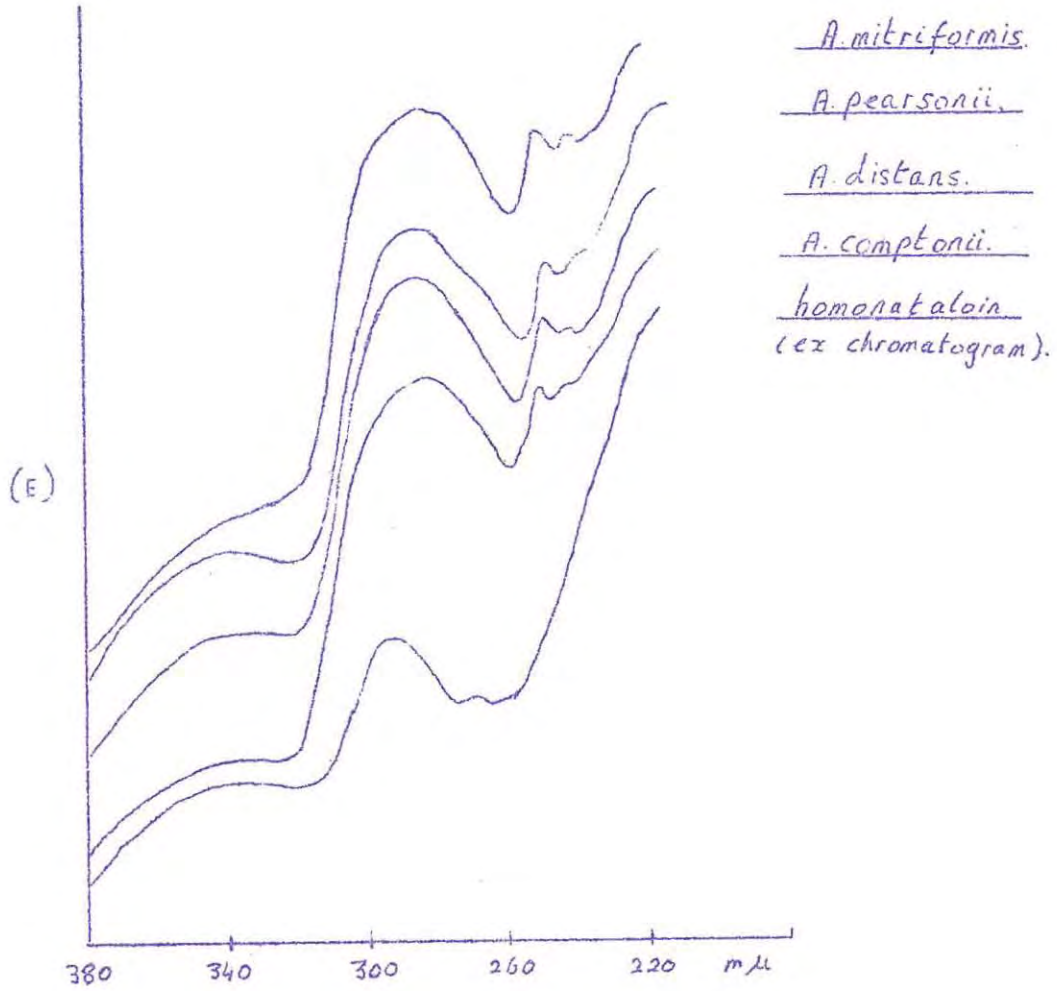


figure 5(iv).

ABSORPTION CURVES.

HOMONATALOIN - RESIN.



Group X, with maxima at 347, 290, 273 (infl), 252, 244, 222 mμ.

The inflection at 273 mμ is weak, while the peaks at 252 and 244 mμ are small but sharp. It has been observed (section 5.3.3) that three of these species i.e. A.mitriformis, A.distans and A.comptonii, belong to the Series Latebracteatae, and had been shown chromatographically to contain a similar principle just below the homonataloin. No such principle was observed in A.pearsonii, however.

Postulating that impurity from a confluent spot was causing the different spectral picture, these four species were run in Solvent P, removed as previously described, and their peaks recorded. It at once became obvious that the spectral pictures for the species in the two solvents were identical. Professor Haynes (personal communication) suggested that since some Aloe species contained isomeric form of aloin, namely aloin (barbaloin) and isobarbaloin, the possibility existed of there being both homonataloin and an isomeric form thereof. Later work, however, showed that these peaks were due to resins. (McCARTHY & PRICE (148)).

EXTRACTION.

In most cases the samples of sap used were from one or two leaves kindly donated from the gardens of Aloe collectors, and in consequence the amount of material available was very

small. Furthermore, those samples underwent repeated usage, while repeated extraction with methanol and other solvents had caused hydrolysis to take place in all samples. As a consequence thereof the method of micromelting point determinations successfully applied to aloin-containing species (section 4.2.5) could not be applied, as the compound turned purple at temperatures circa 170-180°C on the heating block of the melting point apparatus.

5.4.4 A.SPECIOSA.

A.speciosa grows freely both in Port Elizabeth and Grahamstown, and several leaves were collected from an A.speciosa plant growing in a grove of A.speciosa some ten miles outside Grahamstown.

In essence the method of HAYNES and co-workers (99) for the isolation of homonataloin from Natal Aloes was employed as follows: To 8g sap, dried at 100°C, was added 16 ml acetone, and the suspension shaken occasionally during 24 hours.

The suspension was then filtered under reduced pressure through filter paper, and the red filtrate reserved (A).

Undissolved solid (B) (circa 5g) was refluxed with 50 ml of 60% alcohol for 30 minutes on a waterbath and then filtered. The filtrate (C) was set aside overnight to crystallise. The residue (D) which remained on the filter (circa 0.5g) was

insoluble in methanol, ethanol, chloroform, toluene and water.

The solution (C) deposited a crop of circa 100 mgm of shiny yellow crystals, a portion of which was chromatographed after acetone-washing on a filter, together with (A) and (B) which has been reserved.

(A) contained all the components normally found in A.speciosa, while (B) and the mother-liquor from (C) were identical, but differed from (A) in that two spots (brown and grey-brown) were absent. The crystalline homonataloin chromatographed pure, but was re-crystallised once from methanol to give a melting point 201°-204°C (uncorrected).

5.4.5 A.COMPTONII.

Several leaves of this species were kindly supplied by a Port Elizabeth collector. However, as the sap yielded was far less than the amount obtained from the A.speciosa, the acetone extraction used for A.speciosa was omitted.

0.5g dried sap from A.comptonii was refluxed for 1 hour with 20 ml alcohol 60%, filtered hot and the filtrate left overnight. As no precipitate had formed, the filtrate was concentrated to half its volume, whereupon a glue-like precipitate resulted. This was discarded and the clear filtrate left overnight. The resulting crop of yellow-brown crystals was washed with 20 ml water and dried at 50°C. The melting point

of the dried crystals was 199°-204°C, so that they were re-crystallised twice from methanol, whereupon the ultra-violet spectrum and melting point (202°-204°C) of homonataloin were obtained. At a later date it was ascertained that the impurity was in fact resinous in nature.

5.4.6 A.MITRIFORMIS.

Using the method described by VAN OUDSTHOORN (224) 0.32g dried sap (obtained from a plant growing in the Albany Museum grounds) was refluxed with 3 ml methanol for 30 minutes on a water bath, and filtered hot. This sample took several days to crystallise, due to the high resin content, and was obtained impure. Re-crystallisation from methanol was extremely slow with consequent methanolysis, and so the impure crystals were recrystallised from isobutyl alcohol as star-shaped clusters having a melting point of 202-204°C, but showing slight rounding at 198-199°C. The spectra of both A.comptonii and A.mitriformis assumed the form of pure homonataloin after this purification, eliminating the peaks caused by contaminating resin, which has peaks (section 5.4.3) at 295mμ, 252mμ and 244mμ. Although further samples of A.distans and A.pearsonii could not be obtained, there is no doubt that the peaks at 252mμ and 244mμ in these species were also due to the presence of resin which had merged with the homonataloin spot.

Interference due to resin is also reflected in the ultra-violet spectra of A.pachygaster and A.hereroensis, the particular resin being later revealed (by use of Fast Blue B reagent) at the base of the homonataloin spot (figure 8(iii)). By using Solvent O (butanol: chloroform 5:2) it was possible to raise the resin impurity in these species, where it appeared as a blue halo around the homonataloin, which fluoresced reddish instead of its customary brown colour.

5.5.1 DISCUSSION.

The presence of homonataloin in a relatively large number of species shows that, along with aloin and chrysophanol, homonataloin could be used as a basis for a chemical taxonomic grouping of several Aloe species. The intimate mixing of resin and glycanthrone in several species makes direct melting points from an eluted chromatographic (silica gel) spot virtually impossible, in contrast to aloin-containing species. However, in several homonataloin-containing species the resin content is so low that direct extraction and crystallisation of glycanthrone is far easier than with aloin-containing species.

CHAPTER 6.

SCREENING OF ALOE SPECIES NOT CONTAINING ALOIN OR HOMONATALOIN.

6.1.1 INTRODUCTION.

Several *Aloe* species chromatographed in the solvent system chloroform/ethanol 95% (3:1) had shown neither aloin nor homonataloin, which due to their characteristic fluorescent colours under ultra violet light are easily detectable.

The spot test techniques employed using nitrous acid had indicated that apart from those species containing homonataloin, which gave mauve to purple colours with nitrous acid, several *Aloe* species not containing homonataloin similarly gave mauve to purple colourations, and this was further observed with both dithranol and chrysarobin, which contain anthraquinone derivatives.

Consequently, since these *Aloe* species had shown several spots when chromatographed in the solvent employed for aloin and homonataloin, it was decided to screen these species using several solvent systems. The *Aloe* species studied in this manner are as follows:

6.1.2

A.grandidentata Salm Dyck

A.longibracteata Pole Evans

A.simii Pole Evans

A.fosteri Pillans.

A.dyeri Schonl.

A.ammophila Reynolds

A.petrophylla Pillans.

A.branddraaiensis Groenewald

A.saponaria (Ait.) Haw

A.chabaudii Schonl.

A.globuligemma Pole Evans

A.karasbergensis Pillans.

A.longistyla Bak.

A.pratensis Bak.

A.polyphylla Schonl.

A.reynoldsii Letty.

A.broomii Schonl.

None of these samples was freshly collected, and all had frequently been extracted with methanol.

6.1.3 SOLVENT SYSTEMS.

The following solvent systems were employed:

Z Toluene (paper)

Y Benzene:glacial acetic acid 2:1 (T.L.C.)

X Isopropyl ether (T.L.C.)

W Toluene/petrol ether (paper)

V $CHCl_3$/ethanol, 95% 3:1 (T.L.C.)

U Ethyl acetate/n-propanol/water/acetone 50:15:15:30 (T.L.C.)

T Acetic acid/formic acid/ethyl acetate/water 3:1:18:4 (paper)

S Butanol/ethanol 95%/water 40:11:9 (paper)

R n-propanol/ethyl acetate/water 4:4:3 (T.L.C.)

Q Benzene/carbon tetrachloride 1:1 (T.L.C.)

6.1.4 TOLUENE: SOLVENT Z.

Whatman No. 1 paper was used, an accommodation time of 1 hour being employed; (ascending chromatography, at room temperature.) This solvent was used for all the Aloe species listed except A.broomii. It was found that paper chromatography is not as sensitive as the thin-layer chromatography for detecting hydroxyanthraquinones, and visualisation both by spraying and by using ultra-violet light is more difficult with the former. Consequently multiple spotting was used after it had been found that the usual pipette volume (6 µl) gave scarcely-discernable spots. Furthermore it was found that cold methanolic extracts of the saps gave far weaker reactions than when the saps were extracted (cold extraction) with chloroform/ethanol (3:1).

Certain spots were visible which when sprayed with alcoholic KOH turned pink, indicating hydroxyanthraquinones. The Rf values thus obtained are listed in table 6(i).

Table 6(i).

Aloe.	Colour with KOH.	Rf.
A.grandidentata	pink	0.99
A.longibracteata	pink	0.99
A.simii	pink	0.99 & 0.56
A.fosteri	pink	0.99
A.dyeri	pink	0.99 & 0.57
A.ammophila	pink	0.99 & 0.63
A.petrophylla	pink	0.99
A.branddraaiensis	orange-pink	0.99
A.saponaria	pink	0.99
A.chabaudi	-	0.99
A.globuligemma	-	0.99
A.karasbergensis	pink	0.99 & 0.12 (tailing)
A.longistyla	-	0.99
A.pratensis	-	0.99
A.polyphylla	-	0.99
A.reynoldsii	-	0.99
methanolic Rheum extract	pink	0.98

6.1.5 BENZENE: ACETIC ACID GLACIAL (2:1) SOLVENT Y.

Methanolic solutions of the 17 Aloe species were run on Silica gel G in the above solvent system, and sprayed with

alcoholic KOH 5%. Pink-red spots became visible in the following:

Table 6(ii).

Aloe.	Colour with KOH.	Rf.
A.grandidentata	pink (weak)	0.98
A.longibracteata	pink (weak)	0.98
A.simii	pink	0.98
A fosteri	pink-red	0.98 & 0.91
A.dyeri	pink	0.98
A.ammophila	pink	0.98
A.petrophylla	pink	0.98
A.branddraaiensis	pink-red	0.98
A.saponaria	pink	0.98
A.chabaudii	pink-red	0.98 & 0.09
A.karasbergensis	pink-red	0.98 & 0.87
A.polyphylla	pink-red	0.98 & 0.15
A.reynoldsii	pink (weak)	0.98

When these 17 Aloe species were extracted with petrol ether and run in this solvent all ran as a yellow front.

6.1.6 ISOPROPYL ETHER. SOLVENT X.

Methanolic extracts of the 17 Aloe species excluding A.broomii were run on Silica gel G plates, and the plates

sprayed separately with alcoholic KOH 5% and methanolic magnesium acetate 0.5%. The results are recorded in table 6(iii).

Table 6(iii).

Aloe.	Colour with KOH / MgAcetate		Rf.
A.simii	pink	red	0.98
A.fosteri	pink	red	0.98,0.83, 0.16
A.dyeri	pink	red	0.98
A.ammophila	pink	red	0.98
A branddraaiensis	pink	red	0.98
A.chabaudii	pink	red	0.98
A.karasbergensis	pink	red	0.98,0.83
A.polyphylla	pink	red	- , - , 0.12

The 16 species were then extracted with petrol ether (cold), but none ran in Solvent X.

6.1.7 TOLUENE/PETROL ETHER. SOLVENT W.

VAN OUDTSHOORN (227) has reported excellent separation of Aloe constituents on paper using the systems toluene/pentane. The samples are run 5 cm in toluene using ascending chromatography,

the papers removed and dried, and then run a further 5 cm in pentane. In this manner a separation of chrysophanol and physcion can be achieved by the pentane, whereas rhein, aloe-emodin and emodin, which migrate in the toluene, remain stationary in the pentane phase.

As pentane was not available, petrol ether (40-60°) was substituted. Accommodation times of 30 minutes and 15 minutes respectively were used for the toluene and petrol ether, and methandic extracts of Aloes grandidentata, longibracteata, simii, fosteri, dyeri, ammophila, petrophylla, branddraaiensis and saponaria, were spotted onto Whatman No.1 paper which was marked at the 5 cm and 10 cm heights. Using toluene/petrol ether, satisfactory separation was obtained with the toluene, but, there was no further migration using the latter solvent. Rf values are recorded in table 6(iv) for the toluene separation only

Table 6(iv).

Aloe.	Rf.
A.grandidentata	0.99
A.longibracteata	0.99 (weak)
A.simii	0.99 & 0.57
A.fosteri	0.99, 0.32, 0.16
A.dyeri	0.99 & 0.57
A.ammophila	0.99 (weak) & 0.63
A.petrophylla	-
A.branddraaiensis	0.99
A.saponaria	0.99 (weak)

6.1.8 CHLOROFORM/ETHANOL 95% (3:1). SOLVENT V.

Since aglycones had been shown in several of the aforementioned species by means of the spray reagents alcoholic KOH and magnesium acetate, it was decided to examine these species for the presence of sugars and/or glycosides, particularly as VAN OUDTSHOORN (224) had reported a glycoside in A.saponaria which on hydrolysis yielded chrysophanol.

The tabled Aloe species were run on Silica gel G plates in Solvent V, then sprayed with periodate/starch reagent, where sugars and/or glycosides are revealed as white spots on a mauve background, the Rf values of which appear in table6(v).

Table 6(v).

Aloe.	Rf. of sugar/glycoside.
A.grandidentata	0.07
A longibracteata	0.10
A.simii	-
A.fosteri	-
A.dyeri	0 10
A.ammophila	0.10
A.petrophylla	0.10 & 0.21
A.branddraaiensis	0.10
A.saponaria	0.10
A.chabaudii	0 10, 0.15, 0.37
A karasbergensis	-

The above were re-run in Solvent V on Silica gel G and sprayed with p-anisidine HCl, and thereupon heated at 100°C for 5 minutes. Brown spots formed indicating cis-hydroxyl reducing groups, but as no spot exceeded an Rf of 0.10, the 17 species were run on Silica gel G in Solvent U, which also did not give good separation, hence paper chromatography was resorted to for separation of sugars/glycosides.

Thin-layer chromatography on Silica gel G of the remaining six species not appearing in table 6(v) gave interesting results. Solvent V was once again the system employed. Duplicate plates were run, one being sprayed with alcoholic KOH, the other with periodate/starch reagent. In the former case both aglycones (red) and possible glycanthrones (yellow) were seen, while several spots appeared with the periodate/starch reagent, indicating sugars or glycosides.
The results appear in table 6(vi).

Table 6(vi).

Aloe.	Rf.	U.V.	Colour. K.O.H.	eriodate/ starch
A.globuligemma	0.13	-	-	white
	0.32	-	-	white
	0.45	-	-	white
A.longistyla	0.27	yellow	-	-
	0.41	yellow	-	-
	0.60	yellow	-	-
	0.92	yellow	-	-
A.pratensis	0.26	pinky orange	-	-
	0.36	grey	-	-
	0.83	pinky orange	-	-
	0.20	-	-	white
	0.32	-	-	white
	0.45	-	-	white
A.polyphylla	0.32	-	-	white
	0 44	yellow	intense yellow	-
	0.58	yellow	pink	-
A.broomii	0.44	yellow	-	-
	0.59	yellow	yellower	-
A.reynoldsii		No result.		

The reactions of A.polyphylla with alcoholic KOH suggest the presence of both a glycanthrone (Rf 0.44) and an aglycone (Rf 0.58). It is surprising that since A.broomii has spots at almost identical Rf's to those of A.polyphylla, identical results with the KOH spray were not obtained.

6.1.9 SUGARS AND/OR GLYCOSIDES

The previous work had indicated the presence of spots which indicated either sugars, glycosides or both. Where aglycones were also present in a species the possibility of a glycoside having existed, and having been hydrolised by frequent solution in methanol, could not be overlooked. Consequently use was made of the Solvents T, S and R primarily for the separation and detection of sugars or glycosides.

6.1.10 ACETIC/FORMIC/ETHYL ACETATE/WATER (3:1:18:4) SOLVENT T.

Descending chromatography on Whatman No. 1 paper was used, with no accommodation time. The tank was saturated with the vapour phase, there being solvent both in the base of the tank and in the trough for several hours. The papers were allowed to run overnight, with consequent elution of solvent from the base, which gave good separation of the components of the sugar mixture used as a marker. This marker consisted of 2% w/v solutions of each of the following:- D-ribose, D-galactose, D-arabinose and L-rhamnose.

In the Aloes longibracteata, dyeri, ammophila, saponaria, branddraaiensis and petrophylla the reagent p-anisidine HCL showed spots at the base of the galactose marker and almost on a line with the arabinose marker. However, these spots were faint, and the possibility existed that the methanolic

solutions of the other saps run had been too weak to show a positive reaction. The location of the spots near the base of the galactose marker indicated that for this solvent system glucose might be present, and so D-glucose was included in the marker mixture.

When the duplicate paper was sprayed with periodate/starch reagent a colourless zone became apparent in A pratensis between the arabinose and ribose markers, while in A.chabaudi the yellowy white spot was very close to the ribose markers. In the case of A.polyphylla a mauve spot formed with the periodate, as does homonataloin, which this species does not contain.

Two further chromatograms were run in Solvent T by the descending method on Whatman No.1 paper, using the 5-sugar mixture as marker. After about seven hours (ca 40 cm length) the papers were removed, dried, and viewed under ultra-violet light. From this it was clear that the pigmented spots had migrated towards the front, that is, had Rf values exceeding 0.60. Streaking was observed due to the multiple spotting, especially of blue fluorescent pigments. A clear greenish spot was observed at Rf 0.35 in A saponaria. This is probably a flavonoid.

Further visualisation.

a) p-anisidine HCl spray. This reagent appears to be more sensitive than the periodate/starch reagent. The sugar markers had separated well, with the exception of glucose and galactose, which are normally difficult to separate.

Once again a spot not quite on the Rf of the two arabinose markers (which were on either side of the Aloe species) was observed in the following species; A.grandidentata, A.longibracteata, A.simii (weak), A.ammophila, A.petrophylla, A.branddraaiensis and A.saponaria. The colour of these spots was yellow in contrast to the brown of the arabinose markers.

A spot opposite the base of the galactose/glucose markers (and therefore corresponding more to the latter) was observed in each of the species run, namely: A.grandidentata, A.longibracteata, A.simii, A.fosteri, A.dyeri, A.ammophila, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii, A.prantensis and A.broomii. Other spots were observed in some of the species and these are recorded in table 6(vii), where the Rf values quoted are relative to the arabinose markers taken as 100.

Table 6(vii).

Aloe.	Rf arabinose (p-anisidine 100 HCl)			Rf arabinose 100 (periodate/starch)
A.simii (weak)	-	28	-	25
A.fosteri (weak)	-	28	-	25
A.dyeri	-	-	52	-
A.ammophila	-	28	52	-
A.petrophylla (weak)	-	28	-	-
A.branddraaiensis (weak)	14	28	-	25
A.saponaria	14	28	52	25
A.chabaudii	-	-	52	25

b) periodate/starch spray.

Apart from the above spots, periodate/starch reagent revealed spots close to the Rf of arabinose (R94) in A.longibracteata, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii, and A.pratensis, but only three species (A.grandidentata, A.saponaria and A.chabaudii) had spots opposite the galactose/glucose marker, which was less spots than that revealed by the p-anisidine HCl spray. In contrast however, periodate/starch revealed weak spots in both A grandidentata and A.longibracteata (R37) not revealed by the former spray.

6.1.11 BUTANOL/ETHANOL 95%/WATER (40:11:9) SOLVENT S.

Whatman No.1 paper was used with this solvent. Using the descending technique described before, but spotting the galactose and glucose markers separately, it was ascertained with p-anisidine HCl spray reagent that the spots observed previously agreed exactly with the glucose marker, the galactose being clearly seen to be at a lower Rf. Spots at the starting line showed that the other reducing components had not migrated in this solvent system.

6.1.12 PROPANOL/ETHYL ACETATE/WATER (4:4:3) SOLVENT R.

The 17 named Aloe species were run on Silica gel G plates in Solvent R. Spraying with periodate/starch reagent revealed several white spots on a blue background. All the species had a spot directly in line with the D-glucose run as marker. This confirms the results obtained with Solvents T and S. Six of these species had spots at a higher Rf than the glucose marker, and these species were run on Silica gel G in Solvent R in smaller concentration to give smaller spots, especially of those above the Rf of the glucose marker.

Although both plates had been activated under the same conditions, the time taken to spot the first plate with the 17 species and control markers was far longer than for the

second plate, which required only 10 spottings. In consequence the Rf values differ, as do the R glucose values. Consequently the Rf values of the glucose and the other observed reducing sugars are given in table 6(viii), where their positions relative to glucose can be ascertained.

Table 6(viii).

Rf. of observed spot.	Aloe species having this spot.
0.42 (D-glucose)	All 17 species.
0.24-0.26	A.simii, A.dyeri, A.ammophila, A.saponaria, A.globuligemma, A.karasbergensis & Rheum B.P.
0.1-0.12	A.longibracteata, A.simii, A.dyeri, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii, A.globuligemma, A.karasbergensis, A.pratensis A.polyphylla & A.reynoldsii.

In table 6(ix) are recorded the Rf values obtained for the six species run on the second plate, with a cold methanolic extract of Rheum B.P. and glucose as markers.

Table 6(ix).

Rf. of observed spot.	Species having this spot.
0.15	A.petrophylla, A.saponaria, A.chabaudii, A.globuligemma.
0.38	A.petrophylla, A.saponaria.
0.53 (glucose)	A.petrophylla, A.saponaria, A.chabaudii, A.globuligemma, A.pratensis, A.polyphylla, & Rheum B.P. extract.
0.63	A.chabaudii, A.globuligemma, A.pratensis, A.polyphylla.
0.69	A.petrophylla, A.saponaria, A.chabaudii, A.globuligemma, A.pratensis (tailing) & Rheum B.P. extract.
0.79	A petrophylla, A.globuligemma, A.pratensis (tailing)
0.88 & 0.94 (two merged spots) or 0.91	A.petrophylla and Rheum extract A.saponaria, A.chabaudii, A.globuligemma, A.pratensis (tailing)

In the above table 6(ix) all the spots having an Rf higher than 0.53 were initially yellow or as in the case of A.petrophylla, blue under u.v. Furthermore, in all the species except A.grandidentata, A.reynoldsii and A.polyphylla, a bright orange

fluorescent spot was observed at the solvent front.

Anthraquinone derivatives.

The 17 Aloe species were run on Silica gel G in Solvent R and observed under ultra violet light. Several spots were seen to fluoresce yellow or orange. Blue fluorescent spots were noticed only in A.petrophylla, A.globuligemma and A.karasbergensis. This chromatogram was then sprayed with methanolic magnesium acetate spray, heated at 100°C for 10 minutes, and observed both by daylight and under ultra-violet light. Methanolic extracts of cascara and frangula barks and of senna leaves were run as markers.

Result.

Red spots were observed at the fronts of the following Aloe species: A.simii, A.fosteri, A.dyeri, A.ammophila, A.petrophylla (weak), A.branddraaiensis, A.saponaria, A.karasbergensis. Similar spots were observed in the cascara and frangula extracts.

In A.karasbergensis, A.pratensis and in the senna extract, a pink spot at Rf 0.64 was also observed. Deep orange colours were observed in A.longistyla (front) and in A.reynoldsii (Rf 0.90 and 0.85) and in cascara and senna (Rf 0.80). These colours are indicative of hydroxyanthraquinones.

A bright fluorescent yellow colour under u.v. light suggested anthraquinone glycosides in the following species:

A.grandidentata (Rf 0.63)

A.dyeri (Rf 0.64)

A.saponaria (Rf 0.63 and 0.94)

A.polyphylla (Rf 0.79 and 0.70)

6.1.13 BENZENE/CARBON TETRACHLORIDE (1:1). SOLVENT Q.

The 17 species were run in this solvent on two plates of Silica gel G, using chrysophanol (kindly supplied by Dr. van Oudtshoorn) as markers on each end. No similar spot was observed either by daylight, ultra-violet light, or after spraying with alcoholic KOH. This indicates that the anthraquinone derivatives described before are not chrysophanol.

6.2.1 DISCUSSION.

The presence of glucose has been shown in all of the 17 Aloe species discussed in this chapter. It is probably there as a product of plant photosynthesis rather than as a hydrolysis product of unidentified glycosides. Nevertheless the presence of several other sugars or glycosides has been detected in the following species:

A.grandidentata, A.longibracteata, A.dyeri, A.ammophila, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii,

A.polyphylla, A.globuligemma, A.pratensis and A.broomii. By means of the spray reagents alcoholic KOH and methanolic magnesium acetate, possible hydroxyanthraquinones have been shown to exist in A.simii, A.fosteri, A.dyeri, A.ammophila, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii, A.karasbergensis, A.polyphylla and A.reynoldsii.

6.2.2 Rf VALUES.

The Rf values of components on thin-layer chromatograms have not been reproducible, even though use has been made of the same capacity development tanks, and machine-made plates of (presumably) uniform layer thickness, activated for equal periods of time in one oven at 105°C for 30 minutes. It is felt that the main factor causing differences of Rf for the same components on different plates is the length of spotting time required, and until this time is quoted as standard procedure along with the activating time, etc., published figures lose much of their significance.

It is far more satisfactory to be able to run a standard substance as a marker. Unfortunately in the case of Aloe species, insufficient is known about the chemistry, excluding anthraquinone derivatives such as aloin, aloe-emodin, homonataloin, mono-o-methyl nataloe-emodin and chrysophanol.

Work on the Coprosma species by BRIGGS and co-workers (36) - (40) shows the multiplicity of anthraquinone derivatives existing in only relatively few species. Recent work on senna, cascara and rhubarb shows the same picture. It is therefore probable that in Aloe species far more anthraquinone derivatives are yet to be discovered.

6.2.3

With regard to the 17 Aloe species discussed here, it is significant that several species have the same component common to them when run in different solvents. Thus the classification of Aloe species with the assistance of chemical taxonomy may possibly be utilised, since VAN OUDTSHOORN & GERRITSMA (226) have shown by means of chemical taxonomy that the Section Anguialoe Reynolds is homogeneous with respect to anthraquinone accumulation, aloin having been isolated from all five of the South African species contained therein. Apart, therefore, from major anthraquinone derivatives such as aloin, minor anthraquinones, sugars and other components may conceivably assist in the confirmation of, or suggest the re-classification of, the several Sections and Sub-sections of Aloes.

ADDENDUM TO CHAPTER 6.

In addition to the Aloe species screened in Chapters 4 and 5 for aloin and homonataloin respectively, certain species

were obtained during the course of this work which were screened for the presence of glycanthrones and/or anthraquinones. These species were not subjected to all of the solvents used in this Chapter , and are hence listed separately. The following such species contain no known glycanthrones, (but in A.striata (Rf 0.87) a magnesium acetate positive anthraquinone was found using chloroform:ethanol (3:1): A.lineata, A.striata, A.aristata, A.variegata, A.dominella, A.ramosissima, A.claviflora, A.gracilis, A.humilis and A.plicatilis.

In A.variegata three orange spots which turned yellow with alcoholic KOH were observed, as also a pink spot at the solvent front (Ethyl acetate: glacial acetic acid:water 4:1:5). Resins were absent from this species.

C H A P T E R 7.

CHRYSOPHANOL.

7.1 DISTRIBUTION OF CHRYSOPHANOL.

Chrysophanol is not normally a constituent of all commercial aloes (i.e. the boiled juice), but does appear in certain varieties. Thus, for example, HÖRHAMMER et alia (113) report its presence in commercial aloes from both India and the West Indies, while MARY, CHRISTENSEN & BEAL (145) had a decade earlier showed the presence of chrysophanol in the latter variety during a study of Curaçao aloes.

Chrysophanol is more widely distributed in the other anthracene-containing purgative drugs, but in addition has been isolated from Rumex hymenosepalus TORR by HILLIS (102a) and from the root bark of Ventilago viminalis.HOOK by COOKE & JOHNSON (52). POETHKE et alia (171) isolated chrysophanol from Oreoherzogiafallax Boiss, together with rhein, aloe-emodin, frangula emodin and physcion.

Chrysophanol occurs free in frangula bark (SIEPER et alia (198)), but in Rheum occurs both free, and combined as chrysophanol diglycoside and mono-glycoside (HÖRHAMMER et alia (112)). In similar manner it has been reported free in

cascara (BEAL et alia (145)), and also combined as C-glycosyl compound (FAIRBAIRN & MITAL (69)). Chrysophanol does not occur free in senna, but KHORANA & SANGHAVI (120) have isolated two new glycosides from senna pods, one of which is based on chrysophanol, the other on rhein.

With regard to Aloe species, VAN OUDTSHOORN (224) reported the presence of chrysophanol in A.saponaria. Later, using thin-layer chromatography, VAN OUDTSHOORN & GERRITSMA (226) were able to detect at least three anthraquinone glycosides in the species Aecklonis, A.saponaria, A.davyana, A.ammophila and A.transvaalensis. In preparative chromatography one of these glycosides gave chrysophanol on hydrolysis with 1N hydrochloric acid, while free chrysophanol was also detected by extraction methods. This indicated that chrysophanol and other anthraquinones not yet identified are present as ordinary glycosides in both the leaves and roots of these Aloe species.

7.2 CHROMATOGRAPHY OF CHRYSOPHANOL.

The literature contains several references to the chromatographic separation of chrysophanol. Usually these methods are by thin-layer or paper chromatography, but FURAYA et alia (83) have separated chrysophanol from physcion, emodin, aloe-emodin and rhein by gas chromatography. Since physcion (the 6,-methoxy derivative of chrysophanol) so frequently has the

same Rf as chrysophanol, it is also treated here. In certain solvent systems, however, it can be separated from chrysophanol, and furthermore its chemical separation has been described in section 3.6.4.3. Some of the solvent systems used for chrysophanol appear in table 7(i). Of these, solvents 1,2,9,10,11 & 12 were used in this work.

Table 7(i) CHROMATOGRAPHY OF CHRYSOPHANOL.

No.	Solvent.	Type.	Reference.
1 (Z)	Toluene	Paper	BETTS et alia (18)
2 (M)	Petrol ether (40^{o}) 4 Toluene 1 Xylene 1 Methanol 2	Paper	HÖRHAMMER et alia (107)
3	Toluene Pentane	Paper	LOTH et alia (ex(227))
4	Methanol sat^{d} $\tilde{c}$ n-hexane	Paper	HILLIS (102a)
5	Petrol ether (65^{o}) sat^{d} $\tilde{c}$ methanol 97%	Paper	BEAL et alia (145)
6 (P)	Ethyl acet 100 Methanol 16.5 Water 13.5	T.L.C.	HÖRHAMMER et alia (113)
7	Dichloromethane 10 Methanol 3	T.L.C.	SIEPER et alia (198)
8	Dichloromethane 10 Methanol 10.5	T.L.C.	ditto.

9 (Q)	Benzene 1 Carbon tetrachloride 1	T.L.C.	SIEPER et alia (198)
10	Benzene 3 Ethyl acetate 1	T.L.C.	POETHKE et alia (171)
11 (X)	Isopropyl ether	T.L.C.	HÖRHAMMER et alia (109)
12 (Y)	Benzene 2 Glacial acetic acid 1	T.L.C. T.L.C.	MÜLLER et alia (162)

Table 7(ii). Rf VALUES.

Solvent.	Rf Value. Chrysophanol.	Physcion
1	0.96	-
2	0.90	0.88
3	Complete	separation
4	0.71	0.56
5	0.94	-
6	0.97	-
7	0.95	0.95
8	0.85	0.85
9	0.30	0.23
10	Not separated	
11	0.70	0.58
12	0.88	0.88

7.3 CHEMICAL CHARACTERISTICS OF CHRYSOPHANOL.

A methanolic solution of chrysophanol has a yellow colour, fluorescing bright orange-yellow under ultra-violet light. It is interesting to note that when placed on Silica gel G plates

this orange solution momentarily goes red, fading to orange.

The chemical reactions of chrysophanol with acids and alkali have been reported separately by GARDNER (86) and HILLIS (102a). Although differing somewhat in their interpretation of the colours, in the main chrysophanol gives the following reactions:-

(i) with con^{c} H_2SO_4 - red to purple red
(ii) with con^{c} HNO_3 - yellow
(iii) strong ammonia - blue-red to violet
(iv) NaOH 2-5 N - red.

Similar results were obtained in this work using methanolic solutions of chrysophanol. When to the solution in H_2SO_4 is added HNO_3, the mauve colour fades to yellow. With magnesium acetate reagent on chromatograms, chrysophanol and physcion appear orange-red, while rhein and aloe-emodin appear red.

7.4 EXTRACTION OF CHRYSOPHANOL.

Although GARDNER (86) has described a method of synthesising chrysophanol from chrysarobin, the method is complex. Consequently several authors rely on solvent extraction methods to obtain chrysophanol from the plant.

To obtain chrysophanol from Rumex hymenosepalus, HILLIS (102a) used soxhlet extraction with benzene for three days, purification being by sublimation at 125^{o}C at 0.003 mm Hg.

POETHKE et alia (171) refluxed 1g quantities of Oreoherzogia fallax with benzene (10 ml) and H_2SO_4 5% (1 ml) to obtain anthraquinones, including chrysophanol.

7.4.1.

To obtain chrysophanol from A.saponaria VAN OUDTSHOORN (224) extracted approximately 1g of powdered plant material with ether in a soxhlet apparatus until all the colouring matter was extracted. This ethereal extract was purified by shaking with 1N NaOH until the aqueous layer showed no further extraction. The combined aqueous NaOH layers were acidified with hydrochloric acid and the precipitated chrysophanol extracted with ether. This was concentrated down to a small volume for chromatography.

7.4.2 Extraction of free and combined chrysophanol.

The method is that of VAN OUDTSHOORN (227) and is as follows:

5g of powdered plant material is soxhleted with 50 ml ether until the circulating ether is colourless. This extracts free anthraquinones. Of this, 40 ml is reserved for fractionating with 5% $NaHCO_3$ solution (10 ml aliquots), followed by 5% Na_2CO_3

solution, and finally by 5% NaOH solution. Each of these is re-extracted with ether after acidification. The other 10 ml of ether extract contains undifferentiated anthraquinones and is concentrated to a volume of about 2 ml for chromatographic purposes.

7.4.3 Extraction and hydrolysis of anthraquinone glycosides.

Powdered and dried plant material from above (2g) is soxhleted with 25 ml methanol to exhaustion, 20 ml being evaporated to dryness on a waterbath. To the dried residue is added 20 ml of 5% hydrochloric acid and refluxed on a waterbath for one hour. When cool, the hydrolysate is repeatedly extracted with 10 ml ether aliquots to exhaustion, and contains anthraquinones.

The remaining 5 ml of methanolic extract which was not hydrolised is concentrated down to half its volume and used for the chromatography of anthraquinone glycosides.

7.5 OWN WORK.

Initially, a leaf sample of A.saponaria growing in Port Elizabeth was used. This was dried at 50°C and powdered, approximately one gramme resulting. This was soxhleted and extracted in the manner described in section 7.4.1. The concentrated ethereal solution was chromatographed on

Silica gel G plates using Solvent 9 and Solvent 12, and on paper using Solvent 1 (table 7(i)). On spraying with alcoholic KOH only the chromatogram run in Solvent 12 showed a spot at Rf 0.90 (literature 0.88). The presence of chrysophanol thus appeared doubtful.

At a later stage the work of VAN OUDTSHOORN & GERRITSMA (226) showed the presence of chrysophanol glycosides in five species, four of which belonged to the Section Eualoe, Series Saponariae, namely A.saponaria, A.ammophila, A.transvaalensis, and A.davyana, while the fifth (A.ecklonis) belonged to the Section Leptoaloe.

Postulating that chrysophanol could be of chemotaxonomic interest in the Series Saponariae, six species belonging to the Series Saponariae which could be obtained in Port Elizabeth and Uitenhage were examined for chrysophanol and chrysophanol glycosides by the methods described in sections 7.4.3 and 7.4.4. The juices of the six species were also examined for chrysophanol and glycosides, but these proved to be absent from the juices.

The six species used were A.simii, A.fosteri, A.dyeri, A.branddraaiensis, A.saponaria and A.greenii.

For the first three species three gramme quantities of powdered dried leaf were extracted, and for the latter three species, six gramme quantities were used.

7.5.1 CHROMATOGRAPHY.

Interesting pigments were noticed in all six species when chromatographing on Silica gel G using Solvent 2 and Solvent 9. One of these was a red pigment (daylight and ultra violet light) at Rf 0.50 in Solvent 2 and Rf 0.05 in Solvent 9. When sprayed with ammonia this spot remained red in daylight but appeared yellowish under ultra violet light. In Solvent 2 was a colourless principle at Rf 0.65 which fluoresced a brilliant red under ultra violet light. This spot was unchanged by ammonia. Neither of these spots shows typical flavonoid reactions (74a).

The ethereal and hydrolysed methanolic concentrates were chromatographed on Silica gel G in Solvents 9,11 & 12, and sprayed with magnesium acetate reagent. Chrysophanol was run as control.

7.5.2 RESULTS.

It was apparent that chrysophanol was absent from the ethereal extracts of all six species, but in one of the methanolic hydrolysates (A.dyeri), a (red) magnesium acetate positive principle, which fluoresced the same colour as the chrysophanol marker before spraying, was observed at a slightly lower Rf than the control chrysophanol. Since a pure substance often runs higher than a mixture of components (PASTUKA (168)),

it is concluded that chrysophanol exists in A.dyeri in O-glycosidal form. This glycoside of A.dyeri before hydrolysis had an Rf value (T.L.C.) of 0.79 in chloroform:ethanol 95% (3:1) where aloin had an Rf 0.68. Chrysophanol (either free or combined) appears to be absent from the five remaining species.

C H A P T E R 8.

RESINOUS CONSTITUENTS AND PARA-COUMARIC ACID.

8.1.1 INTRODUCTION.

Commercial aloes contain a considerable amount of resin. With regard to the production of aloin from such aloes, precipitation of this resin is a necessity if a good quality crystalline aloin is desired, and even small quantities of resin can delay crystallisaion - conceivably in the absence of electrolytes the resin acts as a lyophobic colloid. Rarely are commercial samples of aloin entirely free from resins, and this may readily be shown chromatographically.

8.1.2 COMPOSITION OF RESINS. (59)

Resins are not single chemical compounds but are usually mixtures of substances of different and complex chemical character.

The following are the three most important groups of compounds present in resins.

1) Resenes - the chemical nature of which is unknown, but which are exceedingly stable.
2) Resin Acids - these are complex, high molecular weight acids usually occurring free.
3) Resin Esters - These consist mostly of resin alcohols combined with aromatic acids, of which benzoic and cinnamic acids are of frequent occurrence.

There are two principal kinds of resin alcohols in combination with these acids, namely:

a) Resinols - these give a negative reaction with iron salts

b) Resinotannols - these are phenolic compounds which give blue, green or violet-coloured compounds with ferric chloride.

Resinotannols are said to be produced on hydrolysis of aloes (TREASE (220)). Thus on hydrolysis the resin of Curaçao aloes yields a resin alcohol (barbaloresinotannol) and cinnamic acid, while the resin of Cape aloes yields capaloresinotannol and p-hydroxycinnamic (p-coumaric) acid. Little is known of these resinotannols, and it has been suggested that they are mixtures of decomposition products formed during the hydrolysis of the original resins. In contrast, RAMSTAD (175) states that most of the anthraquinone drugs contain resinous substances which appear to be condensation products of anthraquinones or anthranols. This latter statement is of interest in relation to the findings described in this chapter, and will be referred to later.

8.1.3 PHARMACOLOGIC ACTION OF RESINS.

Confusion also exists as to the pharmacologic action of the resins. KIEFER (ex 237) isolated three active resins from Cape aloes and attributed the purgative action to the

three resins, saying that aloin and emodin played a minor part. In contrast, TSCHIRCH & HOFFBAUER (222) arrived at the conclusion that only the resin is totally inactive or without purging properties, while aloin, aloe-emodin and the anthraglucosides have a purging effect. A further note to the lack of exact knowledge regarding these resins is the fact that, while some pharmacopoeias discard the resinous material, others make preferential use thereof for making galenic preparations (RAMSTAD (175)).

8.2.1 ISOLATION OF RESIN.

Pure resin from Cape aloes has been isolated by AUTERHOFF & BALL (4) as a greyish-brown powder, soluble in about 15 parts of ethyl alcohol. They report this resin as giving an ultraviolet absorption spectrum, with a strong maximum at 300 mμ. The resin separated by BÖHME & KREUTZIG (28) had a broad peak at about 295 mμ, but two sharper, more intense peaks at 253 mμ and 245 mμ.

8.2.2 Hydrolysis of the resin isolated by AUTERHOFF & BALL (4) produced p-coumaric acid (12%) and acetic acid. The brown residue, which was insoluble in both ether and water, remained in the reaction fluid and was presumably the resinotannol. It was furthermore found that hydrolysis by mineral acid broke the

glycosidal bond and yielded arabinose, and in conjunction with alkaline hydrolysis removing the acids, left what was called a "tannol-aglycone" of phenolic character.

8.2.3 The acid character of the resins isolated from Cape aloes by KIEFER (237) is reflected in the fact that these resins were soluble in sodium bicarbonate solution, and were described by the author as two, possibly identical, very active, bright yellow resins, (each about 30%) and another very active resin, (6.8%).

8.3.1 CHROMATOGRAPHY OF RESINS.

Solvent choice is of utmost importance in the complete separation of mixtures of constituents by chromatographic means, and this applies equally well to chromatography of the resins of Aloe species. Where earlier workers had reported one resin spot, later workers using different solvent systems could resolve the resin into further spots, while BÖHME & KREUTZIG (28) recommend two-dimensional chromatography for complete resolution.

8.3.2 PAPER CHROMATOGRAPHY.

In 1954, AUTERHOFF & BALL (4), in a paper chromatographic study of Cape aloes, using water-saturated butanol as running solvent, regarded the spot located at Rf 0.80 as the resin.

This spot was invisible before alkali treatment, but blue-violet after spraying with alkali, and when viewed under ultra-violet light.

In 1957 KRAUS (124) utilised as solvent the upper phase of butanol: acetic acid: water (4:1:5). His findings were as follows: aloin Rf 0.65, p-coumaric acid Rf 0.49, resinous product Rf 0.80. Using the same solvent system, AWE, AUTER-HOFF & WACHSMUTH-MELM (5) (1958) reported further spots after alkali, and the following were observed:

Rf 0.22:	a green spot
Rf 0.50:	an unidentified anthracene derivative possibly of 1:8-dihydroxyanthraquinone structure
Rf 0.65:	Cape aloin
Rf 0.81:	p-coumaric acid and resin
Rf 0.88:	Aloe-emodin.

It will be noticed that the Rf claimed for p-coumaric acid by KRAUS (124) and by AWE et alia (5) respectively, differs widely.

Further work by AWE & KUMMELL (1962) (6) on the juice of A.ferox and A.vera resulted in similar chromatograms, except that under ultra-violet light an orange spot was observed at Rf 0.56, which fluoresced blue in ultra-violet light after alkali treatment, and they felt that this might be isobarbaloin.

8.3.3 A detailed chromatographic study of Cape, Socotra, Curaçao and Natal aloes by BÖHME & KREUTZIG (1963)(28) followed. These authors pointed out that several previous workers had used Partridge's solvent (that is, the upper phase of butanol: glacial acetic acid: water) but due to difference in methods and types of paper used, the given Rf values were not comparable.

These authors were mainly interested in paper chromatography, because it afforded complete and quantitative separation of aloin, as shown by AWE & WACHSMUTH-MELM (7), but the Partridge's solvent used by the latter was found to be far from ideal, and use was made of the following formula - ethyl acetate: glacial acetic acid: water (4:1:5) (upper phase), and the softer S & S 2043b Mgl paper was replaced by the harder S & S 2045b Gl paper to decrease the running time and improve the Rf values.

The authors state that the Rf values reported are the average value of many experiments, but stress that these values invariably depend on method, paper, saturation etc. The sequence of separation should be noted however, as this remains constant, and chromatograms in which the Rf values deviate can be orientated by reference to aloin or other suitable known constituent.

8.3.4 In the four commercial varieties of aloes examined by BÖHME & KREUTZIG (28) (Cape, Socotra, Curaçao and Natal) following spots were common to all four varieties -

a) Rf 0.07, a yellowish-green fluorescent spot, before and after treatment with KOH, under ultra-violet light which could be an anthocyanin.

b) Rf 0.31, this spot appears as a blue fluorescence (quartz lamp 254 mμ) which becomes a strong light blue after treatment with KOH, and is visible as a light yellow colour in daylight. This substance is a crude resin (German "Rohharz"). This spot was previously reported in Cape aloes by AWE et alia (5) as an anthracene derivative, and in Curaçao aloes as isobarbaloin by AWE et alia (5), and PARIS & DURAND (166). This, according to BÖHME & KREUTZIG (28) is due to the incomplete separation afforded by Partridge's solution, wherewith several substances appear at the same Rf. The resin of Rf 0.31 has peaks at 295, 253 and 245 mμ.

c) Rf 0.55, this can also be classified as crude resin, and gives a blue fluorescence under ultra-violet light after alkali treatment. Only in Cape aloes at this Rf the reaction is blue-violet, and by means of thin-layer chromatography it is possible to differentiate the resin spot from a spot which gives a (fading) blue colour with periodate.

d) Rf 0.78, this spot after treatment with alcoholic KOH gives under ultra-violet light a blue colour identical with that of Rf 0.55, and both give a light yellowish colour in daylight. BÖHME & KREUTZIG (28) refer to this spot as the "main resin spot".

e) Rf 0.88, this spot, like the "main resin apot" only becomes visible after treatment with KOH as dark blue under ultra-violet light. This spot was absent from Curaçao aloes.

In brief, the hydroxyanthraquinone derivatives appear at the following Rf values:

Rf 0.43:	isobarbaloin (Curaçao only)
Rf 0.40 & 0.48:	aloinoside (Cape and Socotra only)
Rf 0.67:	aloin (absent from Natal)
Rf 0.69:	homonataloin (present only in Natal)

8.4.1 THIN-LAYER CHROMATOGRAPHY.

Thin-layer chromatography allows the identification of further substances which are present in such minute traces so as not to become visible on paper chromatograms (BÖHME & KREUTZIG (28)). These workers used the solvent system ethyl acetate: methanol: water (100:16.5:13.5) (HÖRHAMMER et alia (108)) and Silica gel G plates to obtain differentiation of the resin spot (Rf 0.55) described previously.

8.4.2 This same system was used by HÖRHAMMER, WAGNER & BITTNER (110) in an investigation of Cape and Natal aloes. They similarly differentiated three resins, although the chemical composition of one such spot is described as "unknown", even though it gives identical reactions to the two named resin spots. The approximate Rf values of these three resin spots are 0.26, 0.50 and 0.66, where aloin was 0.45.

8.5.1 COLOUR REACTIONS OF RESINS.

Several spray reagents exist which give characteristic colours with resins, and consequently aid in their identification in chromatography. These are described below.

8.5.2 (a) ALCOHOLIC KOH OR NaOH (2-5%).

Several spots fluoresce varying shades of blue under ultra-violet light. As described before (section 8.3.4), certain spots become lighter blue and more intense on spraying (e.g., Rf 0.31), dark blue spots appear to deepen, while spots not visible under ultra-violet light before spraying develop after spraying as a dark blue colour (e.g., Rf 0.88). Some of these spots show as light yellow in daylight. (Caution: p-coumaric acid gives a royal-blue to violet colour in ultra-violet light after this treatment, the colour being dull light brown in daylight).

8.5.3 (b) FAST BLUE B (ECHTBLAUSALZ B) 0.05% AQUEOUS.

This reagent is also described as True Blue Salt B (Merck (153)), who describe it as a reagent for phenols and amines capable of coupling (diazo-reagent). The reagent should be freshly prepared, and deposits after a few days. With resins it gives orange to red colours in daylight, these colours being unaffected by after-treatment with alcoholic KOH (HÖRHAMMER et alia (111)). However, these workers state that aloin, isobarbaloin and homonataloin, which are unaffected by the Fast Blue B reagent, go green (dark-green for homonataloin) when oversprayed with methanolic KOH.

(Note: under Experimental it will be noted that chromatograms were first sprayed with methanolic KOH to reveal hydroxyanthraquinones and then oversprayed with Fast Blue B reagent. This in no way interfered with the effectiveness of the Fast Blue B reagent, although aloin took several days to form a dull green and homonataloin formed a faint mauve, later changing to greenish.

8.5.4 (c) ANTIMONY TRICHLORIDE REAGENT.

BÖHME & KREUTZIG (28) used a solution of antimony trichloride 4% in chloroform as an afterspray following the use of Fast Blue B reagent, whereupon the orange resin spots show haloes and change to a strong violet-red colour. They recommend

this aftertreatment to differentiate p-coumaric acid from resin, the former giving a strong ultra-marine colour, which easily distinguishes the acid from the resin.

8.5.5 (d) 2,6-DIBROMOCINCHONINE CHLORIMIDE REAGENT.

This reagent, used to identify phenols, was used by BÖHME & KREUTZIG (28) as a 0.4% methanolic solution, followed by spraying with a 10% solution of Na_2CO_3 in 30% methanol. This gave the following colours in daylight:-

a) resins - appeared brown
b) aloin, substances like aloin, and periodate - reactive substances gave blue-green or violet colours.

8.6.1 PARA-COUMARIC ACID.

As this substance is so closely connected with the resins, it seems approriate to deal with it here, as both resins and p-coumaric acid were on several occasions investigated conjointly, particularly as the spray reagents described above react with both substances.

8.6.2 A number of aromatic acids are widely distributed in plants, but by no means as universally as the aliphatic acids. Although in some instances they occur free (e.g. benzoic acid), they more generally occur as esters in oils and resins, and as

glycosides. Commonly occurring aromatic acids are salicylic, O-hydroxybenzoic, cinnamic, p-coumaric, hydrocoumaric, protocatechuic, gallic, and caffeic acids. Para-coumaric acid is said to occur both free and combined in Aloe species (BONNER (30)).

8.6.3 There is no measure of agreement in the literature regarding the occurrence of free para-coumaric acid in Aloe species. Normally the analyses were performed on commercial aloes, but AUTERHOFF & BALL (4) claimed to have identified the substance both in commercial Cape aloes and in the fresh juice of a plant from the gardens of the Würzburg University.

8.6.4 Employing paper chromatography and using water-saturated butanol as their running solvent, p-coumaric acid had an Rf value of 0.38. Paper chromatography was also used by AWE, AUTERHOFF & WACHSMUTH-MELM (5), who utilised the upper phase of butanol: glacial acetic acid: water (4:1:5) as solvent. In their opinion p-coumaric acid appeared together with resin at Rf 0.81. They also described the finding of an unidentified anthracene derivative at Rf 0.50, and cited earlier workers as having reported p-coumaric acid at this Rf, while a figure of 0.49 was obtained by KRAUS (124) using the same chromagraphic procedure.

In the opinion of BOHME & KREUTZIG (28), the above solvent was unsatisfactory for separation of certain Aloe constituents, several spots frequently being superimposed at the same Rf. In consequence they made use of the solvent system ethyl acetate: glacial acetic acid: water (4:1:5), and with the harder paper described before, obtained p-coumaric acid with resin at Rf 0.88. The p-coumaric acid could be differentiated by use of antimony trichloride reagent, which, when used after Fast Blue B reagent, revealed p-coumaric acid as ultra-marine and resin as violet-red. They further stated that with their solvent, p-coumaric acid and resin could be separated using thin-layer chromatography, but no further details were given in the paper (28).

The conclusion they reached was that free p-coumaric acid was absent from the commercial aloes that they investigated (Cape, Curaçao, Socotra and Natal), and similar conclusion was reached by other workers (166) & (111). DÖFF (60), however, whilst investigating the tuberculostatic activity of Aloe extracts, claimed that p-coumaric acid was the most active compound, effective even in dilutions of 1-50,000 to 1-100,000 (Sauton medium).

In view of the uncertainty which existed with regard to p-coumaric acid in Aloe species, it was decided to investigate the juices of fifty-five Aloe species that had been collected

since 1962, both with regard to p-coumaric acid, and also to investigate the distribution of resins in the species, some of which yielded a viscous resinous juice when the leaf was incised, others a thin, watery liquid. Furthermore, with the exception of work performed on the resins found in a limited number of commercial aloes, very little work had been done on resins in their more natural form, as occurs in the unboiled juices obtained direct from leaves of Aloe species. These investigations are described under experimental.

8.7.1 EXPERIMENTAL.

The usefulness of chromatographic separation is that, provided a suitable solvent system can be chosen, separation of constituents differing in chemical characteristics can be achieved. Where this can be followed by visualisation using selected chemical (spray) reagents, not only can the presence or absence of sought substances be established, but also the chemical relationship of several components in one species.

8.7.2 (a) CHOICE OF SOLVENT SYSTEM.

Initially, use was made of solvent systems described in the literature by AWE et alia (5) and HÖRHAMMER et alia (111). Although resins were visible, the separation of p-coumaric acid was problematical. Other solvent systems were employed,

the most satisfactory being that of BÖHME & KREUTZIG (28), thin-layer separation being substituted for paper chromatography.

SOLVENT SYSTEMS.

N	Butanol/glacial acetic acid/water (4:1:5)	- paper
M	Petrol ether (40°): toluene:xylene:methanol (4:1:1:2)	- paper
R	Propanol:ethyl acetate:water (4:4:3)	- T.L.C.
P	Ethyl acetate:methanol:water (100:16.5:13.5)	- T.L.C.
V	Chloroform:ethanol 95% (3:1)	- T.L.C.
Q	Benzene:carbon tetrachloride (1:1)	- T.L.C.
L	Ethyl acetate:glacial acetic acid:water (4:1:5)	- T.L.C.

8.7.3 SPRAY REAGENTS.

(i) Alcoholic KOH 5% w/v

(ii) Fast Blue B 0.5% w/v (aqueous) (HOECHST AG)

(iii) Antimony trichloride 4% w/v in chloroform.

8.7.4 PROCEDURE.

Methanolic solutions of the juices of the following Aloe species were examined, using paper and thin-layer chromatography and utilising visualisation by spray reagents, and ultra-violet light - as an attempt at classification, the 55 species have been sub-divided into the following chemical classification:-

Group A - Species containing aloin

Group B - Species containing homonataloin

Group C - Species containing neither aloin nor homonataloin

GROUP A (aloin)

A.ferox Mill.
A.excelsa (Rhodesia)
A.arborescens Mill.,
A.cameronii (Rhodesia)
A.marlothii Berger
A.africana Mill.,
A.castanea Schonl.,
A.candelabrum Berger,
A.sessiliflora Pole Evans,
A.reitzii Reynolds,
A.aculeata Pole Evans,
A.vryheidensis Groenewald,
A.dolomitica Groenewald,
A.vanbalenii Pillans

GROUP B (homonataloin)

A.suprafoliata Pole Evans,
A.cryptopoda Bak.,
A.lutescens Groenewald,
A.pachygaster Dinter,
A.melanacantha Berger
A.hereroensis Engler,
A.comptonii Reynolds,
A.mitriformis Mill.
A.speciosa Bak.,
A.wickensii (var.lutea) Reynolds
A.angelica Pole Evans,
A.mutabilis Pillans,
A.munchii Christian,
A.pearsonii Schonl.,
A.distans Haw,

GROUP C.

A.grandidentata Salm Dyck.,	A.longibracteata Pole Evans,
A.simii Pole Evans,	A.fosteri Pillans
A.dyeri Schonl.,	A.ammophila Reynolds,
A.petrophylla Pillans,	A.branddraaiensis Groenewald,
A.saponaria (Ait) Haw,	A.chabaudii Schonl.,
A.globuligemma Pole Evans,	A.karasbergensis Pillans,
A.longistyla Bak.,	A.pratensis Bak.,
A.polyphylla Schonl.,	A.reynoldsii Letty,
A.broomii Schonl.,	A.peglerae Schonl.,
A.succotrina Lam.,	A.dominella Reynolds,
A.lineata (Ait) Haw.,	A.striata Haw.,
A.ramosissima Pillans,	A.plicatilis (L) Mill.,
A.pretoriensis Pole Evans	A.glauca Mill.,

8.7.5 PAPER CHROMATOGRAPHY.

All the species in Group A were chromatographed on Whatman No.1 paper by the cylinder method, using Partridge's solvent (henceforth referred to here as Solvent N), with accommodation time 2 hours, at a temperature of 18-20°C. As marker was used a 0.2% w/v methanolic solution of p-coumaric acid, an equivalent of 10µg of acid being used.

After suitable development, the paper was removed and dried at room temperature, sprayed with methanolic alkali and

viewed under ultra-violet light. The control p-coumaric acid showed vividly as a royal blue at an Rf of 0.95, but at this Rf blues of similar colour were also visible in most Aloe species, while pink colours were observed at the fronts of A.arborescens, A.vanbalenii, A.marlothii and A.castanea, while A.africana had a greenish front.

As it was obvious that differentiation of p-coumaric acid under these circumstances would be difficult, the Group A species were again chromatographed as before and examined carefully under ultra-violet light before spraying. In some species a light blue fluorescence was observed at Rf 0.95, in other not. These findings were recorded, and on spraying with alkali, deep blue colours were observed in A.ferox, A.castanea and A.marlothii but only a weak reaction in A.dolomitica, A.candelabrum and A.sessiliflora. However, as a precaution, p-coumaric acid (10 µg) had been added to a duplicate of A.ferox, and not only was this marker not differentiated, but the intensity of the blue spot of the two samples of A.ferox appeared very similar. Furthermore, as certain resins appear only after treatment with alkali as blue spots, it became obvious that this method as used by AWE et alia (5) was impractical.

Use was next made of Solvent M, by ascending chromatography, after an accommodation period of one hour (Whatman No.1 paper,

temperature 18-20°C). After spraying with alkali it became immediately clear that overlapping of constituents had occurred. In the case of A.mitriformis the homonataloin appeared as a reddish colour through a blue resin spot. Para-coumaric acid had an Rf of 0.4 (tailing), and was not visible in the sample of A.ferox to which it had been added, while at this Rf both the sample of A.speciosa and A.mitriformis had the brownish-red colour of homonataloin, which also had tailed. The free anthraquinones aloe-emodin and mono-o-methylnataloin-emodin which were present, and which according to the literature (107) have Rf values of 0.42 and 0.46 respectively, were not visible on spraying. Since with thin-layer chromatography (and especially with the solvent system of HÖRHAMMER et alia (111) - here called Solvent P) excellent differentiation of the constituents of aloes could be obtained, this means of analysis was resorted to in place of the relatively unsuccessful paper method.

8.7.5.1 THIN-LAYER CHROMATOGRAPHY.

The Aloe species of Group A were run in Solvent P on non-machine spread plates of Silica gel G. In consequence the Rf values of the aloin in the species differ.

When observed under ultra-violet light, all species except A.arborescens had a fluorescent, whitish blue suspected

resin spot which appeared mid-way between the starting point and aloin (Rf values are not quoted due to the obvious differences in layer thickness on this plate). An idea of the colour of this spot, which appears in so many species, may be gained by reference to the photograph in figure 5(i), where the spot lies midway between the starting point and homonataloin. Later work showed this compound to be aloesin (section 8.7.12).

The plate was then sprayed with alkali to reveal both hydroxyanthraquinones and p-coumaric acid. With regard to the former, aloe-emodin was evident in all species, and after standing several weeks, the spots immediately above and below aloin in A.candelabrum had turned purple. (KINGET (122) describes purple compounds obtained by heating - for 5 minutes at 100°C - chromatograms of Rhamnus purshiana sprayed with KOH 5% alcoholic, these compounds being reduced forms of the anthraquinone aglycones.) Furthermore, pink spots just above aloin were evident in A.reitzii, A.africana and A.marlothii at Rf 0.72 ± 0.03.

Para-coumaric acid was revealed by the alkali treatment as a concise blue spot of a similar Rf to aloin, while the sample of p-coumaric acid added to A.marlothii could just be distinguished, as a fraction of it showed blue at the base of the yellow-orange aloin spot. A resin spot deep blue in colour appeared after alkali treatment in A.castanea, A.dolomitica

and A.sessiliflora, and this was only slightly higher than the p-coumaric acid markers, and could serve as a further possible source of mistaken identity. Solvents V and R were then tried, and in each case the p-coumaric acid marker appeared on the Rf of the "resin" spot which fluoresced whitish blue, and the sample of p-coumaric acid added to A.ferox was completely masked by this resin spot in both solvents. The following Rf values were recorded:

Solvent V - aloin 0.42, p-coumaric acid and resin 0.25

Solvent P - aloin 0.87, p-coumaric acid and resin 0.75

As had been anticipated from its non-polar nature, in Solvent Q no migration of either resins, p-coumaric acid or glycanthrones occurred.

At this stage close attention was paid to the work of BÖHME & KREUTZIG (28), who, as mentioned before, had reported that p-coumaric acid could be separated from resins chromatographically, using their solvent system (Solvent L) and thin-layer chromatography, although no actual details were given. Furthermore, their claim that the acid and resins could be distinguished by use of the Fast Blue B and antimony trichloride reagents seemed to offer a solution even if the chromatographic separation proved as perplexing as previous attempts.

To judge the effectiveness of the spray combination, use was made of Solvent P and Silica gel G plates, and fresh juices

of A.africana, A.speciosa, A.arborescens and A.ferox. Several plates were run, p-coumaric acid being added to the Aloes in certain instances.

Spraying with Fast Blue B reagent was performed for the first time, and produced interesting results - several spots which had been observed as blue under ultra-violet light immediately turned bright orange (daylight colour), while mauve-purple colours also appeared instantaneously in A.speciosa. Both aloin and homonataloin were unaffected by this treatment, but resins were effectively revealed.

Overspraying with the antimony trichloride reagent changed the orange and mauve observed before to deep red and deep purple respectively, while the p-coumaric acid which had thus far not been revealed, appeared as a greeny blue colour (daylight) directly in line with the mauve spots. This colour was not permanent, but was visible at the actual moment of spraying in the species to which it had been added. (The colour was improved to give the ultra-marine quoted in the literature by spraying with dilute hydrochloric acid). It was also observed that the antimony trichloride spray reagent produced purplish spots below aloin, particularly in A.ferox.

The use of Solvent L proved even more satisfactory, inasmuch that p-coumaric acid in this solvent travelled almost to the front, and appeared as a very deep purple spot, clearly

visible under ultra-violet light both as the marker and in the juices to which it had been added. Spraying with methanolic alkali revealed the spot even more clearly as a rich blue. Thus it was clearly seen that p-coumaric acid was either absent from Aloes africana, speciosa, arborescens and ferox, or present in equivalent quantity less than 10 µg. What was of even greater significance was that, when the plates were over-sprayed with Fast Blue B reagent, the p-coumaric acid was unaffected and retained its blue colour. The resins became evident as orange spots (daylight) while a mauve spot appeared in A.speciosa.

Table 8(i) COMPARISON OF Rf VALUES OBTAINED.

Solvent P.	Resins.					
A.africana	0.33,	0.41,	0.65,	0.73,	0.80	-
A.arborescens	-	-	0.65	-	-	-
A.speciosa	-	0.42	0.66	-	-	0.89
A.ferox	-	0.42	0.66	-	-	-

(aloin appeared at Rf 0.62, homonataloin at Rf 0.73, p-coumaric acid at Rf 0.54)

Solvent L.	Resins.				
A.ferox	0.10,	0.25,	-	0.53	-
A.speciosa	-	0.25	0.41	0.53	0.90

(aloin appeared at Rf 0.48, homonataloin at Rf 0.64, p-coumaric acid at Rf 0.97)

8.7.6 APPRAISAL OF SPRAY REAGENTS.

The use of methanolic KOH followed by Fast Blue B reagent was reversal of the method used by HÖRHAMMER et alia (111), who used alkali after Fast Blue B to reveal glycanthrones. Prior use of alkali had several advantages inasmuch as its use revealed p-coumaric acid, and differentiated resins not visible before spraying from those visible (ultra-violet light), and furthermore revealed hydroxyanthraquinones as pink or red spots.

The antimony trichloride reagent had one serious disadvantage, namely, it precipitated a white precipitate almost immediately after preparation, and particularly on contact with water or polar liquids. Thus spraying-apparatus in particular became easily blocked, even though thoroughly washed before use with chloroform.

Attempts at improving the antimony trichloride (which is notoriously hygroscopic) by solvent washing and drying evinced no improvement, and an industrial firm confirmed that this problem troubled them as well. This precipitate was slowly soluble in dilute hydrochloric acid, but considering the fact that the p-coumaric acid did not give a permanent colour with this reagent, the use of alkali seemed preferable in view of its other obvious advantages.

One grave disadvantage had to be eliminated, namely, whether the aglycone (e.g. aloe-emodin) which ran with the

front in this Solvent L, would mask the p-coumaric acid when sprayed with alkali, since the 55 species to be examined were known to have undergone some hydrolysis.

Four species which had produced relatively large amounts of aglycone on storage were used, namely A.ferox, A.reitzii, A.speciosa and A.comptonii. To each of those was added 10 µg of p-coumaric acid, and after chromatography in Solvent L they were examined under ultra-violet light, where the p-coumaric acid was clearly seen as a thin elliptical dark purple spot just below the aglycones. Immediately under this acid, blues of varying shades were visible, in all species except A.ferox, where the spot was yellowy green. The plates were then sprayed with alkali, which revealed the aglycones as orange-red spots, and the p-coumaric acid as royal blue under ultra-violet light. When oversprayed with Fast Blue B reagent, purples appeared above p-coumaric acid (in daylight) in A.reitzii and A.speciosa, and below in A.reitzii, A.ferox and A.comptonii. The aglycones were unaffected by this after-treatment, and the p-coumaric acid remained clearly visible, although some masking occurred in A.speciosa due to diffusion.

To check the cause of the purple colours a duplicate plate was sprayed firstly with Fast Blue B reagent, followed by antimony trichloride reagent. It became evident that the purples were the result of Fast Blue B reagent alone, while

the p-coumaric acid was not revealed by the aftertreatment with antimony trichloride on this occasion.

8.7.7 CHROMATOGRAPHY USING SOLVENT L (Ethyl acetate:glacial acetic acid:water-4:1:5)

Each of the Groups A, B and C were run on machinespread Silica gel G plates (20cmx20cm) in Solvent L, and upon removal, sprayed first with alcoholic KOH, this being followed by Fast Blue B reagent. Methanolic solutions of the juices were used in a volume of 0.01 ml, while as marker 5 µg and 10 µg of p-coumaric acid (0.2% methanolic solution) was used both free, and added to selected species.

8.7.8 GROUP A (Aloe species containing aloin).

The fourteen species of this group are re-listed to facilitate study of the chromatogram appearing in figure 8(ii). From left to right on the plate, the spotting was as follows:- p-coumaric acid 5 µg, A.ferox, A.candelabrum, A.excelsa, A.sessiliflora, A.arborescens, A.reitzii, A.cameronii, p-coumaric acid 5 µg, A.aculeata, A.marlothii, A.vryheidensis, A.africana plus p-coumaric acid 10 µg, A.africana, A.dolomitica, A.castanea, A.vanbalenii, A.vanbalenii plus p-coumaric acid 5 µg, and p-coumaric acid 10 µg.

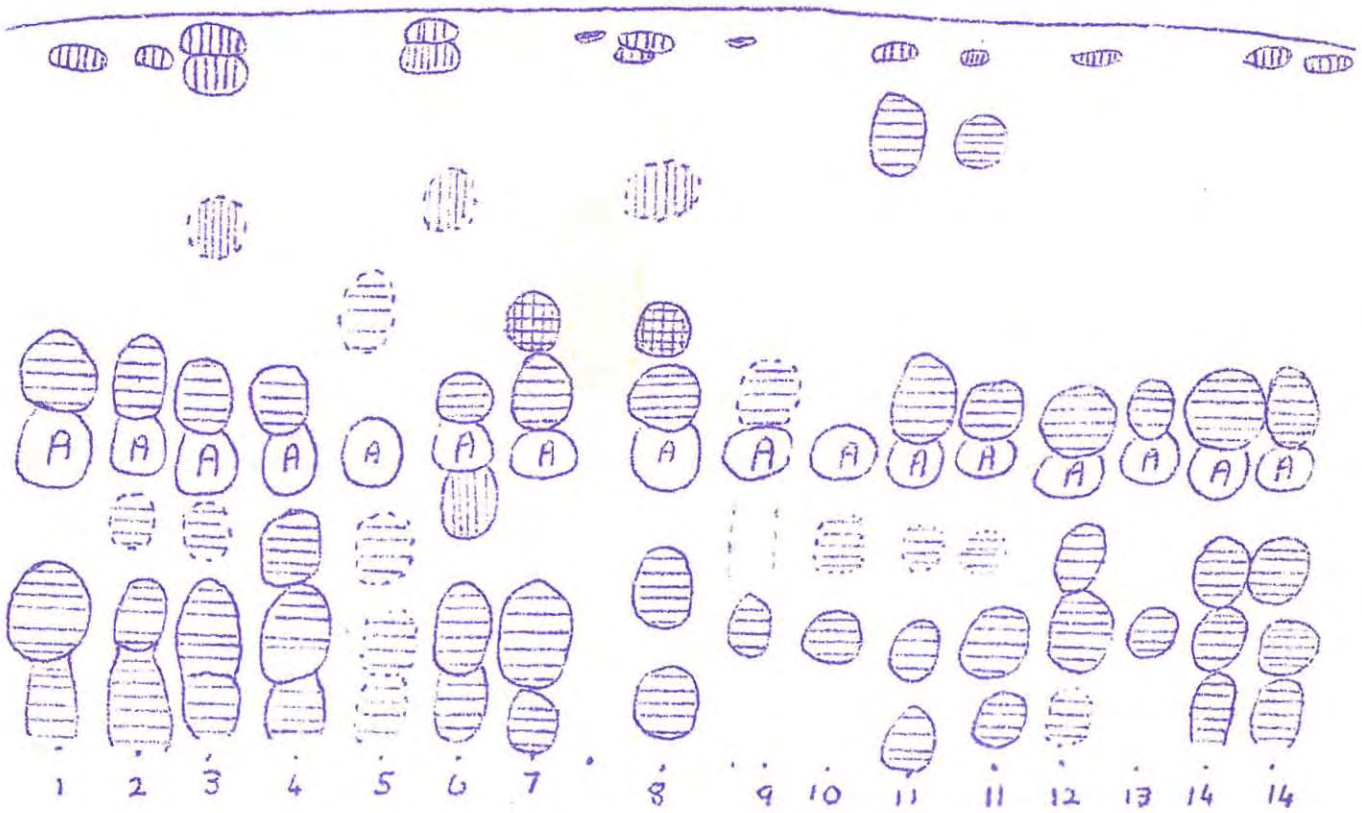
The plate was developed to a height of 10 cm, removed and dried thoroughly in air to remove acetic acid vapours which interfere with the alkali spray.

The plate was observed under ultra-violet light and it is interesting to note that the resin spot which normally fluoresces pale bluish-white (aloesin) in other solvents appears to be somewhat quenched by this solvent.

After spraying with alcoholic alkali the p-coumaric acid markers were clearly visible under ultra-violet light as royal blue spots, while orange-pinks (aloe-emodin) were visible in all species to varying extents. Overspraying with Fast Blue B produced purple fronts in all except the following, but had no effect on the p-coumaric acid:
A.arborescens, A.cameronii, A.marlothii, A.vryheidensis and A.castanea. Reference to figure 8(ii), traced from the plate, will show the relative position of the resins, whichappeared as orange spots in daylight upon treatment with the Fast Blue B. It will be observed that aloin appears clearly in all species at Rf 0.44, but that aloinoside appeared only in A.africana and A.reitzii, at Rf 0.19. An orange spot before spraying was observed under ultra-violet light at Rf 0.59 in the following species: A.cameronii and A.aculeata.

figure 8(ii).

Group A.



Colour code

A = Aloin (yellow).

= orange.

= weak orange.

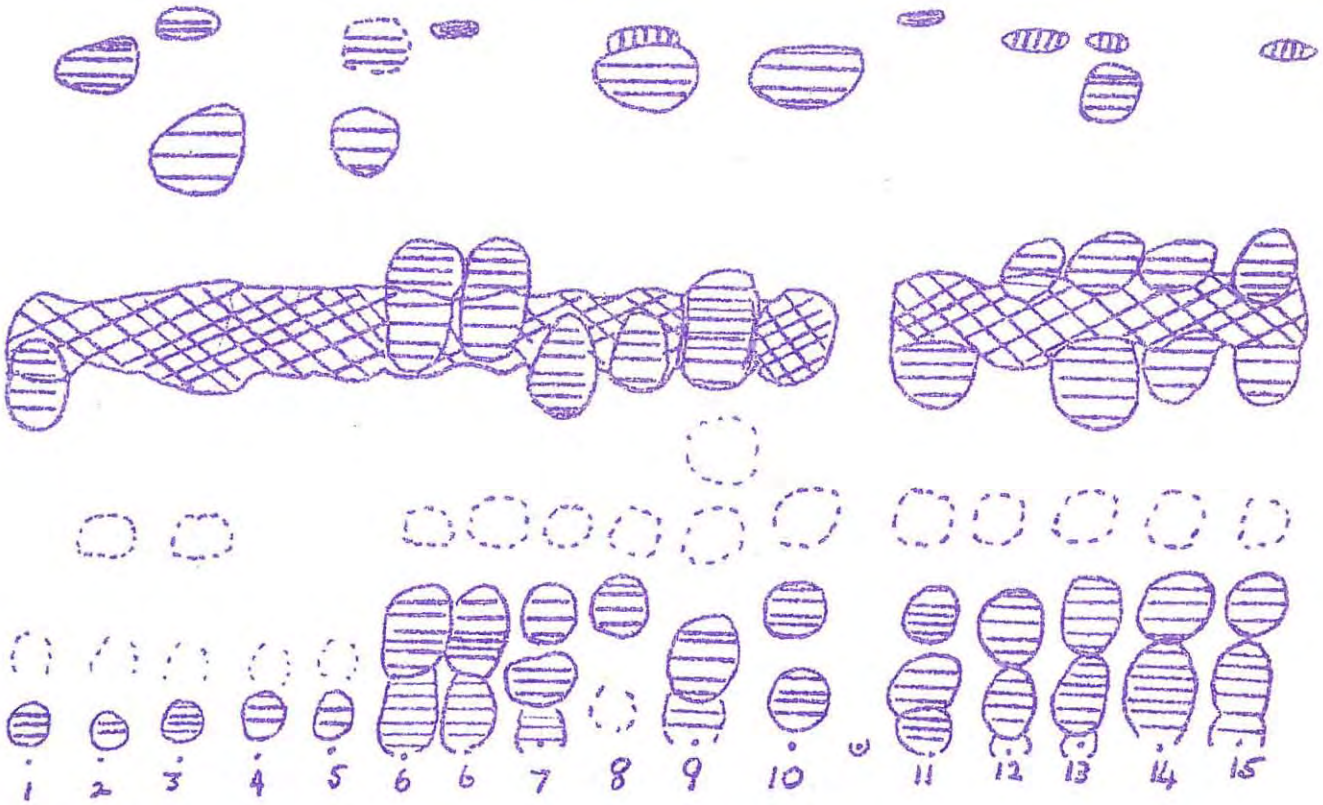
= mauve (pale).

= orange (uv) before spraying.

= p-coumaric acid.

Figure 8(iii)

Group B



1 A. suprafoliata.
2 A. speciosa.
3 A. cryptopoda.
4 A. wickensii (lutea).
5 A. lutescens.
6 A. angelica. plus p-c-acid.
6 A. pachygaster.
7 A. mutabilis.
8 A. melanacantha.
9 A. munchii.
10 p-c acid.
11 A. hererocensis.
12 A. pearsonii.
13 A. comptonii.
14 A. distans.
15 A. mitriformis.

Colour Code.

= homonataloin (later mauve)

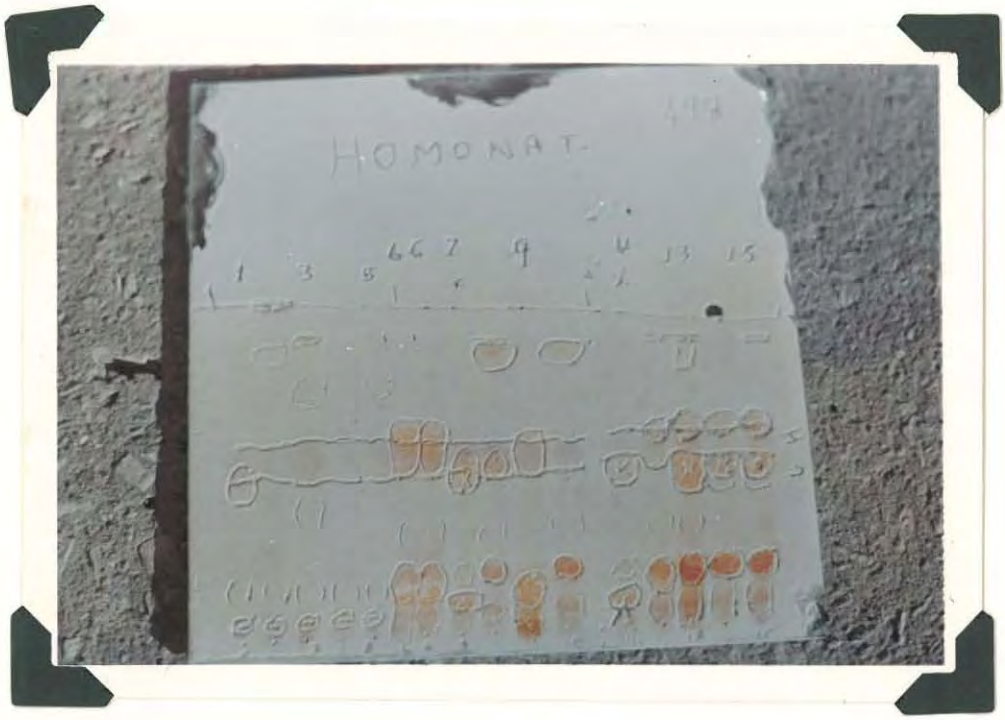
= orange or orange-brown

= purple-mauve

= brown (weak)

= p-coumaric acid.

figure 8(i).



Group B.

Group C(ii).

RESINS IN GROUP A.

The resin distribution in the species is remarkably constant with few exceptions: A.arborescens gives very weak reactions for any resins, while the resin spot normally found immediately above aloin is weak or absent in the following species: A.arborescens, A.reitzii, A.aculeata, A.marlothii, A.vryheidensis, and A.africana. Further to this, there appeared a thin mustard yellow spot under and adjoining the aloin spot of the species A.candelabrum, A.excelsa and A.aculeata.

CONCLUSION.

Para-coumaric acid was absent from the species of Group A, or present in concentration less than 5 µg, that is, 0.5% w/v in the sap. Furthermore, with the exception of A.arborescens, all species had several resin spots of high concentration, judging by the colour reaction. This would appear to confirm the statement (RAMSTAD (175)) that the resins appear to be the condensation products of anthraquinones or anthranols.

8.7.9 GROUP B.

Exactly the same procedure was applied as before, and the plate from left to right was spotted as follows -

p-coumaric acid 5 µg, A.suprafoliata, A.speciosa, A.cryptopoda, A.wickensii var. lutea, A.lutescens, p-coumaric acid 10 µg, A.angelica, plus p-coumaric acid 10 µg, A.pachygaster, A.mutabilis, A.melanacantha, A.munchii, A.hereroensis, A.pearsonii, A.comptonii, A.distans, A.mitriformis.

When viewed under ultra-violet light the close chromatographic similarity of certain species became obvious, for example, A.comptonii, A.distans and A.mitriformis bear close resemblance to one another, as do A.pachygaster and A.hereroensis, both of the latter producing a deep blue spot (Rf 0.07) with alkali, which no other species possessed. Following the use of both spray reagents, the remarkable similarity between A.cryptopoda, A.speciosa and A.lutescens becomes apparent, while A.wickensii var. lutea is similar to these three but lacks certain spots. It will be recalled that these four species also have a common factor, inasmuch as they alone of the homonataloin group possess a spot which goes rose-pink with nitrous acid (see section 5.3.4). The four species were consequently run in the above Solvent L (as distinct from Solvent V used in section 5.3.4) and sprayed with the nitrous acid reagent, whereupon the pink spots appeared just above the starting line (Rf 0.06). This plate was oversprayed with Fast Blue B and resins appeared slowly as weakly orange spots, while purple spots also appeared. The bright purple of

homonataloin was unchanged by this aftertreatment.

RESINS IN GROUP B.

Reference to figures 8(i) & 8(iii) will show the relative distribution of resins. Certain of these fluoresced deep blue after alkali treatment, including the one mentioned previously (Rf 0.07), and a spot just below homonataloin in the following species:- A.pachygaster and A.hereroensis (both of which had the spot at Rf 0.07), A.comptonii, A.distans and A.mitriformis. The alkali treatment revealed the p-coumaric acid markers and confirmed the absence of this acid from the Group B species.

Treatment with Fast Blue B revealed several orange, brown or purple spots. Intense orange spots were observed as follows:

(a) immediately above homonataloin (frequently merging with it) A.angelica, A.melanacantha, A.pearsonii, A.comptonii, A.distans and A.mitriformis

(b) immediately below homonataloin (frequently merging with it) A.suprafoliata, A.pachygaster, A.mutabilis, A.hereroensis, A.comptonii, A.distans and A.mitriformis.

(c) Rf 0.17 ($\pm$ 0.01)
A.angelica, A.mutabilis, A.munchii, A.pearsonii, A.comptonii, A.distans and A.mitriformis.

(d) other spots

Several other spots appear, the positions of which are best seen in figure 8(iii). The absence of resinous spots below the homonataloin level (Rf 0.52) is most noticeable in the following four species viz. A.speciosa, A.cryptopoda, A.wickensii var. lutea and A.lutescens, although two of these (A.cryptopoda and A.lutescens) have very similar resins above homonataloin at Rf's 0.76 and 0.88. The spot at Rf 0.88 is common also to the following - A.speciosa, A.mutabilis, A.munchii and A.comptonii.

CONCLUSION.

As for the aloin group, the fact that the homonataloin group contains several resins points to the possible origin of resins as being anthranolic condensation products. Para-coumaric acid was absent from this group, or present below a concentration of 0.5% w/v in the sap.

8.7.10 GROUP C.

For convenience, the 26 species in this group were divided into two groups, 15 being run on one plate and the remaining 11 species on another plate.

Group C (i)

from left to right were the following:

A.grandidentata, A.longibracteata, A.simii, A.fosteri, A.dyeri, p-coumaric acid 10 µg, A.ammophila, A.petrophylla, A.branddraaiensis, A.saponaria, A.chabaudii, A.globuligemma, A.karasbergensis, A.longistyla, A.pratensis, A.polyphylla.

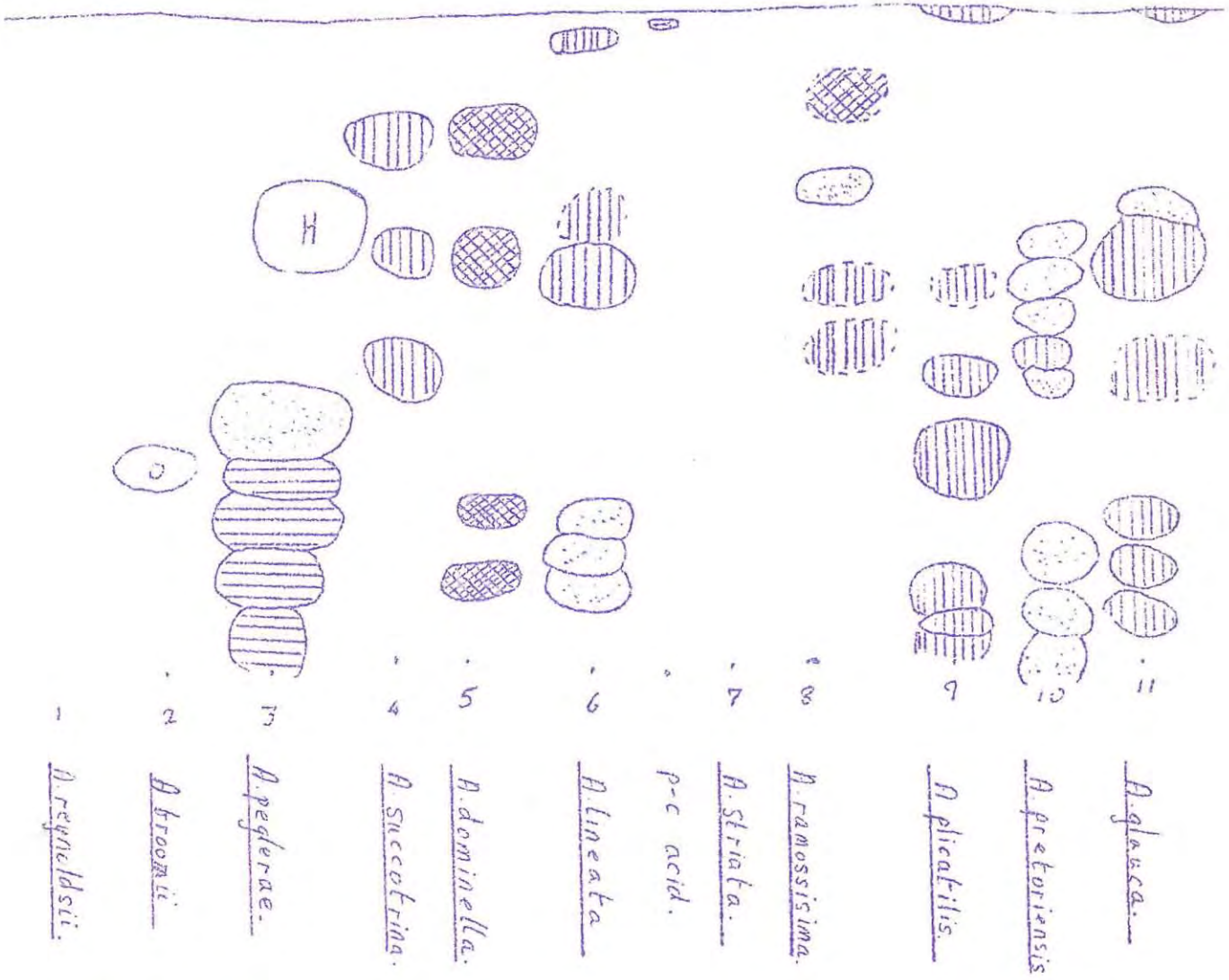
When sprayed with alkali it was observed that the p-coumaric acid was on a lower Rf than normal, which is accounted for by the longer activating time used. Pink spots (anthraquinones) in daylight were observed only in A.chabaudii, appearing at Rf 0.28 and 0.89, while orange fronts were observed in the following:- A.longibracteata, A.simii, A.fosteri, A.ammophila, A.petrophylla and A.karasbergensis.

Overspraying with Fast Blue B revealed that only a few resins were present, notably in A.petrophylla, A.globuligemma and A.chabaudii, but several purple spots appeared, as is shown in figure 8(iv).

According to HÖRHAMMER, WAGNER, BITTNER & GRAF (113) certain orange coloured spots with Fast Blue B are p-coumaric acid-containing resins, or cinnamic acid resins, but purple spots have not been classified. In view of the appearance of coloured spots at the same Rf as p-coumaric acid in A.fosteri, A.chabaudii, A.karasbergensis, A.pratensis and A.polyphylla these species were re-run in Solvent L, where it was seen that

figure 8(iv).
Group C(i).

Figure 8(v).
Group C(ii).



Colour Code.

= orange

= mauve or purple.

= buff

= reddish-brown

H = homonataloin.

O = orange (KOH).

= p-Coumaric acid.

p-coumaric acid was absent from these species.

Group C (ii).

was as follows from left to right on the plate - p-coumaric acid 5 µg, A.reynoldsii, A.broomii, A.peglerae, A.succotrina, A.domimella, A.lineata, p-coumaric 10 µg A.striata, A.ramosissima, A.plicatilis, A.pretoriensis and A.glauca.

When sprayed with alcoholic KOH the p-coumaric acid markers stood out clearly just below the front, and p-coumaric acid was absent from all species. This reagent produced a dark yellow spot in A.peglerae at Rf 0.70 both by ultra-violet and daylight.

Overspraying with Fast Blue B produced several spots, mainly purple, redish brown or buff-coloured. These colours were recorded immediately when traced, but several purples had faded entirely or changed to a buff colour not unlike pale orange, and it is thus difficult to say if these are resinous substances. Their inactivity with alkali would suggest that they are not.

Only in A.peglerae were several orange spots observed, while the spot at Rf 0.70 which had been dark yellow with alkali turned orange with Fast Blue B and finally deep blackish purple. As this species had not been critically examined before, it was re-run at a later date and from its chromatographic appearance, and reaction with nitrous acid, appeared to

contain homonataloin. This spot when eluted and examined spectrophotometrically had peaks at 347, 295, 273, 250 and 222 mμ which confirms the presence of homonataloin in this species.

Reference to figures 8(iv) & 8(v) shows the several coloured spots that appeared on spraying with Fast blue B, while an idea of the colour intensity can be gauged from the colour photo of Group C(ii) in figure 8(i). From this it will be observed that a similar purple spot occurs at the front of A.lineata, A.plicatilis and A.glauca.

It should be noted that purple spots appeared in the following species - A.chabaudii, A.pratensis, A.succotrina, A.lineata, A.plicatilis, A.pretoriensis, A.polyphylla and A.glauca, while purple tailing colouration from the base upward (varying heights) appeared in A.longibracteata, A.branddraaiensis, and weakly in A.simii, A.fosteri and A.saponaria. These colours faded rapidly.

8.7.11 CHROMATOGRAPHY USING SOLVENT P (ethyl acetate:methanol: water (100:16.5:13.5)

Solvent P suffers from the disadvantage that with this solvent p-coumaric acid cannot be seen distinctly, as it is hidden by other constituents. However, using this solvent HÖRHAMMER, WAGNER, BITTNER & GRAF (113) were able to differentiate certain of the resins found in commercial aloes. These

aloes were Cape, East African, Socotra, Indian, West Indian and Natal aloes, and the resins described by them were as follows:

(i) a resin immediately above aloin, which was described as "p-coumaric acid-containing,"

(ii) a resin immediately above this (in Indian and West Indies aloes) described as "cinnamic acid-containing"

(iii) a resin at approximately Rf 0.25 on Silica gel G plates, which is the basic resin or "harzgrundcörper". They state that all of these are coloured orange by Fast Blue B, but only the first two are coloured blue with alkali.

Consequently the Aloe species of Groups A, B & C were run in this solvent on Silica gel G plates, whereupon they were sprayed first with alcoholic KOH, followed by Fast Blue B reagent. The resin spots common to the species of Groups A & B are set out below according to Rf in Solvent P:

Rf 0.35: resin (bright light blue under u.v., increased by KOH, and orange with Fast Blue B in daylight). This resin was common to many species of Groups A & B, and is aloesin.

Rf 0.53: resin, (blue with KOH under u.v., orange with Fast Blue B in daylight). This spot, located just under aloin or homonataloin, was present in the following species: (Group A) A.dolomitica and A.vanbalenii; (Group B) A.angelica, A.pachygaster, A.melanacantha, A.mutabilis, A.comptonii,

A.distans and A.mitriformis. In the case of the last three the resin encroaches into the homonataloin.

Rf 0.61: aloin

Rf 0.64: homonataloin

Rf 0.67: resin, (blue with KOH under u.v. light and orange with Fast Blue B in daylight). HÖRHAMMER, WAGNER, BITTNER & GRAF (113) found this resin only in Cape and Natal commercial aloes. This is said to be a p-coumaric acid-containing resin and was present in all the species of Group A & B except the following: A.reitzii, A.marlothii, A.vryheidensis, A.suprafoliata, A.speciosa and A.munchii. It was absent from Group C.

Rf 0.77: resin, (blue with KOH under u.v., orange with Fast Blue B in daylight). This resin has a similar location to the cinnamic acid-containing resin described in Indian and West Indies aloes by HÖRHAMMER et alia (113) but they do not describe the blue colour with KOH. This resin was present only in Group B in the species A.speciosa, A.cryptopoda, A.lutescens, A.mutabilis and A.munchii.

Several purple spots at higher Rf's than aloin were described by HÖRHAMMER et alia (113) in East African, Socotra and West Indies aloes. No attempt was made by them to differentiate these purple spots. Purple spots were observed here in A.speciosa, A.candelabrum, A.excelsa, A.sessiliflora,

A.reitzii and A.aculeata, and in several of the aloes of Group C.

8.7.12 ALOESIN.

Aloesin is the name given to a new C-glycosyl chromone from aloes, the structure of which has just been elucidated by HAYNES & co-workers (to be published).

8.7.12.1 CHEMICAL REACTIONS OF ALOESIN.

A sample of aloesin (kindly supplied by the above) had the following characteristics: It was a pale yellow micro-crystalline powder, readily soluble in methanol to give a yellow solution which fluoresced luminous whitish blue under ultra violet light.

This methanolic solution was spotted onto filter paper and treated with certain reagents, as shown in table 8(ii).

Table 8(ii) CHEMICAL REACTIONS OF ALOESIN.

Reagent.	Instantaneous. Daylight.	Instantaneous. U.V.	After 7 days. Daylight.	After 7 days. U.V.
1) KIO_4	Nil	Nil	pale yellow	pink-mauve
2) HNO_2	Nil	greenish tinge	yellow	greenish-blue
3) Mg acetate	Nil	Nil	Nil	Nil
4) KOH 5% alcoholic	yellow	bright blue	yellow-brown	bright blue
KOH 10% aq.	yellow	deeper blue	yellow-brown	bright blue
5) Fast Blue B	orange	orange-red	orange	orange-red

These reactions were highly suggestive of the "resin" spot common to many species of Group A and Group B which had been studied some months previously. A record had been kept of this luminous whitish blue spot, but it was decided to re-run all the Aloe species of Groups A, B & C in various solvents, using aloesin as control, since it had been observed previously that spots on the same Rf as the luminous whitish blue spot, but of a greyish appearance, had reacted positively with Fast Blue B, suggesting masking by other compounds.

8.7.12.2 CHROMATOGRAPHY.

Chromatography was performed on Silica gel G plates using the following solvent systems:-

Solvent V - chloroform: ethanol 95% (3:1)

Solvent P - ethyl acetate:methanol:water (100:16.5:13.5)

Solvent R - Propanol:ethyl acetate:water (4:4:3)

Solvent L - ethyl acetic: glacial acetic acid:water (4:1:5)

Solvent M - petrol ether (40^{o}):toluene:xylene:methanol (4:1:1:2)

Chromatography of the aloesin sample in the above solvents showed it not to be a pure component, but as the appearance under ultra-violet light of the two spots was identical, they are possibly isomers. One spot is far larger than the other, and this is the spot which corresponds to the principle found in

many Aloe species, and which is referred to here as aloesin. Since the aloesin was not a single entity, no melting point determination was made.

The species of Groups A, B &C (section 8.7.4) were run initially in Solvent P. The plates were then observed under ultra-violet light, and aloesin was obvious in certain species. At the same Rf was frequently observed a greyish spot not having the same luminous appearance. These species were run in the other solvents to try to differentiate this greyish spot, which is frequently Fast Blue B positive. Solvent M caused distortion of the spots and was discarded, while Solvent L caused a definite quenching of the luminous spot, it appearing bluer in this solvent. (This effect was useful in the case of A.angelica, since in Solvent L the principle at the Rf of aloesin was identical in colour to the control, whereas in the other solvents it fluoresced a deeper blue.)

The positive reaction of aloesin with Fast Blue B is interesting. Although the reaction is attributed almost exclusively in the literature to resins, the reagent actually indicates phenolics and amines capable of coupling (section 8.5.3). It is doubtful if aloesin is in fact a resin, and the publication by HAYNES on its structure is awaited with interest.

RESULTS.

Aloesin was positively present in the following species, on grounds of Rf identical to the control, ultra-violet appearance and reaction with Fast Blue B:
A.ferox, A.candelabrum, A.excelsa, A.cameronii, A.sessiliflora, A.reitzii, A.aculeata, A.marlothii, A.vryheidensis, A.africana, A.dolomitica, A.castanea, A.vanbalenii (i.e. all of Group A except A.arborescens), A.mutabilis, A.munchii, A.pearsonii, A.comptonii, A.distans, A.mitriformis.

In the following species a spot occurred at the same Rf as aloesin but differing in colour. This spot, which was Fast Blue B positive, occurred in the following: A.angelica, A.melanacantha, A.peglerae, and A.petrophylla. From the appearance in the above solvents, aloesin is probably present in A.angelica and A.petrophylla and possibly present in A.melanacantha and A.peglerae.

Table 8(iii) Rf VALUES OF ALOESIN.

Solvent.	Aloesin.	Aloin.	Homonataloin.
P	0.37	0.60	-
V	0.21	-	0.65
R	0.64	-	0.86
L	0.23	-	0.60

8.7.12.3 ULTRA-VIOLET SPECTRUM OF ALOESIN.

When chromatographed in Solvent V (T.L.C.) aloesin formed two spots, the larger lower spot being aloesin, and an upper smaller spot of identical appearance. These were cut off and spectra were run on a Beckman D.B. spectrophotometer fitted with a recorder (1cm cells, solvent methanol).

Aloesin had a broad clearly-defined peak at 296 mμ, with a valley at 256 mμ rising sharply to give two small, sharp peaks at 252 mμ and 244 mμ. These peaks are identical to those described by BÖHME & KREUTZIG (28) for their "Harzsubstanz Rf 0.31" (section 8.3.4). The smaller upper dot had no clearly-defined peaks, increasing steadily in optical density from 360 mμ to 220 mμ, with a hump between 320 and 280 mμ.

8.7.12.4 HYDROLYSIS OF ALOESIN.

Seeing that aloesin is a C-glycosyl compound it was decided to hydrolyse the compound to see if the aglycone was an anthraquinone. To this end a few milligrammes of aloesin were refluxed with 10 ml of 5N HCl containing 20% ferric chloride for four hours on a boiling waterbath.

After this the solution was extracted separately with 5x5ml portions of ether and later ethyl acetate. Each was washed free of ferric ions, concentrated to a small volume and chromatographed in polar and non-polar solvents.

It was evident that aloesin no longer remained in either the hydrolysate or the ethereal or ethyl acetate extracts. Only a few grey spots became evident under ultra-violet light, and no spot reacted either with ammonia solution or magnesium acetate reagent. The solutions were similarly negative - hence the aglycone is not an anthraquinone derivative.

8.7.13 ELECTROPHORESIS OF RESINS.

Two species, A.ferox and A.speciosa, were subjected to electrophoresis to ascertain the charge on the resins, which in turn might give an indication of chemical groups on the molecules.

Dilute methanolic solution (0.01 ml) of the two Aloe species were transferred to Whatman No.1 paper, a 2 cm 'line' being made with the micro-pipette at the centre of the paper, which was 30 cm long.

Use was made of the Shandon Unikit apparatus (350 volts, 10 mA) and separate runs were performed in acid buffer, neutral buffer, and alkaline buffer. In acid buffer very little migration had occurred even after 25 hours, but in alkaline buffer good separation was achieved in $4\frac{1}{2}$ hours. The papers were well-dried before a heater immediately on removal to curtail diffusion, then viewed under ultra-violet light. This was followed by spraying with alcoholic KOH, and when dried,

by spraying with Fast Blue B reagent.

ACID BUFFER:

Equal volumes of acetic acid N/10 and ammonium acetate N/25 were used (pH 3.5). In this buffer the glycanthrones migrated further than the resins, with the exception of a resin in A.ferox which appeared on spraying with alcoholic KOH. Migration was towards the negative pole, with the exception of a flavonol in A.speciosa, which migrated towards the positive pole. The results are set out in table 8(iv), distances being measured from the starting line to the centre of the spot.

ALKALINE BUFFER:

Equal volumes of ammonium hydroxide N/10 and ammonium acetate N/25 were used (pH 9.7). In this buffer good separation occurred, with several resin spots appearing. Despite the shorter running time, far greater migration of the resins occurred, but migration of the glycanthrones was small.

Table 8(iv).

Distance in cm	u.v.	KOH (uv)	Fast Blue B	CHARGE	compound
A.ferox	(Acid buffer)				
0.7	light blue	blue	-	+ ve	resin
1.6	orange	yellow-orange	brown yellow	+ ve	aloin
2.4	-	light blue	-	+ ve	resin
A.speciosa	(Acid buffer)				
1.2	blue	blue	-	+ ve	resin
2.0	brick red	red	mauve	+ ve	homonataloin
10.0	yellowy green	greenish	-	+ ve	flavonol
A.ferox	(Alkaline buffer)				
0.3	orange	yellow orange	-	+ ve	aloin
0.9	pale orange	pale orange	-	+ ve	?
4.8	blue-violet	bright blue	orange	+ ve	resin
6.6	lichen green	blue	orange	+ ve	resin
(8.0)	-	blue	orange	+ ve	diffused resin
9.3	lichen green	pinkish	purple-orange	+ ve	resin (?)
(11.0)	-	pinkish	faint orange	+ ve	diffused resin

Distance in cm	u.v.	KOH (uv)	Fast Blue B	CHARGE	compound
A.speciosa	(Alkaline buffer).				
0.8	yellowy green	greenish	-	+ ve	flavonol
1.5	brick red	red	mauve	- ve	homonataloin
3.4	violet	violet	faint mauve	- ve	resin
6.1	dull mustard	-	mauve	- ve	resin (?)
8.1	dull mustard	-	deep mauve	- ve	resin (?)
A.ferox	(Neutral Buffer)				
1.4	-	dull green-brown	pale orange	- ve	resin
2.3	pale beige-grey	-	faint mauve	- ve	resin (?)
2.1	orange	yellowy-orange	-	+ ve	aloin
A.speciosa	(Neutral Buffer)				
1.6	yellow	greenish yellow	-	- ve	flavonol
2	brick red	red	deep purple	+ ve	homonataloin

In this buffer the resins were strongly electronegative, homonataloin migrated 1.5 cm towards the positive pole, but aloin appeared to migrate (0.3 cm) towards the negative pole. However, this figure is so small that diffusion could have occurred. The principle observed before in A.speciosa (flavonol) had moved 0.8 cm towards the negative pole in contrast to its movement of 10 cm towards the positive pole in acid buffer. These results also appear in table 8(iv).

NEUTRAL BUFFER:

Use was made of Beckman buffer of pH 7.0. In this buffer the main resin spots (as shown by Fast Blue B reagent) had diffused on either side of the starting streak to a diatance of approximately 1 cm during the 23 hours running time. This resin spot gave with Fast Blue B a mauve colour (A.speciosa) and orange (A.ferox). The glycanthrones had migrated a short distance towards the negative pole, while the suspected flavonol occurring in A.speciosa, and a weakly-reacting spot which tailed in A.ferox, had migrated towards the positive pole for short distances. The exact readings appear in table 8(iv).

Both in acid and in neutral buffer the movement of glycanthrones was small even after a protracted run exceeding 20 hours, and was of the order of 1.5-2 cm. In alkaline buffer

their movement was negligible.

Movement of the resins was even smaller in acid buffer and negligible in neutral buffer, but in alkaline buffer the movement of resins was large, being of the order of 8-10 cm in the relatively short time of 4½ hours. Their migration towards the positive pole in alkaline buffer is suggestive of carboxyl groups, which would be in accordance with Kiefer's (ex 237) isolation of three resins due to their solubility in sodium bicarbonate solution.

8.7.14 DISCUSSION OF THIS CHAPTER.

It is clear that more than one resin is common to species found in both Group A and Group B studied here, but not in Group C. (McCARTHY & PRICE (150)). Since Groups A & B are anthrone-containing, and Group C is not, it would seem that there may be some substance in the theory that resins are condensation products of anthranols or anthraquinones (RAMSTAD (175)). The prevalence of aloesin in aloin-containers is striking, and one wonders if it could be a precursor. However, it does not appear to be an anthraquinone derivatives.

Para-coumaric acid.

The method described here using Solvent L and thin-layer chromatography is suitable for identifying p-coumaric acid, especially in fresh saps where no masking anthraquinone

aglycone is present, provided the concentration is high enough. Using 5 µg or a 0.2% w/v solution of p-coumaric as marker it was shown that at that concentration p-coumaric acid acid was absent from all these Aloe species (with the possible exception of A.chabaudii). (McCARTHY & PRICE (150)). However, since p-coumaric acid is tuberculostatic below a concentration of 0.01% (DÖFF (60)), chromatographic methods cannot be relied upon to show complete absence of this compound from Aloe juice, microbiological methods being more sensitive. What can be shown, however, is that reports by earlier workers, who claim to show the presence of p-coumaric acid in Aloe species by chromatographic methods, are erroneous.

CHAPTER 9.

METABOLISM OF ANTHRACENE DERIVATIVES.

9.1 INTRODUCTION.

In recent years several papers have been published regarding the metabolism of anthracene derivatives, the plants concerned being Rheum palmatum, Rhamnus purshiana, Rhamnus frangula and Rumex alpinus. Apart from work by McCARTHY & VAN OUDTSHOORN (149) on two Aloe species (A.ferox and A.marlothii), no work has been performed on Aloe species. Consequently in this work the monthly variation of the anthracene derivatives in four Aloe species has been studied, simultaneously with the monthly variation of organic acids in those same species. The reason for this is that previous work on A.ferox by McCARTHY (146) seemed to indicate that wind had a profound effect on aloin content of the species, measured monthly, and it was thought that the simultaneous study of both anthracene derivatives and organic acids in the species might reveal a patter, possibly associated with water balance in the plant.

9.2 WORK ON OTHER ANTHRACENE-CONTAINING SPECIES.

A study of the significance of anthracene derivatives to the living plant of Rhamnus purshiana has been made by

BETTS & FAIRBAIRN (17). For analysis, use was made of leaves, and the bark of shoots, and analysis was of C-glycosides, O-glycosides and of anthraquinones, all calculated as aloe-emodin. Marked variation in the classes and totals of anthracene derivatives occurred throughout the year, suggesting that these compounds are concerned with metabolic processes, since if they were merely waste products, one would expect a gradual accumulation or a fairly steady concentration throughout the year. In the opinion of the authors, the C-glycosides represent the storage form of the anthracenes, since the proportion of C-glycosides to total anthracenes increases markedly from the leaf and young shoots to the oldest bark.

A rapid increase in total anthracenes in the older and young barks was observed from January to April (Winter to Spring in the Northern hemisphere), whereas, apart from an initial drop (in Spring), the anthracene derivatives in the leaves did not vary much during the season. Thus mature leaves may act as synthesising sites, the products being removed constantly to the shoot and older bark for storage until the following Spring.

Working on the closely related species Rhamnus frangula, NIHOULE-GHENNE (164) came to similar conclusions. She showed that there was a drop in combined anthracene derivatives of

the bark in the Winter, suggesting migration to storage organs. There was a marked rise in the Spring which coincided with early development of the leaves, and later the photosynthetic activity of the leaves enabled the stores to be replenished.

SCHRATZ & NIEWÖHNER (188) have studied the shoots of Rheum palmatum at all stages of development both for type and quantity of anthraquinones. They found that the distribution of anthraquinones was even more complicated than in the previously described species.

Thus the embryo was found to contain a small amount of chrysophanol and rheum-emodin, while the cotyledons contained, in addition, aloe-emodin. Aloe-emodin remained only in the first primary leaves but re-appeared later in the fruit alone.

With the exception of the actual development stages, the main constituent of the shoot represents rheum-emodin, with chrysophanol as the secondary constituent. The shoots thus differed from the roots in their lack of rhein.

The distribution of anthraquinone in the fully grown leaf is also interesting, since the content increased uniformly and intensely from the petiole towards the tips of the main ribs. According to the authors the ageing and dying of the leaf is accompanied by an enrichment in anthraquinones.

The work of LUKIC (144) on Rumex alpinus is conc rned more with monthly content variation than with distribution throughout the plant of specific anthraquinones. In contrast to the findings of BETTS & FAIRBAIRN and NIHOUL-GHENNE, the anthraquinone content of the root and rootstock, in this case, fell in Summer, but similarly had its highest point in Spring. However, a study of the oxidised glycosidally-bound form showed a rise in the Summer months, although the anthraquinone by contrast, showed almost the complete antithesis. In fact, the action of the two forms seems almost complementary inasmuch as when one falls the other rises. The fact is stressed, as a similar occurrence will be demonstrated later for the C-glycoside and O-glycoside of A.africana studied here. A rise in the Summer months of the C-glycoside, aloin, has been observed by McCARTHY & VAN OUDTSHOORN (149) for the species Aloe ferox and Aloe marlothii.

Aloe species differ from the previously named species, inasmuch as the anthracene derivatives, which are mainly C-glycosyl compounds (or glycanthrones), are almost invariably confined to the leaf, and more specifically to the leaf sap, since well-studied species such as A.ferox and A.vera show well-defined aloinaceous cells in the leaf. Apart from the glycanthrones aloin and homonataloin, VAN OUDTSHOORN (227) has shown chrysophanol and chrysophanol glycosides to exist

both in the leaves and roots of five Aloe species.

Consequently in this work not only the leaf sap of the four species has been examined, but also the seeds, fruits, flowers, stem bark and outer stem wood.

9.3. THE FUNCTION FO GLYCOSIDES.

Since the leaf sap of the four species studied is known to contain only glycosides, the question arises as to what may be the possible function of the glycosides in the plants. It has been mentioned that BETTS & FAIRBAIRN (17) consider the C-glycosides as storage forms of the anthracenes, yet A.ferox, A.africana, A.arborescens and A.speciosa appear to contain no anthracene derivative apart from the glycanthrones.

According to MEYER & ANDERSON (155) the role of glycosides in the metabolism of plants is obscure although it is possible that they may serve in a minor way as storage foods.

STEELE (207) has several suggestions for the functions of glycosides, a) that they are reserve food materials, since some glycosides have a definite seasonal variation, often accumulating in Autumn and Winter and disappearing in Spring when more active metabolism sets in, e.g. salicin and arbutin; b) the removal of injurious substances. This is thought to beprobably the most general function of the glycosides, namely the conversion of aglycones which are insoluble

(and susceptible to oxidation and polymerisation), into soluble chemically stable and inert substances; c) that they act as antiseptics and hormones. STEELE points out that many aglycones are antiseptic and bactericidal and as a corresponding enzyme is so often adjacent to the glycoside, their presence may effect some control of invasion by disease or injury; d) biological function. Since many glycosides are toxic to animals they may protect the plant from raids by animals until the appropriate time for distribution of the seeds, which often coincides with a decrease in glycosidal content as fruits mature.

Whatever the function of glycosides in the plant, there seems no doubt that the glycosidal form of anthraquinones is more effective pharmacologically in man than the aglycone. According to FAIRBAIRN (74), it has been established that the presence of sugar groups is essential for the purgative activity of anthraquinone glycosides. Further, increase in sugar groups seems to lead to even greater activity. The sugars may also protect the aglycone from destructive enzymes as has been shown to occur with certain flavonoid glycosides.

9.4 THE EFFECT OF WIND.

Earlier work by McCARTHY (146) on A.ferox seemed to indicate that wind had a profound effect on aloin production

inasmuch as aloin content fell in months of high wind and rose as wind velocity dropped. No reason for this could be put forward unless shrinkage of the leaf surface, which is known to occur, could possibly be interfering with the aloinaceous cells lying just beneath the cuticle.

A leading authority on the effect of wind on plants, Dr. Whitehead, suggested in a private communication that possibly what was being reflected was the plant's efforts to maintain water balance during periods of high wind, water migrating away from the leaves, which are organs of transpiration.

WHITEHEAD (239) and WHITEHEAD & LUTI (240) have studied the effects of wind on the growth of, respectively, Heliaanthus annuus and Zea mays. In the case of the former it was shown that higher wind speeds tended to produce greater water loss from the shoots, which consequently shrank to reduce shoot area while root area increased, allowing greater moisture uptake and restoring the water balance of the plant. With Zea mays it was found that wind-treated leaves were much thicker and wider than controls of plants grown in absence of wind. Furthermore, stems were thicker and shorter, roots more elongated, so that water lost by transpiration was much less than that of the controls. Another method of conserving plant moisture is by shedding of leaves (to reduce the

transpiring surface), by the closing of stomates, or by incipient dying of the cell membranes (TRANQUILLINI (219)). Aloe plants frequently bear dead leaves.

According to MEYER & ANDERSON (155) a gentle breeze is relatively much more effective in increasing the transpiration rate than winds of great velocity, probably due to stomatal closure in the latter case. Thus months of low wind velocity would be in concordance with high aloin concentrations in the leaf, and vice versa.

9.5 EXPERIMENTAL.

In view of the foregoing arguments it was decided to study the monthly variation in both glycanthrone content and in organic acid content. It was assumed that if wind caused moisture loss and consequent increase in concentration of glycanthrones, a similar rise should occur in organic acid content, always bearing in mind that no knowledge was held of the possible migration or acceptance across the cell membranes, of one or other compound due to osmotic effects.

To this end four species were selected, the first two named having a thick hard cuticle, the latter two having far softer, more pliable leaf tissues:

A.ferox	-	containing aloin
A.africana	-	containing aloin and aloinoside
A.arborescens	-	containing aloin
A.speciosa	-	containing homonataloin.

Each month a leaf was cut from each species and the sap analysed for glycanthrone content in the manner described below, and for acid content in the manner described in section 10.5.8. Collection was at 8 a.m. to eliminate possible diurnal variation in leaf content.

9.6 ANALYTICAL PROCEDURE FOR GLYCANTHRONES.

A weighed amount of fresh sap was made up to a set volume with methanol. The glycanthrones were separated by thin-layer chromotography on Silica gel G in chloroform: ethanol 95% (3:1). The spots were localised under ultra-violet light and transferred to centrifuge tubes, 5 ml of methanol added, then centrifuged and finally filtered through fine filter paper. This solution was read in a spectrophotometer at 360 mμ for aloin and aloinoside, and at 294 mμ for homonataloin, using 1 cm quartz cells, and methanol as blank. For the former determinations use was made of a Unicam S.P.600 spectrophotometer, and for the latter a Beckman D.B spectrophotometer. For each species the test was performed in triplicate.

To obtain the glycanthrone present as a percentage of the dried sap, an aliquot of the fresh sap was dried to constant weight at 105°C at the same time to determine its moisture content.

9.6.1 PREPARATION OF STANDARD GRAPHS.

Standard graphs for homonataloin and for aloin were prepared, but not for aloinoside, as VAN OUDTSHOORN (227) had shown that the two latter substances have similar extinctions at 360 mμ. Consequently aloinoside is actually calculated as aloin in this work. The pure substance in each case, was dissolved in methanol, and graded dilutions were prepared to contain the microgramme quantities per 5 ml methanol as depicted in table 9(i).
(The graph for aloin is that of McCARTHY (146) (1964).

Table 9(i). CONCENTRATIONS AND EXTINCTIONS FOR STANDARD GRAPHS.

Glycanthrone Concentration	Extinction. 294 mμ (homonataloin)	360 mμ (aloin)
20	0.195	0.122
40	0.292	0.244
60	0.375	0.328
80	0.488	0.428
109	0.682	0.577
120	0.720	0.633

9.7 DESCRIPTION OF ALOE SPECIES USED FOR MONTHLY VARIATION.

A.africana was unbranched, 4-5 feet in height and grew on a rocky terrace in a small public garden in Port Elizabeth.

A.arborescens was some 3 feet high growing on flat ground ith several other A.arborescens plants. It grew in a private garden, but the area was unfortunately shaded for a large part of the day which might account for both the low aloin and organic acid content. (It had been found that A.africana grown in the shade had a very low aloin content.) It was used after a previously selected A.arborescens was destroyed by vandals three months after work had commenced.

A.ferox was some 5 feet in height, growing on a rocky terrace in a Port Elizabeth park. It was selected because it had been studied previously for aloin variation (McCARTHY 1964 (146)) and it was thought that the comparison of the two years would be interesting. Unfortunately it had developed leaf scale, and this grew progressively worse, infecting nearly every leaf.

A.speciosa grew in the same park as A.ferox, but on flat ground. It was a magnificent specimen, having healthy leaves. It was some 9-10 feet high, and branched into nine 'heads' of leaves. These same plants were also used for the monthly variation in organic acid content. (Chapter 10)

9.7.1 SELECTION OF LEAVES.

Many variables exist in plant analysis, included amongst which is the possible variation in content of active constituent either in various plant parts, or in the case of leaves, at varying levels of the plant.

Thus VICKERY (232) & (233) found that the content of organic acids in tobacco leaves varied throughout the plant, but that the middle leaves had the most uniform distribution of acids. For A.ferox McCARTHY (146) had found that aloin content varied least for leaves on the same level in the plant, while BETTS & FAIRBAIRN (17), to reduce sampling

error as much as possible, collected samples at equivalent positions on the tree of Rhamnus purshiana.

Nevertheless, it was decided that before work could be performed on the species A.africana, A.arborescens and A.speciosa, their glycanthrone content at a fixed level on the plant would have to be determined. The results of these analyses appear in table 9(ii).

(Had it not been for the fact that the same leaf was used for both glycanthrone and acid determination, the method of VAN OUDTSHOORN (see McCARTHY & VAN OUDTSHOORN (149)) of incising the same leaf monthly would have been resorted to as being the method least likely to produce untoward variation.)

Table 9(ii) GLYCANTHRONE CONTENT OF NEIGHBOURING LEAVES.

Species.	Glycanthrone % in sap.
A.arborescens	a) 8.2, 8.7, 8.9 b) 10.2, 8.9, 8.7, 9.3, 8.7, 8.1
A.speciosa	10.4, 10.5, 12.8
A.africana	a) 5.9, 5.3, 6.1 b) 4.1, 2.0, 2.1
A.ferox (1964)	14.0, 13.6, 12.8.

Thus it will be seen that although a close agreement is generally reached, in some instances a neighbouring leaf may vary somewhat considerably, which makes the interpretation of the final graphs all the more difficult.

9.7.2 QUANTITIES OF SAP USED AND CALCULATION OF RESULTS.

Simce a micro-pipette of accurate calibration 0.006 ml was used, samples were made up to 6 ml with methanol, which simplified calculation of results as follows:

$$\text{Percentage glycanthrone} = \frac{\text{micrograms (from graph)} \times 100}{\text{mgm of sap in 6 ml methanol.}}$$

For extinction readings in the regions of 0.3-0.4, the following weights of sap were found suitable:

A.ferox	- 0.6g in 6 ml methanol.
A.africana	- 1.0g in 6 ml methanol.
A.arborescens	- 0.2g in 2 ml methanol. (= 0.6g in 6 ml methanol)
A.speciosa	- 0.4g in 6 ml methanol.

In the case of A.arborescens it should be noted that rarely could more than 0.2g be obtained from a leaf weighing circa 80g. In the case of A.speciosa the sap "gels" very quickly, and in all cases the saps were used within 30 minutes of collection.

9.8 RESULTS OF MONTHLY ANALYSES.

The results obtained are tabulated in tables 9(iii) to table 9(v). The relevant graphs appear in figure 9(i).

At each monthly analysis, A.arborescens and A.africana were analysed together, one day before A.ferox and A.speciosa. For simplicity they have been grouped in the same manner together with the weather statistics for each month (kindly supplied by the Meteorological Office, Port Elizabeth) to allow rapid correlation. In figures 9(i) & 10(iv) appear comparative graphs of the four species for glycanthrone content and for acid content. The table for the acid content is to be found, however, in table 10(vi).

A record was kept throughout of the weather on the day of collection of samples, but as this does not seem to have had any bearing on the results, these are not reported.

figure 9(i)

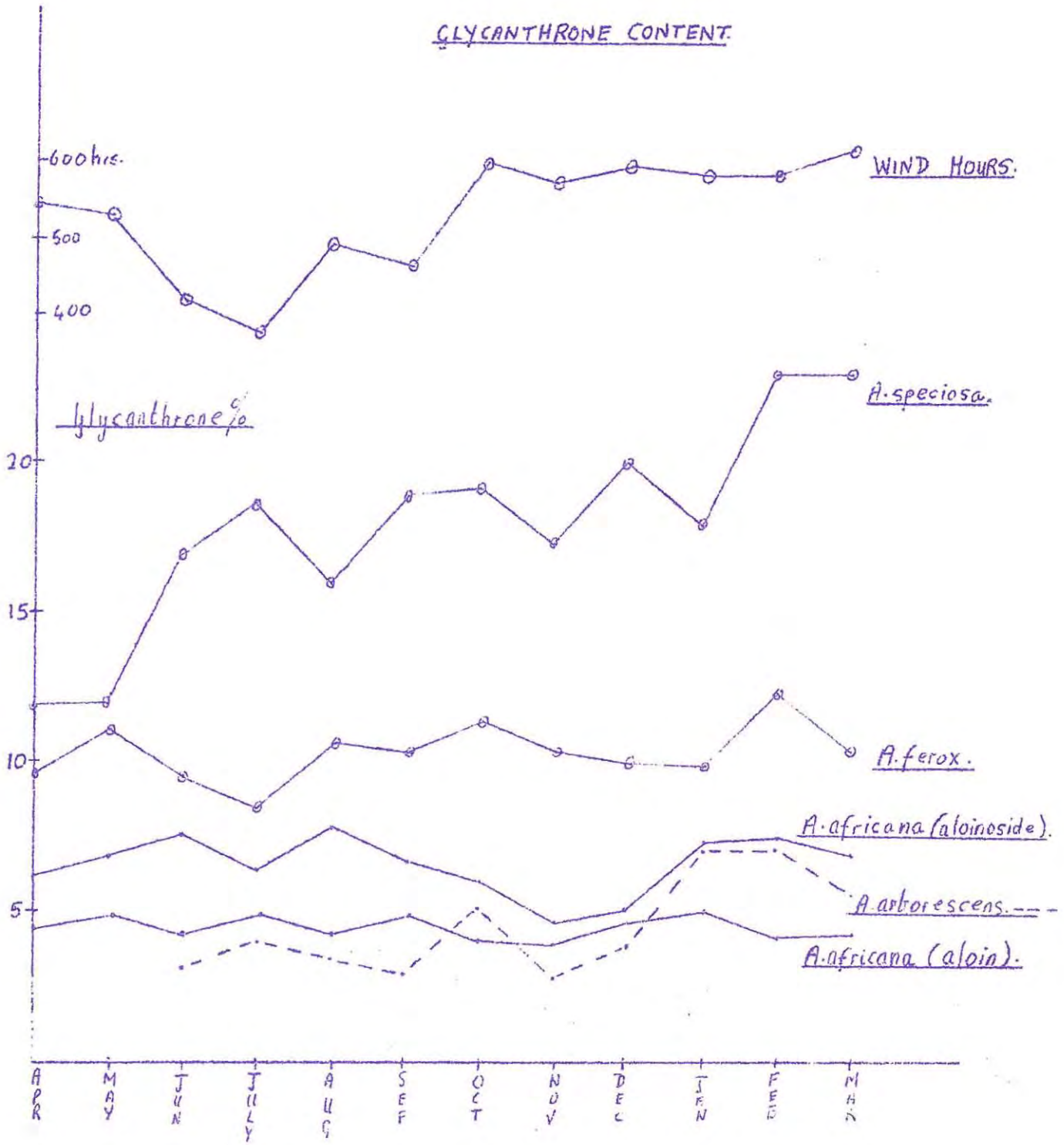

GLYCANTHRONE CONTENT.
600hrs.
500
400
WIND HOURS.
Glycanthrone %
20
15
10
5
A. speciosa.
A. ferox.
A. africana (aloinoside).
A. arborescens.
A. africana (aloin).
APR
MAY
JUN
JULY
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR

Table 9(iii). GLYCANTHRONE CONTENT OF A.ARBORESCENS & A.AFRICANA.

Date.	Glycanthrone content % A.arborescens aloin	A.africana aloin	A.africana aloinoside	Aver.Temp. C°.	Relative Humidity %	Rainfall mm.	Wind mph.	Wind Monthly hours.
March	-	-	-	20.8	76	37.3	7.6	-
April (1/5/65)	-	4.3	6.4	18.3	77	34.8	8.9	553
May (28/5/65)	-	4.9	7.4	15.2	77	84.9	8.0	539
June (29/6/65)	3.0	4.1	7.7	12.1	74	52.3	4.7	419
July (30/7/65)	4.0	4.8	6.4	14.1	82	29.9	4.9	382
August (1/9/65)	3.4	4.2	7.9	14.5	81	21.2	7.3	502
September (27/9/65)	2.9	4.8	6.7	15.2	80	27.2	7.1	470
October (28/10/65)	5.2	4.0	6.1	15.9	81	118	10.1	608
November (24/11/65)	2.8	3.8	4.7	17.2	79	92.7	10.5	579
December (29/12/65)	4.0	4.6	5.1	18.3	72	25.7	13.7	601
January (28/1/66)	7.1	5.2	7.3	21.2	81	20.3	8.0	588
February (25/2/66)	7.2	4.3	7.6	23.9	81	81.0	12.4	590
March (1/4/66)	5.6	4.4	6.8	21.1	84	27.3	10.7	624

Table 9(iv).

Date.	Glycanthrone content % A.ferox Aloin	A.speciosa Homonataloin	Aver.Temp. C°.	Relative Humidity %	Rainfall mm.	Wind mph.	Wind Monthly hours.
March 1965	-	-	20.8	76	37.3	7.6	-
April (1/5/65)	9.6	11.8	18.3	77	34.8	8.9	553
May (29/5/65)	11.2	11.9	15.2	77	84.9	8.0	539
June (30/6/65)	9.4	16.8	12.1	74	52.3	4.7	419
July (31/7/65)	7.9	18.7	14.1	82	29.9	4.9	382
August (2/9/65)	10.6	15.7	14.5	81	21.2	7.3	502
September (28/9/65)	10.5	19.0	15.2	80	27.2	7.1	470
October (29/10/65)	11.4	19.3	15.9	81	118.0	10.1	608
November (25/11/65)	10.4	17.3	17.2	79	92.7	10.5	579
December (30/12/65)	10.0	20.4	18.3	72	25.7	13.7	601
January (29/1/66)	9.8	18.1	21.2	81	20.3	8.0	588
February (26/2/66)	12.5	23.3	23.9	81	81.0	12.4	590
March (1/4/66)	10.4	23.2	21.1	84	27.3	10.7	624

Table 9(v). A.AFRICANA: PERCENTAGE GLYCANTHRONES IN FRESH SAP.

Month.	Aloin % -oside	Aloin %.	Total %.	Ratio aloinoside / aloin
1965				
April	4.3	6.4	10.7	1.49
May	4.9	7.4	12.3	1.51
June	4.1	7.7	11.8	1.87
July	4.8	6.4	11.2	1.33
August	4.2	7.9	12.1	1.88
September	4.8	6.7	11.5	1.39
October	4.0	6.1	10.1	1.51
November	3.8	4.7	8.5	1.23
December	4.6	5.1	9.7	1.11
1966				
January	5.2	7.3	12.5	1.41
February	4.3	7.6	11.9	1.76
March	4.4	6.8	11.2	1.54

9.9 ANALYSES OF PLANT PORTIONS OTHER THAN THE SAP.

a) Fruits and seeds.

Fruits from the four species were collected in June/July and allowed to dry spontaneously. They were then divested

of their seeds.

The seeds and portions of the fruits were separately placed in a mixture of chloroform/ethanol (3:1) and with the exception of the seeds of A.ferox and A.speciosa, immediately gave yellow coloured solutions.

Further seeds were taken for these two species and after cutting with a fine blade, these were ground with a glass rod. These were then extracted with hot chloroform/ethanol.

Each solution was then chromatographed on Silica gel G plates in the solvent chloroform:ethanol (95%) (3:1). On removal the plates were dried and sprayed separately with alcoholic KOH followed by Fast Blue B reagent, and also with magnesium acetate reagent, followed by heating at 105°C for 5 minutes.

Results. It was observed that the seeds gave no chromatographic picture apart from a spot at the solvent front for A.africana and A.arborescens which turned red with both the alkali and magnesium acetate reagents, suggesting an anthraquinone derivative.

All four fruits, however, produced several spots which, from their reactions with the sprays, appeared to be pigments. However, at the front of each fruit was a spot which reacted

like an anthraquinone. Resins were absent from both fruit and seed.

Consequently, since aloe-emodin also runs to or near the front in this solvent, they were re-run in benzene/acetic acid glacial (2:1), in which solvent aloe-emodin has an Rf of 0.62.

Once again the fruits of all four species and the seeds of A.africana and A.arborescens had a spot at the front, which went red after alkali treatment and after magnesium acetate, which suggests an anthraquinone reaction, but eliminates aloe-emodin. A.speciosa (fruit) had a red principle tailing from Rf 0.50 which turned mustard-yellow with magnesium acetate, but this is probably a pigment.

b) Flowers.

The flowers of each species except A.africana were formed and unopened or partly opened when collected. Those of A.africana were unformed and green. Extraction was with hot chloroform/ethanol, and the extracts were chromatographed as before, alternated with the sap of each species as a comparison.

Result. When viewed under ultra-violet light the four flower extracts had identical chromatographic pictures, namely a small round light-blue-grey spot at Rf 0 17 and a

light blue spot at Rf 0.34, the aloin spots having an Rf 0.35. These spots were unaffected by alkali treatment, and magnesium acetate and consequently anthraquinone derivatives are absent from the flowers.

c) Stem-bark and wood.

A small portion sliced from each plant was finely cut and extracted with boiling chloroform/ethanol. All except the sample from A.speciosa, which was a very young plant, formed deep yellow solutions. The extracts were chromatographed as before in chloroform/ethanol (3:1), and apart from the A.speciosa which had not run, showed identical pictures, with a fluorescent light green spot both at the front and opposite the aloin run as control. When sprayed with alkali a blue spot appeared for each of the three species at Rf 0.18 (aloin Rf 0.52). After spraying with Fast Blue B showed resins to be absent.

Repetition of the above tests with the bark and stem wood from a larger and older sample of A.speciosa showed a similar chromatographic picture as for the three species above, but there appeared a dark orange spot beneath the fluorescent light green spot at the front, while the fluorescent green spot at Rf 0.52 was absent from A.speciosa. Thus anthraquinone derivatives are absent from the bark and outer stem wood of the four species. The green spots react like a flavonol.

9.10 DISCUSSION.

Reference to the graphs in figure 9(i) reveal that the glycanthrone variation of no two species is the same. (Neither is there any apparent correlation between the variation of organic acid, either relative to the four species, or to the glycanthrone content of a species (section 10.6.8.1) In other words each species has acted independently of the others, and consequently variables such as soil drainage, soil pH and such-like factors probably predominate over weather factors for each individual plant. In a study of the physical properties of the sap of A.arborescens var. Natalensis, BEWS & BAYER (19) found that the physical properties (osmotic pressure, viscosity and density) of the sap were not subject to much variation in response to such external factors as rainfall and temperature during the months April to October.

For A.ferox it was shown (McCARTHY (146) 1964) that weather factors apart from wind had no effect on the aloin variation of the plant. From the results obtained here it appears doubtful if even wind has any effect although it will be noted that for A.africana the aloin variation is almost a mirror-image of the wind graph, which was the case with A.ferox in 1964. This shows how misleading can be the results obtained from a single plant and this same criticism

must apply to the results obtained by the workers on the other anthracene-containing plants reviewed in section 9.2. The problem of variables is not an easy one to overcome. To some extent small plants grown in pots in a hot-house can give very similar results, but as has been shown under Acid Metabolism, (section 10.6.7), Aloe plants grown in drums metabolise relatively poorly with respect to acids when not growing in their normal habitat. This may well apply to glycanthrone metabolism as well.

In this work the A.speciosa and A.ferox were located some fifty yards apart in a small park; the two other species came from different locales. Thus it was not possible to reproduce exactly the growth conditions of the four species.

Reference to the graph in figure 9(i) shows an interesting monthly correlation for the combined glycoside aloinoside and the C-glycoside aloin in A.africana. Once again it is dangerous to be dogmatic for only one sample but it would appear that there is a correlation between the two, inasmuch as when the one increases the other decreases. LUKIC'S (144) work on Rumex alpinus shows an identical relationship between the free anthraquinone and the glycosidal form (both in the oxidised state).

9.10.1 SEASONAL VARIATION.

Referring to the work of BETTS & FAIRBAIRN, LUKIC, NIHOULE-GHENNE and SCHRATZ & NIEWÖHNER described in section 9.2, it will be seen that for these plants, translocation of constituents occurs to a large degree, there being apparent synthetic sites and storage sites. With the exception of roots which were not tested here, it would appear that the metabolic activity of the Aloe species is confined to the leaf, anthracene derivatives being absent from stem bark and outer stem wood and from flowers, although as has been shown in section 9.9(a), a principle reacting like an anthraquinone was present in the fruits of all four species and in the seeds of A.arborescens and A.africana.

As with the previously mentioned species, the continual fluctuation in the monthly content of the Aloe species indicates that the glycanthrones are actively concerned with metabolic processes, since if they were waste products one would expect a gradual accumulation or a fairly steady concentration throughout the year (BETTS & FAIRBAIRN (17)). Their exact role in the plant is not known.

It is difficult to compare such dissimilar species as the Aloe, Rhamnus and Rumex species. In the case of the latter species it was found that the seasonal picture was one of a drop in anthraquinone content in the Winter months,

followed by a rise (usually rapid) in the Spring months.

Weather conditions are far more equable in South Africa, especially in Port Elizabeth, and furthermore the Aloe species flower in June (Winter) and not in Spring. Thus the results differ for Aloe species. Working six hundred miles apart on the two species A.ferox and A.marlothii respectively, McCARTHY & VAN OUDTSHOORN (149) found that the graphs of the seasonal variation of the two species were alike, both showing a steady rise from the Winter to the Summer months, followed by a slow fall as Winter returned.

The findings here have not been as clear-cut, although comparisons of the weather for 1962-1963 and 1965-1966 show vast differences. Thus for the latter period extremely high wind and heavy rainfall were experienced in October-November, and it will be noted that in all four species this has resulted in a drop in glycanthrone content for that period. However, A.speciosa has shown a steady rise throughout the year as has A.arborescens, if October-November's weather be taken into account. The total glycoside content of A.africana fell steadily from the flowering period to December, but then showed a rapid rise in the Summer months.

Only A.ferox has not followed a pattern similar to the other three species, although a belated rise is observed for February. Since this was the same plant used in 1962-1963,

and for which a definite rise in the Summer months was obtained, there are two possible causes contributing to this rather untoward result; a) the plant was badly diseased with white scale and this has possibly affected the normal metabolism of the plant. This scale spread alarmingly over the last few months but the high February result was obtained using an unblemished leaf; b) due to this scale it was not always possible to take undiseased or partly diseased leaves from the same level on the plant, which could cause untoward variation.

Nevertheless the overall picture in these four Aloe species and of A.marlothii is of a rise in glycanthrone content in the Summer months, followed by a slow decline as Winter approaches. No translocation of anthracene derivatives throughout the aerial portions of the plants appears to occur seasonally, with the possible exception of the flowering parts of the plants.

C H A P T E R 10.

ACID METABOLISM OF ALOE SPECIES.

10.1.1 The metabolism of acids in plants has been the subject of much research, and in particular, the acid metabolism of succulents. Included in the latter group are the Aloe species, the metabolism of which has been virtually left uninvestigated, although the identification of acids in Aloe species has in recent years been a subject of research (SODERSTROM (202) VAN OUDTSHOORN (225) SHKOL'NIK (197)).

10.1.2 SUCCULENTS.

Succulents constitute a considerable proportion of the vegetation of most semi-arid regions and are frequently found in locally dry habitats such as sand dunes and beaches in regions of humid climate (MEYER & ANDERSON (155)). The more important families of plants which embrace succulents are the Cactaceae, Euphorbiaceae, Liliaceae, Crassulaceae, Compositae, Aizoaceae, Amaryllidaceae, and Begoniaceae.

The succulents are a distinctive group of plants not only in structure, but in metabolism and water economy as well. Species of the succulent habit of growth are able to survive dry periods because of the relatively large reserves of water which accumulate in the inner tissues of the

fleshy stems or (in some species) in the fleshy leaves. A relatively thick cuticle, and the fact that in many succulents the stomata are generally open only at night, are important factors in permitting the conservation of water by such species. Many cacti can live for months on this stored water even if entirely uprooted from the soil (MEYER & ANDERSON (155)).

10.1.3 OCCURRENCE OF ACIDS.

Apart from amino acids, such as glutamic, aspartic and amino-acetic acids, several organic acids exist in plants, the most important being oxalic, tartaric, succinic, fumaric, malic, oxaloacetic, pyruvic, aconitic, citric, isocitric and α-ketoglutaric acids. Some of these (oxalic, malic, tartaric and citric) are present in considerable quantities in some tissues, others (e.g. pyruvic, oxaloacetic, succinic, fumaric and aconitic) are seldom present in appreciable amounts, but are important metabolites. To these may be added the aromatic acids, namely, benzoic, cinnamic, salicylic, o- and p-coumaric, melilotic, protocatechuic, gallic, rufigallic and caffeic acids, the nucleic acids (and what is referred to as 'traumatic acid' (FREYTAG (80)).

10.1.4 DISTRIBUTION OF ORGANIC ACIDS.

Oxalic acid and oxalates are probably present in almost all plants, although in most cases the quantity present is small. The withering autumn leaves of Rheum (Polygonales) are rich in oxalate. Malic acid is universally distributed, and fourteen natural orders have remarkably large accumulation of malate. Three of these, (Liliflorae, Polygonales and Rhamnales) are important sources of anthraquinones drugs. Tartaric acid is characteristic of the leaves and fruits of the Vitaceae, while citric acid is found in some Solanaceae, the Rutaceae, and in many Aspergilli and Penicillia. Lactic acid is found in species of Rubus, Agave, Solanum and Glaucium, while gluconic acid is found in many mould fungi (BENNET-CLARK (14)).

10.1.5 METABOLISM OF ACIDS.

Although the occurrence of organic acids has been known for many years, the function of these compounds in the plant remained obscure until relatively recently. It is now clear that the plant acids play a central role in cellular respiration both in plants and in animals. The series of reactions which are involved in the biochemical interrelations between the various acids, are known as the KREBS CYCLE.

10.1.6 KREBS CYCLE.

General evidence for the operation of this cycle in plants includes the fact that the enzymes for the component steps of the Krebs cycle have been found widely in higher plant tissue, and that the acids involved are all known as widely distributed plant products. The component acids of the Krebs cycle include all the plant acids except tartaric and oxalic. As a first assumption it may be suggested that it is through the reactions of the Krebs cycle that the several plant acids are formed and interconverted.

Accumulation of an individual plant acid to high concentration in a plant is of very usual occurrence and implies at once that the rate of production of this acid exceeds the rate of withdrawal. On the assumption that the Krebs cycle is operative in the plant tissue involved, accumulation of the plant acid in this tissue must be due to a block or partial block of a particular step in the cycle. Thus the accumulation of malic acid in fruits and leaves must be taken to indicate that the malic dehydrogenase of that tissue is not as active as the other enzymes of the earlier links in the cycle. Similarly the accumulation of citric or isocitric acids must be owing to individual blocks in the aconitase or isocitric dehydrogenase systems. These blocks might be due to low enzyme concentration or to the presence of

inhibitors, but at present the information necessary to explain such preferential accumulations is not available (BONNER (30)).

10.2.1 METABOLISM OF ORGANIC ACIDS IN EXCISED LEAVES.

The changes in organic acids which occur in excised leaves during starvation have been followed in tobacco and in rhubarb by PUCHER, WAKEMAN AND VICKERY (172). In both cases oxalic acid is unaffected by any treatment and is not re-utilised even in extreme starvation. In tobacco leaves cultured in the dark, malic acid rapidly disappears while citric acid appears, although in lesser concentration, since approximately one mol of citrate is formed for each two mols of malate utilised. This transformation might be understandable on the basis of the reactions of the Krebs cycle, since it would be possible for malate to be transformed to oxaloacetate and this in turn converted to pyruvate by the oxaloacetate decarboxylase which is known in higher plant tissues (GOLLUB & VENNESLAND (91)).

In excised leaves of rhubarb both malic and citric acids slowly disappear during incubation in the dark. An unidentified acid accumulates in rhubarb just as citric acid does in tobacco, and it has been suggested that malic acid may be converted in this case to the unknown acid. With

excised leaves of sudan grass and oats, however, both citrate and malate accumulate at the expense of unidentified precursors (WOOD et alia (249)), showing the wide range of possibilities that exist.

In this work the metabolism of excised leaves of Aloe species has been examined, but as these species fall under "succulents", discussion of these results is dealt with later under Experimental.

10.2.2 METABOLISM OF SUCCULENTS.

It has been shown that many species of plants accumulate acid in high concentration in their leaves. With the great bulk of plant species this acid, once formed, is relatively stable and does not disappear except under conditions of stress, as in starvation of the leaf.

With a few species, on the contrary, acids are formed primarily during the night and disappear again during the day. This diurnal fluctuation in acid content is characteristic of the group of plants known as succulents, and the remarkable metabolism which they exhibit is known as succulent or crassulacean metabolism (BENNET-CLARK (14)).

The synthesis of organic acids in the leaves of some succulents is directly related to the carbon dioxide concentration of the atmosphere. In Bryophyllum crenatum,

for example, virtually no organic acids are synthesised in the dark in the absence of carbon dioxide, but the greater the carbon dioxide concentration in the air up to 10%, the greater the quantity of organic acids synthesised (BONNER & BONNER (29)). The principal acids formed are citric, malic and isocitric.

The diurnal variation in the organic acid content of certain succulents is therefore probably to be explained on the basis that, during the daytime, carbon dioxide in the photosynthetic tissue is maintained at a low value, and little or no synthesis of organic acids occurs. At night the carbon dioxide content of the tissues builds up as a result of respiration, and synthesis of organic acids is favoured (MEYER & ANDERSON)(155). It has been shown that leaves of tomato, tobacco or barley, which are non-succulents, take up radio-active carbon dioxide to form radio-active malic or succinic acids, showing that carbon dioxide fixation in non-succulents also involves organic acids (STUTZ & BURRIS)(213).

The succulent plants are a morphological rather than a taxonomic group. They have in common leaves or photosynthetic stems which consist of thickened spongy tissue. (Whilst discussing stems it is of interest to note that although most monocotyledenous stems do not increase much

in thickness as they increase in age, in a few tree-like species (e.g. Yucca and Aloe) there is true secondary thickening, resulting in the formation of vascular bundles.) (HOLMAN & ROBBINS (103)).

10.2.3 EFFECTS OF TEMPERATURE AND LIGHT.

As long ago as 1819, HEYNE found that with Bryophyllum calycinum, leaves tasted more acid at the end of the night than at the end of the day. Thus it has since been found that the leaves of succulents, either on the plants or excised from it, form acids when they are maintained in the dark, and especially at low temperatures, such as 10°C or lower. When such leaves are removed to the light the acids disappear. Disappearance of acids may also be accomplished in the dark at high temperatures such as 30°C.

A complication in the study of the metabolic reactions involved has been the fact that the leaves of many succulents contained an unknown acid, referred to in earlier works as isomalic or crassulacean malic acid. This acid, which forms a large part of the total, is now known to be isocitric acid, at least in the case of Bryophyllum, Sedum and other typical succulent species (BONNER & BONNER (29)).

10.2.4 THEORIES REGARDING ACID FORMATION.

Early theories regarding the mode of acid formation in succulents related these compounds to protein metabolism. Working on Rheum hybridum, RUHLAND & WETZEL (182) & (183) claimed that the large concentration of malic acid in Rheum originated from protein sources, or that it was associated with an abnormally active protein metabolism. According to BENNET-CLARK & WOODRUFF (16) their results indicated rather that the malic acid production was a process concomitant with the carbohydrate metabolism of the plant. Later RUHLAND & WOLF (184)(1934) suggested that carbohydrates are the source of plant acids and suggested that in succulents, pyruvic acid might be diverted from its normal pathway of oxidation in respiration, and be transformed to succinic acid through diketoadipic acid. The scheme of RUHLAND & WETZEL is unsatisfactory, however, in that it explains neither the origin of isocitric or citric acids, nor the reason for diurnal fluctuation in acid content. In addition it supposes reactions involving diketoadipic acid, a substance not known to occur in plant tissue.

A further suggestion as to the nature of the crassulacean metabolism made by BENNET-CLARK (15) is based on the fact that leaves of various crassulean species contain the seven-carbon

sugar, sedoheptose. BENNET-CLARK supposed that this sugar is itself converted to malic acid. The idea suffers from numerous inconsistencies, including the fact that we now know that isocitric acid is the major acid of the succulents.

10.2.5 TRANSLOCATION OF ACID.

The translocation of acids in Rheum is interesting. BENNET-CLARK & WOODRUFF (16) showed that malic acid is newly formed in Rheum only during the Summer months when photosynthesis is proceeding, and further that the acid is l-malic.

In Autumn the acid is translocated to the rhizome and converted to optically active acid(s). These are transported to the young leaves when the buds sprout in the Spring. This sprouting is not associated with increase in quantity of ammonium or 'malate'. There is therefore no reason to suppose that active deamination of amino acids, with formation of ammonium malate, occurs in connection with the transport of nitrogen to growing parts. Translocation of 'malate' occurs from rhizomes so that the concentration in the leaves rises. This and the fact that the period of active l-malic acid formation is the height of Summer and not during sprouting of the rhizome, support the view that malic acid is associated with carbohydrate - rather than protein metabolism.

(Rheum is of interest here in that, like the Aloe species, it contains both anthraquinones and metabolising acids).

10.2.6 ISOCITRIC ACID.

The importance of isocitric acid is plant metabolism has already been mentioned. It is consequently important to describe the latest findings with regard to isocitric acid, particularly where it occurs in Aloe species. In 1962 SODERSTROM (202) found isocitric acid in 38 out of 39 species of Crassulaceae, and in 8 out of 9 species of Liliaceae, with a record concentration of 19.4% in the dry matter of Aloe saponaria. VAN OUDTSHOORN (225), using paper chromatography, showed A.saponaria, A.mitriformis, A.pretoriensis, and A.speciosa to contain isocitric acid, as well as citric, malic and succinic acids. Later, VAN OUDTSHOORN (227) showed citric, isocitric and malic acids to be present in the following Aloe species, namely A.striatula, A.arborescens, A.pluridens, A.dolomitica, A.alooides, A.aculeata, A.ferox, A.marlothii, A.angelica and A.globuligemma. Difficulty is experienced with citric and isocitric acids as they have very similar Rf values in several solvents. However, isocitric acid lactone can be satisfactorily separated with propanol/ammonia, it forming a complex with ammonia. Thus

SHKOL'NIK (178) was unable to show isocitric acid in A.arborescens using a solvent of n-butanol saturated with formic acid and water, although in addition to the commonly occurring oxalic, tartaric, citric and malic acids, he was able to show glycollic, succinic and fumaric acids present. A.arborescens if official in the pharmacopoeia of the U.S.S.R. (206).

VICKERY (229) found that isocitric acid content of excised leaves of Bryophyllum calycinum did not change significantly when subject to water culture under greenhouse conditions of illumination. There appears to be a mutual interconversion of starch and malic acid during diurnal fluctuation, depending upon light conditions. Further to this, VICKERY (230) found that water cultures in the dark of the above leaves decreased steadily in organic acid content for some 140 hours, and then showed a slight rise at about 200 hours. He was also able to show an increase in starch during the same periods. (A similar slight rise following an initial drop in acid content of Aloe species has also been noted). VICKERY (231) has also been able to modify the behaviour of these leaves by growing them under a physiological stress, namely light, for long periods. After this the leaves could not synthesise to the full extent when grown in darkness.

10.3.1 DISCUSSION OF ANALYTICAL PROCEDURES TO BE FOLLOWED.

It was decided to investigate selected Aloe species with regard to whether or not they acted as true succulents, that is, showed diurnal/nocturnal fluctuation, responded to the effect of darkness and how they reacted to varying temperatures. Furthermore it was decided to follow the monthly variations in acid content of the four species which were selected for study with regard to variations in glycanthrone content during the course of a year. It was hoped that comparison of the monthly variations in content of acid and glycanthrone could indicate whether there was a complementary metabolic balance between the two, and whether variations due to weather were an indication of the water balance of the plant. The latter is dealt with in Chapter 9. For the selection of an analytical procedure, the following points had to be borne in mind:-

a) the extraction medium, and whether hot or cold
b) the portion of leaf or leaves to be utilised and the particle size suitable for optimum extraction
c) the method of analysis, and the effect of plant buffers if volumetric analysis were used.

10.3.2 a) THE EXTRACTION MEDIUM.

The various media used for extraction of organic acids have been well described by VAN OUDTSHOORN (227). Suffice to say that these may be classified into

i) organic media hydrophobic in nature
ii) organic media hydrophilic in nature
iii) aqueous media.

No single type is perfect since organic non-polar media will extract fats and waxes, while polar solvents will leach out gums, albumens, sugars etc. Ether has long been used for the extraction of organic acids, but for quantitative work extraction by this method is extremely tedious.

Alcohol 80% either cold or hot has also been widely used, as also acidified alcoholic solutions of various strengths. Alcohol 80% is also used to differentiate the salts of organic acids precipitated by calcium ions - thus most acid salts of calcium, except those of calcium acetate, calcium glycollate, calcium glyoxalate and calcium lactate, are very sparingly soluble in 80% alcohol. However, 80% alcohol added to a neutral calcium solution of precipitated organic acids also precipitates polysaccharide and some proteins, and also polybasic salts of calcium, so that gravimetric determinations of the precipitate would be faulty (BENNET-CLARK (14)).

Although water suffers from the disadvantage of dissolving many plant constituents other than organic acids, it has the advantages that the process is quicker and can, if required, be conducted at low temperatures. Furthermore, if the method used is volumetric, utilising alkali, the presence of other extractive matter is obviated, unless it is itself acidic in character, or is so highly coloured that the colorimetric end-point is masked.

10.3.3 b) THE LEAF PORTION UTILISED.

So little work has been performed on Aloe leaves that the selection of analytical methods had to be largely empirical. Since the leaves of the various Aloe species differ widely in their size, it happens that a required 100g weight of leaf would represent several leaves of a small species like Aloe variegata, and only a small portion of the leaf of large species like A.ferox and A.africana. Thus it was decided to utilise the tips of large leaves, and the entire leaf or leaves of smaller species, minus a portion of the base of the leaf. This was to overcome the acid loss due to drainage of the sap, since it was found that the sap, and in particular aloetic juice, was highly acidic. It was further found by experiment that the tip of the leaf of the larger species contained relatively more acid than the rest of the

leaf, and that the epidermis contained more acid than the pulp. The two facts are inter-relating since the tip contains relatively less pulp than the remainder of the leaf. However, this fact does not give rise to error since in all cases the variation in content was studied, and not the precise quantity of acid per leaf. Furthermore, leaves were taken from the same plant for each test, consequently diminishing leaf variation even further.

The size suitable for extraction also had to be determined empirically, and the methods of comminution used are detailed under EXPERIMENTAL, together with the various comminuting apparatus tested in an attempt to obtain maximum extraction.

10.3.4 c) THE METHOD OF EXTRACTION.

Several methods exist for the estimation of organic acids, some of these being extremely difficult techniques. Frequently oxalic acid is estimated as calcium oxalate using potassium permanganate. A further aliquot is used for the determination of citric and malic acids, the fraction being oxidised by permanganate in the presence of potassium bromide, whereupon citrate yields pentabromoacetone, which may be removed and determined, whereas malate yields an unknown steam-volatile compound which is precipitated by dinitrophenylhydrazine, and determined colorimetrically against a standard

(PUCHER & VICKERY (173)). Enzyme methods have to be used for isocitric acid, aconitase being used, which results in an increase in citric acid (KREBS & EGGLESTON (123)). Similarly succinic acid is determined manometrically in a Warburg apparatus using the enzyme succinic dehydrogenase. Manometric methods are also available for oxaloacetic and α-ketoglutaric acids.

The determination by gravimetric methods as calcium (or lead) salts has already been outlined, as also the disadvantages of the method. However, the deposition of other matter apart from calcium salts need not interfere if the precipitate is ignited and determined as calcium oxide or sulphate. However, many plants rich in malate contain substances which inhibit the precipitation of calcium malate, e.g. Liliaceae, Cactaceae, and Orchidaceae contain pentosans. Hence, according to BENNET-CLARK (14), total acid records can be suspect, although no such suspicion attaches to the recorded values of the quantities of standard alkali required to neutralise a given quantity of plant material, but in some cases there is difficulty in interpreting these results. Thus some substances such as carbon dioxide, phosphate and aluminium malate may interfere: for example, at pH7 about 40% aluminium malate is hydrolysed, but this increases to approximately 90% at pH 9.3 (which is the end point of

phenolphthalein). Consequently at the latter pH a far higher acid titre is obtained.

BENNET-CLARK (13) says further that almost all succulent plants show the crassulacean effect except some of the order Centrospermae. Many non-succulents show similar behaviour. The phenomenon is widespread, but, as the data collected shows, a much more extended survey of the plant kingdom is desirable.

10.4.1 PLANT BUFFERS.

From what has been said before, it is obviously important to have a knowledge of the buffer effects of plant extracts, and the practical aspects hereof are described in the Experimental section under "choice of indicator".

It was as early as 1917 that HEMPEL (200) made an extensive investigation of succulents. Without exception, the plants she used showed an increase of pH (decrease of acidity) on illumination, varying from +0 04 to +1.5pH units. The range of pH values extended from pH 3.95-pH 5.68. The usual pH range for succulents is pH 5.2-4.8, but pH values above 5.2 for cell sap in succulents occur in Aloe variegata, Gasteria verrucosa, and in some others. A number of stone crops and cacti have tissues below pH 4.8, down to pH 4.0.

HONEGGER (104) says that the leaves of Aloe species contain only monoclinic calcium oxalate crystals. This is significant, since SMALL (199) states that calcium oxalate can give an indication of the pH during their formation, in that a large proportion of monoclinic crystals indicate a pH during formation of 6.0 or less, while a pH of above 6.0 is indicated by a large proportion of tetragonal crystals.

Regarding the pH of saps, expressed saps of plants normally have pH values of 5.2 to 6.2, while fruit juices and those from succulents tend to be between pH 3.0 and pH 4.2. However, SMALL (199) warns that in only slightly buffered saps such as from normal plants, incorrect values can be obtained due to the CO_2 effects of injury, crushing and subsequent elimination of CO_2 from the juice, although with succulents, pH values generally have a real significance. According the SPOEHR (203) succulence is said to be associated with a pentose metabolism, which in turn is associated with relatively low pH values, but low pH values do not necessarily mean succulence.

Earlier works gave the buffers of living plants as mainly phosphates, carbonates and a few of the many organic salts, usually in molar concentrations in the range 0.05M to 0.001M. VICKERY (229) obtained values in terms of organic acids namely 0.29M to 0.17M malic, but about one-

third of this total was isocitric acid apparently held in the mucilage cells and not likely to be present in pressed or filtered sap. About one-fifth was "undetermined acids" leaving about seven-fifteenths as mainly malic acid, two-fifteenths of which was citric acid. Isocitric acid is said not to vary much, while the other acids vary extensively.

10.4.2 CHANGES ACCORDING TO ILLUMINATION.

According to SPOEHR'S (203) observations dilute malic acid is decomposed in sunlight forming oxalic, glycollic, formic and acetic acids, which would give an increased buffer capacity below pH 4.5 and also a daylight fall in pH, as in Mesembryanthemum.

Changes of pH and buffer-index in these directions do take place in other plants, but in the dark, not in the light; and the indications are that normally more malic acid is produced in the dark. The molar concentrations of malate vary directly with the buffer-indexes, and the increase in malate from light to darkness may be more than five-fold. If corresponding base is available for the formation of malates rather than free malic acid, there will be little or no fall in pH values. This change in concentration in malate is important in view of what has been said about the

hydrolysability of aluminium malate at varying pH values. Consequently it will be shown under Experimental that the indicator range (and buffering effect of the Aloe extract) were determined potentiometrically due to the difficulty experienced initially using acid/base indicators.

10.5.1 EXPERIMENTAL.

The experimental procedure for analysis of various Aloe leaves will be discussed under the following headings:

(i) choice of species
(ii) subdivision of leaf
(iii) choice of solvent
(iv) selection of indicator.

10.5.2 CHOICE OF SPECIES.

It was decided to make a duplicate study regarding the monthly variation of glycanthrone and acid metabolism in the following four species, namely

A.africana Mill. containing aloin and aloinoside
A.arborescens Mill. containing aloin
A.ferox Mill. containing aloin
A.speciosa Bak. containing homonataloin.

Both A.africana and A.ferox had similar leaves which weighed

approximately 500g and 800g respectively. These leaves were thick, containing much mucilaginous tissue, and had hard exterior walls or epidermes.

Conversely, the leaves of A.arborescens and A.speciosa weighed approximately 100g and 300g respectively, the outer epidermes were thin, and the mucilaginous tissue far less than in the other two species, resulting in a thinner, flatter leaf.

10.5.3 DESCRIPTION OF PLANTS USED FOR MONTHLY VARIATION.

These plants have been described fully under section 9.7. It should be noted that the A.ferox plant was also used for the experiments on acid metabolism at different tem. eratures (section 10.6.3)

10.5.4 SUB-DIVISION OF LEAF.

Initial investigation of the juice of A.ferox showed it to be acid, and since the juice 'bleeds' copiously from the cut leaf, this could introduce a deficit error. Consequently it was decided to utilise the tips of large leaves (e.g. A.ferox and A.africana) and an aliquot portion of the leaves of A.speciosa and A.arborescens, discarding the lower (stem-side) portion to counteract juice loss. However, in the latter two cases juice loss is insignificant as the juice of

A.speciosa rapidly coagulates and is hence 'self-sealing' while A.arborescens yields less than 0.5 ml of juice per 100g of leaf.

Where the leaf contained dead portions, or dry withered portions, these were discarded as analysis was only of fresh (metabolising) leaves.

It was further found that the leaf epidermis of A.ferox contained some four times as much acid as the excised pulp of the leaf. When an A.ferox leaf was cut into several 100g portions, these contained very similar volumes of acid, except the tip, which had the highest value (due no doubt to the relative excess of epidermis). The base portion had the lowest, due to drainage of aloetic juice from that region.

10.5.5 METHODS AND APPARATUS USED FOR SUB-DIVISION.

Four methods of sub-division were tried, using the following apparatus:-

a) sliced - using a sharp knife

b) milled - using a hand-mincing machine
- using a hydro-separator
- using a cutting mill (liquidiser).

To see which method would give the greatest yield, a leaf was taken and split longitudinally into two equal portions. One hundred gramme portions were taken, and some sliced into

1 cm thick slices, others milled in a Braun liquidiser. After extraction these were titrated and it was found that the sliced portions yielded only half the volume of the milled portions.

The difference between the hydro-separator and the liquidiser was next compared, and it was found that the liquidiser gave a slightly higher yield, and in addition, it was far easier to transfer the comminuted drug (marc) to the filter, since the perforated plastic screen of the hydro-separator had to be removed without loss of leaf particles, which was not easy. In the case of the liquidiser, the suspension obtained after milling is merely decanted, and any remaining particles washed out with a wash-bottle.

For economic reasons it was decided to compare the relative merits of a hand-mincing machine and the liquidiser, and the former gave a surprisingly high result, yielding 90% of that achieved by the mill. One serious disadvantage, however, was the fact that with the mincer the final solution had an olive colour, probably due to interaction between the drug constituents and the cast iron of the mincer. This made the end-point extremely difficult to gauge.

Consequently it was decided to use the Braun liquidiser for all experiments. The Braun liquidiser has six cutting blades which rotate at the base of a 1 litre removable glass

vessel, fitted with a plastic lid. It has two speeds, and the lower speed was used, a length of 1 minute being used for milling.

10.5.6 CHOICE OF SOLVENT.

Since the volumetric method of analysis is recommended by BENNET-CLARK (14) it was decided to extract the plant material with water. It was decided to use boiling water after it had been found that two portions of a longitudinally sliced leaf yielded slightly more when boiled. Furthermore, the cold-extracted marc had to be washed with several volumes of water before all acids had been extracted. This was very time-consuming.

The advantage of boiling the extract with water is that cellulose is broken down, thus releasing trapped acids (bearing in mind that isocitric acid is often held in this way). Furthermore carbon dioxide is driven off, and enzyme reactions are stopped. The only disadvantage is that volatile acids can be driven off, but acids such as formic appear in such low concentration, if at all, that no significant loss results.

10.5.7 SELECTION OF INDICATOR.

For the titration of Rheum hydridum extracts, BENNET-CLARK

& WOODRUFF (16) used brom-thymol blue as indicator to the end-point of pH 7. In general the extract was diluted to avoid interference with the end-point by colour changes due to anthraquinone derivatives, or anthocyan pigments. Since the Aloe species also contain anthraquinone derivatives, brom-thymol blue was tried as indicator. However, the colours of the Aloe extracts were found to interfere, being orange for A.ferox and A.africana, pale yellow for A.arborescens and beige for A.speciosa. With the two latter species the green end-point of brom-thymol blue was good, particularly in the case of A.arborescens (pH range 6.0 - 7.6, yellow in weakly acid solution, blue in weakly al .line and green at neutrality). for A.ferox and A.africana the end-point was greenish-brown.

Since the green colour of brom-thymol blue was masked by the orange colour of A.ferox in particular, an indicator having a similar range but different colour change was sought. Hence phenol red was used (yellow in neutral and weakly acid solution, and red in weakly alkaline.) However, it was observed that the anthraquinone itself turned dark orange at neutral pH and that the red colour of the end-point tended to fade, suggesting buffer effects. Consequently a potentiometric plot was made (using a Beckman model G pH metre.).

From the resulting curve it was observed that definite buffering occurred between pH 6.0 and pH 9.6, whereafter the curve steepened rapidly. Consequently the end-point was taken to be between pH 9.6 and 9.7. Consequently phenolphthalein was employed (colourless in acid and weakly alkaline solutions, but red in more strongly alkaline solutions. pH range 8.3 - 10.0). Since A.arborescens contains malic acid (SHKOL'NIK (197)) this would be almost completely hydrolysed at this pH if present as the aluminium salt (BENNET-CLARK (14)).

10.5.8 ANALYTICAL PROCEDURE.

For all analyses that follow the following analytical procedure was observed:

the Aloe leaf was cut and weighed as 100g ($\pm$ 0.1) portions or fractions thereof. In the case of A.ferox and A.africana the tip was used, while for A.speciosa and A.arborescens the whole leaf minus base was used (occasionally in the case of A.arborescens two leaves were used).

The leaf was then sliced thinly and placed in the Braun liquidiser with 200ml of distilled water. This was milled at speed one for exactly one minute, and transferred to a 1 litre flask, a volume of approximately 500ml resulting. This was brought to the boil on a bunsen burner and boiled

for ten minutes, a total heating time of 25-30 minutes. Thereafter it was allowed to cool and transferred to a filter (initially a sintered glass funnel, AG 225#4 was used, but this was discarded due to blockage by fine particles. Thereafter a cotton wool plug was found to be quite adequate.)

The filtered solution was made up to 1 litre with distilled water and 25 ml aliquots were titrated with N/10 NaOH (Volucon) against phenolphthalein, using a micro-burette graduated in 0.02 ml. Near the end-point the solutions turned a darker orange-green, and when necessary, these were diluted with distilled water. The end-point was orange - pink in colour.

The marc remaining in the filter was washed through with sufficient distilled water until no further acid remained. Sometimes as much as 1 litre was passed through, but invariably more than 95% of the acid was contained in the first litre of extract collected. This procedure was performed within 30 minutes of collection of the sample.

10.6.1 ACID CONTENT OF ALOE SPECIES.

Using the above method, four species other than the four named above were collected at 8.30 a.m., and all eight species were assayed for acid content, results thereof appearing in table 10(i). Only from later work was it

discovered that acid content of a species differs considerably according to location of collection; thus three samples of A.arborescens contained 36.4ml, 66.4ml and 118.1ml, all collected at 8.30 a.m. Thus the few quoted acid-content figures noted in the literature can be misleading.

Table 10(i) ACID CONTENT OF VARIOUS ALOE LEAVES.

Soft leaf.	Vol. of acid.	Firm leaf.	Vol. of acid.
A.striata	80.6ml	A.ferox	122.9ml
A.lineata	54.5ml	A.africana	67.8ml
A.speciosa	64.0ml	A.pluridens	87.7ml
A.arborescens	66.4ml	A.marlothii	89.2ml

10.6.2 CHANGES IN ACID CONTENT OF EXCISED LEAVES.

During the course of analyses for the monthly variation in acid content it was decided to analyse the remaining leaf potions after a period of time. It was then found that acid content had dropped appreciably in a relatively short time, as shown in table 10(ii).

Table 10(ii) ACID CONTENT OF STORED EXCISED LEAVES.

Species	A.africana	A.ferox
Time		
0 hours	69.1ml	115.8ml
48 hours	-	36.5ml
72 hours	25.6ml	40.4ml
96 hours	31.2ml	-

Thus in each case a rapid drop followed by a slight rise was observed. A search of the literature suggested that since the leaves had been stored in a room which was subject both to fluctuations in light and dark, and to fluctuations in temperature, the slight rise in acid content after 3-4 days was probably due to the non-rigid conditions during the test. Consequently it was decided to investigate the acid content of leaves stored entirely in the dark at different fixed temperatures.

It was WOLF (241)-(245),(who carried on a most thorough study of succulent metabolism), who re-established the fact that acid formation in leaves of Bryophyllum is a function of light and temperature. With regard to temperature, two factors are involved; the maximum amount of acid is accumulated by excised leaves at a temperature of 7°C, but

this maximum level is attained only after long periods in the dark (96 hours). At higher temperatures acid formation takes place at a faster rate but never attains as high a concentration level. Over a period of 18 hours, a temperature of 20°C was found to be optimum. At temperatures of 30°-35°C, the leaves actually lost acid. The optimum temperature for acid removal in the light was found to be about 25°C. A minimum of 400 meter-candles was needed to effect acid removal, and light saturation of the process was attained only with intensities of about 1000 meter-candles. These intensities are of the same order as are needed to effect photosynthesis in the succulent leaf, and it would appear possible then that photosynthesis is involved in acid removal. Further to this, BHARUCHA & JOSHI (21) found that the acid content of leaves of A.vera increased daily, reaching a maximum after 5 days (120 hours) of darkness followed by a sharp decrease. At the maximum, 134.9ml of 0.1N NaOH were required to neutralise the acid in 100g of leaf tissue.

10.6.3 ACID CONTENT OF LEAVES STORED IN DARKNESS AT SPECIFIED TEMPERATURES.

It was decided for this experiment to operate at four temperatures, namely 30°C, 20°C, 10°C and 0°C, all samples to be stored in the dark. An Aloe ferox leaf was cut into seven 100g portions and three were stored at 30°C ($\pm$ 1°),

and three at 20°C (± 1°). The seventh portion was used to assess the acid content at nought hours.

Two further A.ferox leaves, smaller in size than the previous leaf and from a younger Aloe plant, were each subdivided into four 66.6g portions. Three portions from the same leaf were stored at 10°C (± 1°) and three at 0°C (-3°). The fourth portion from each leaf was used to determine the acid content at nought hours. At specified times the beakers containing the leaf portions were removed, the portion sliced and milled, and the beakers carefully washed out, and the washings added to the bulk. Thereafter the procedure was that detailed in Analytical Procedure (section 10.5.8). The results obtained appear in table 10(iii) and are depicted graphically in figure 10(i) (McCARTHY & PRICE (151)).

figure 10(i)

ACID VARIATION WITH TEMPERATURE

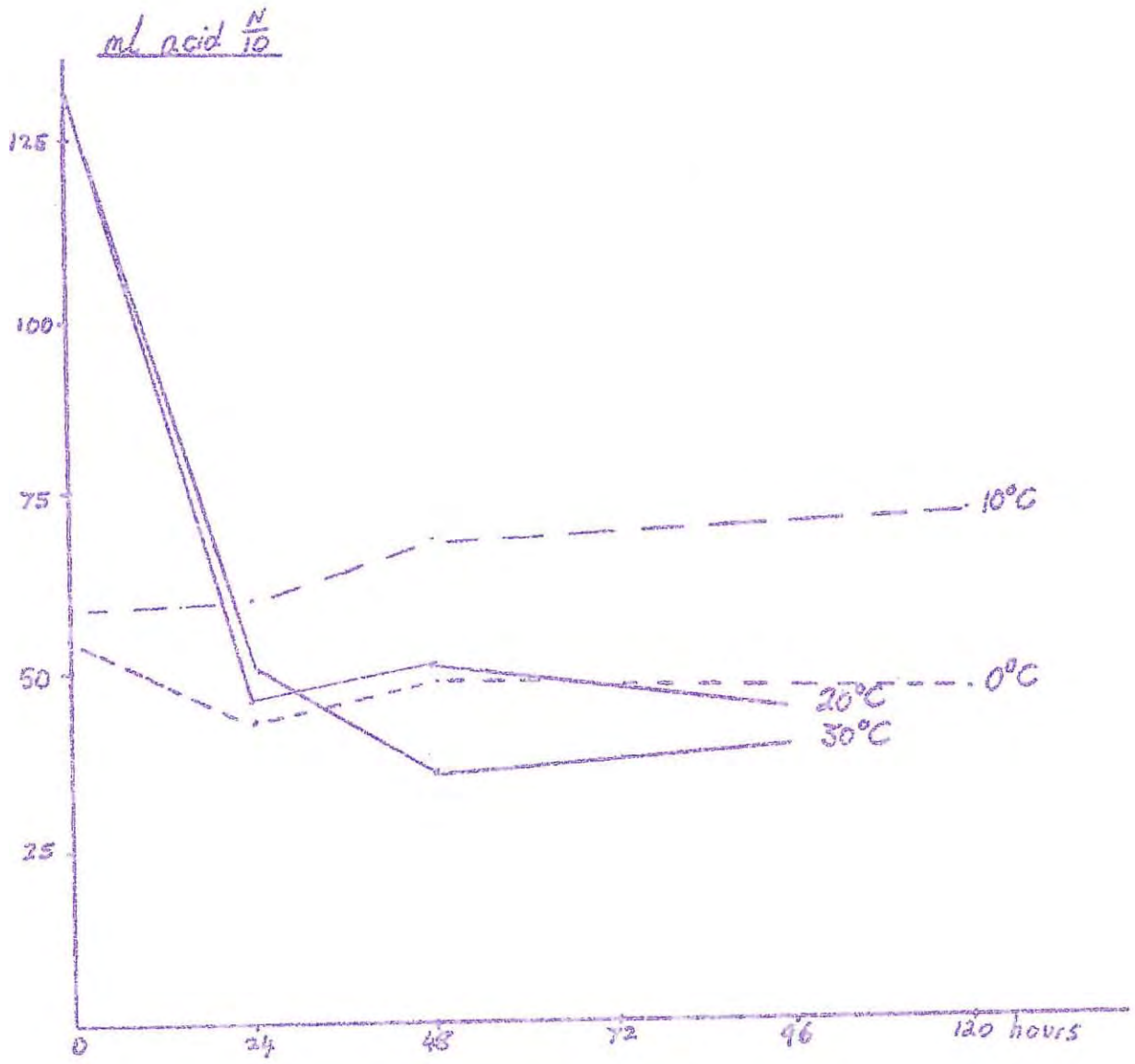


Table 10(iii) ACID CONTENT AT SPECIFIED TEMPERATURES.

Temperature.	Time in hours.	Vol. of $\frac{N}{10}$ alkali per 100g leaf.
30°C	0	129.2ml
"	24	50.1ml
"	96	39.4ml
20°C	0	129.2ml
"	24	45.0ml
"	96	43.8ml
10°C	0	58.3ml
"	21	59.8ml
"	45	67.8ml
"	116	71.3ml
0°C	0	55.2ml
"	21	43.4ml
"	45	48.5ml
"	116	46.8ml

10.6.4 DISCUSSION OF RESULTS.

By reference to table 10(iii) or to figure 10(i) it will be seen that in Aloe ferox the acid content drops sharply at 30°C which is in accordance with WOLF'F (241-245)

findings for Bryophyllum leaves, but acid content also drops sharply in leaves stored at 20°C, whereas in Bryophyllum leaves acid accumulates at this temperature. With A.ferox, both at 20°C and 30°C the initial drop within 24 hours is the greatest, followed by a slight decrease over the next 72 hours.

With Bryophyllum leaves it was found that maximum accumulation occurred at 7°C over 96 hours. Similarly, at 10°C over 116 hours A.ferox leaves show a steady rise of some 20% in acid content. No figures are given for the behaviour of Bryophyllum at 0°C, but for Aloe ferox leaves the concentration remains fairly steady after an initial small drop (21 hours) due probably to inactivation of enzymes as the leaf freezes. It is interesting to note that in the 4th portion of Aloe leaf used for the 10°C experiment (i.e. 116 hours), a red rim of aloe-emodin had formed around the cut rims of the leaf portion. This had not occurred in the equivalent portion stored at 0°C, showing that freezing of the sample had inhibited hydrolysis of aloin.

10.6.5 ACID CONTENT OF EXCISED LEAVES SUBJECTED TO LIGHT VARIATION. (diurnal/nocturnal variation.)

For this experiment use was made of the four species used for the monthly variation in acid content of leaves,

Figure 10(ii).

DIURNAL VARIATION.

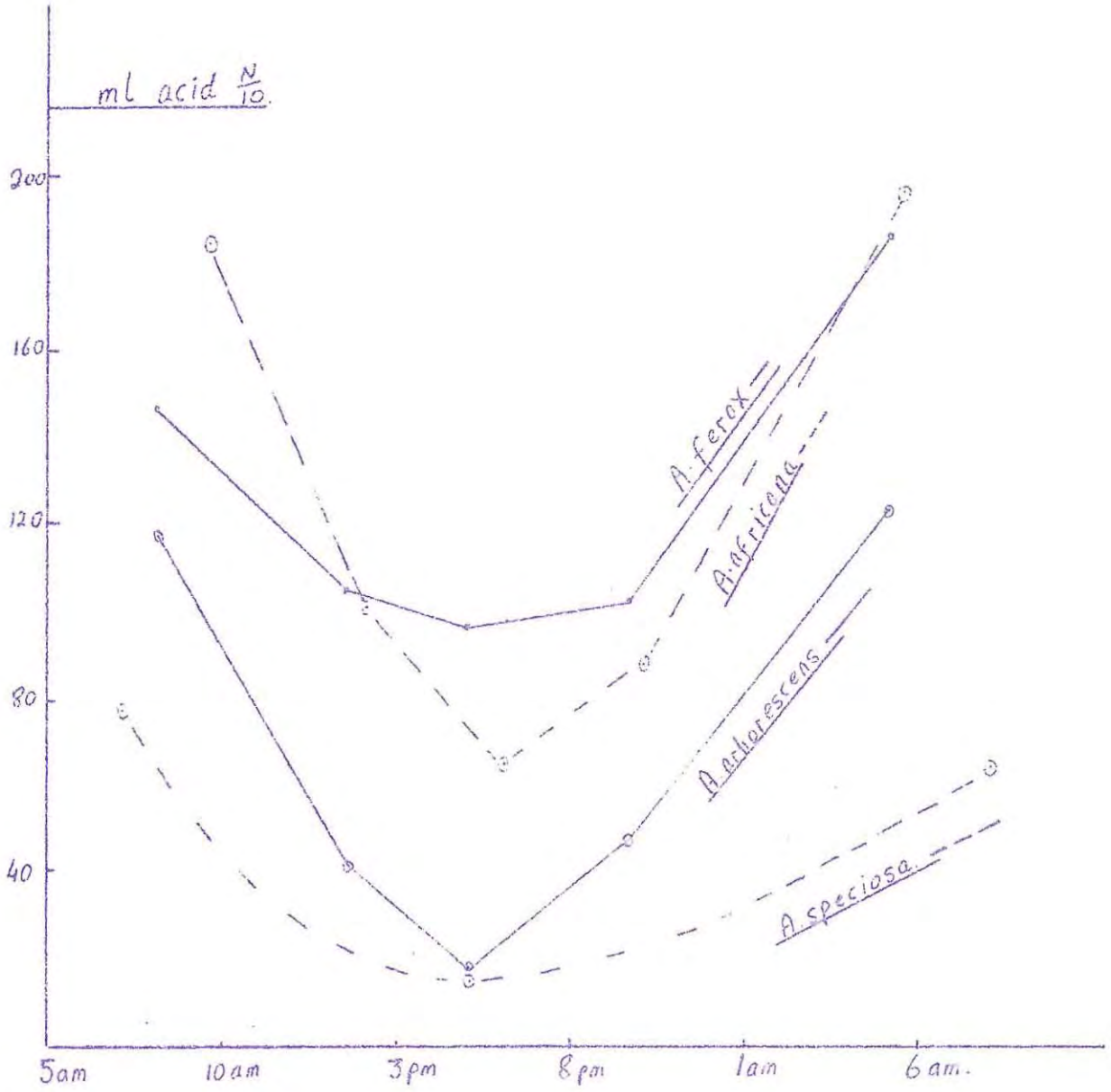
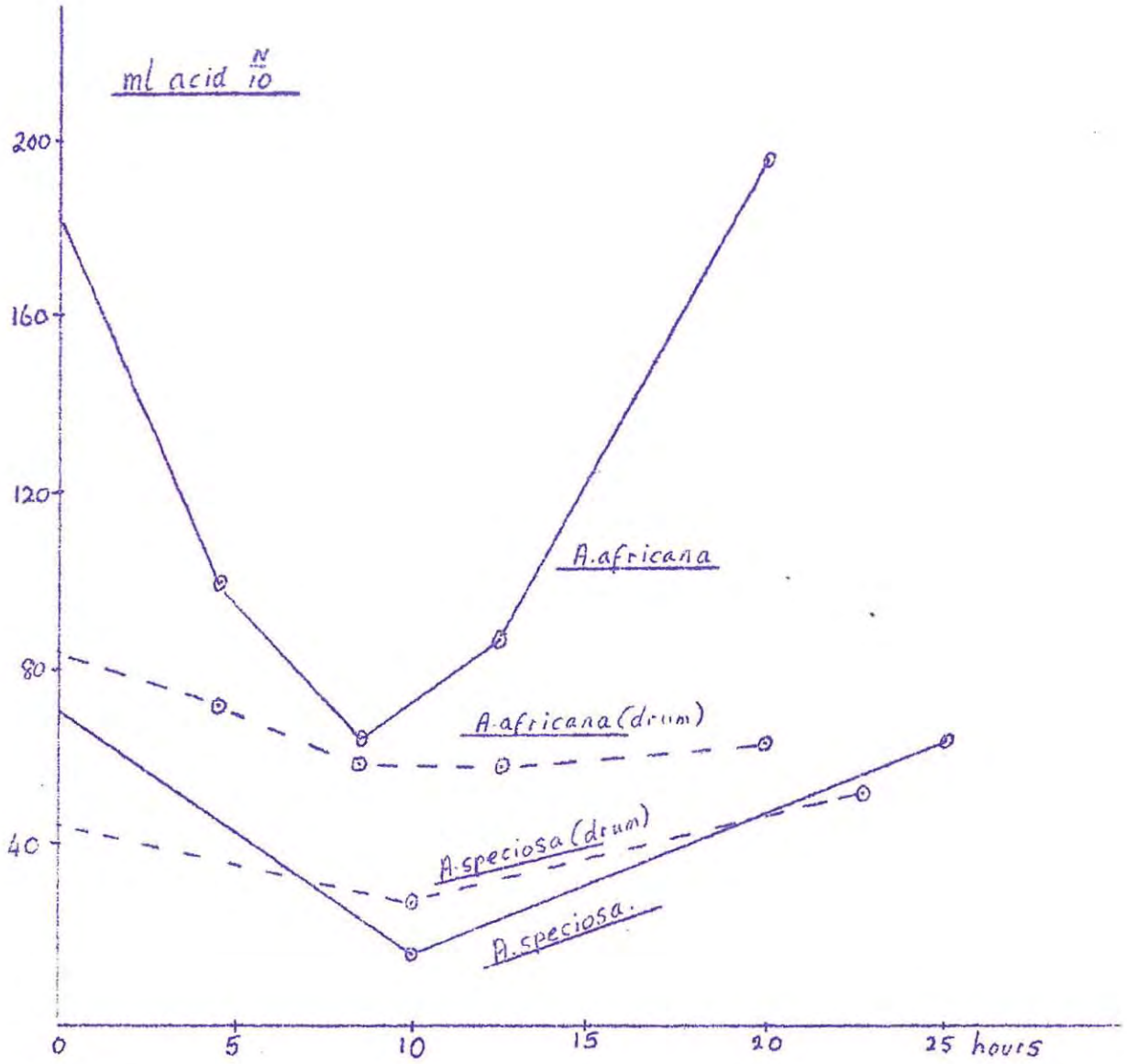


figure 10(iii).

DIURNAL VARIATION.



namely, A.africana, A.arborescens, A.ferox and A.speciosa, but different plants were used. These plants were growing in the writer's garden, and in the case of both A.africana and A.speciosa there were two plants of each, one growing naturally in the ground, the other growing in a four-gallon drum.

For the analyses, a leaf was cut from each of the six plants at prescribed intervals of time, the leaves being cut from the same approximate level on the plant, since it had been found by VICKERY (232) & (233) that the middle leaves of the tobacco plant had the most uniform division of acids, which differed widely with respect to the upper and lower leaves, while McCARTHY (146) found least variation in aloin content of neighbouring leaves of A.ferox (see also section 10.6.8). Once the leaf had been cut it was immediately treated as under Analytical Procedure, section 10.5.8. Results of these analyses appear in table 10(iv), these results being depicted graphically in figures 10(ii) & 10(iii). (McCARTHY & PRICE (151)).

10.6.6 DESCRIPTION OF ALOE SPECIES.

No plant was above 4 feet in height, while A.speciosa (in the ground) was only 1 foot high with a dozen leaves. The A.africana and A.speciosa in drums both had red leaves,

due to long exposure to sunlight, said by HAAS & HILL (94) to be due to the formation of carotin in the chloroplasts. Further, the leaves of A africana (in drum) were covered in white scale.

Table 10(iv) DIURNAL VARIATION IN ACID CONTENT.

Species.	Period.	Time.	Vol of N/10 alkali per 100g leaf.	Weather
A.arborescens	0 hrs	8.30 a.m.	118.1 ml	overcast but warm
	5 hrs	1.30 p.m.	41.0 ml	cloudy
	8½ hrs	5.00 p.m.	17.4 ml	drizzling
	13 hrs	9.30 p.m.	45.9 ml	windy
	22½ hrs	5.00 a.m.	123.3 ml	partially overcas
A.ferox	0 hrs	8.30 a.m.	146.8 ml	overcast but warm
	5 hrs	1.30 p.m.	105.1 ml	cloudy
	8½ hrs	5.00 p.m.	96.3 ml	drizzling
	13 hrs	9.30 p.m.	101.6 ml	windy
	22½ hrs	5.00 a.m.	186.5 ml	partially overcas
A.africana (in ground)	0 hrs	9.30 a.m.	185.1 ml	cloudy but sunny
	4½ hrs	2.00 p.m.	100.2 ml	ditto.
	8½ hrs	6.00 p.m.	64.6 ml	sunny
	12½ hrs	10.00 p.m.	87.8 ml	windy
	20 hrs	5.30 a.m.	196.4 ml	cloudy but sunny
A.africana (in drum)	0 hrs	9.30 a.m.	84.1 ml	cloudy but sunny
	4½ hrs	2.00 p.m.	72.5 ml	ditto.
	8½ hrs	6.00 p.m.	59.2 ml	sunny
	12½ hrs	10.00 p.m.	58.1 ml	windy
	20 hrs	5.30 a.m.	64.4 ml	cloudy but sunny
A.speciosa (in ground)	0 hrs	7.00 a.m.	78.1 ml	cloudy but sunny
	10 hrs	5.00 p.m.	15.2 ml	sunny
	25 hrs	8.00 a.m.	64.3 ml	cloudy but sunny
A.speciosa (in drum)	0 hrs	7.00 a.m.	45.8 ml	cloudy but sunny
	10 hrs	5.00 p.m.	28.2 ml	sunny
	23 hrs	6.00 a.m.	54.6 ml	cloudy but sunny

10.6.7 DISCUSSION OF RESULTS.

From the graphs (figures 10(ii) & 10(iii)) it will be seen that in all species the typical Crassulacean effect is observed, namely an increase in acid during the night, while in the daytime the reverse occurs. The theoretical aspects of this behaviour have been discussed previously.

It is interesting to note that in both the plants grown in drums the curve, relative to their other sample grown in the ground, is far flatter. Now the A.africana was a diseased plant, but since both species have similar curves it suggests that their growth in drums may be the cause of their relatively poor acid metabolism. The extreme flatness of the A.africana drum sample may also be due to the white scale infection on the leaves of this plant. It does bring home the fact, however, that it is important to have plants which are to be used for analyses, growing in their most natural environment, particularly with respect to water acquisition.

STEINMANN (ex 14) gives a table of percentage change in titratable acidity in 44 plants during the night, included amongst which are two Aloe species, A.arborescens, which increased by 2%, and A.cymbaefolia which increased by 340%. In this work A.arborescens increased in the dark by 700%, and the work of HEMPEL (1917) on A.arborescens quoted by

STEINMAN is vastly different from the findings reported here. Most species here increased from 150% to 500%

10.6.8 MONTHLY VARIATION IN ACID CONTENT.

As explained in section 9.5 it was decided to make a simultaneous study of the glycanthrone content and of the organic acid content of each leaf of the four species used for the estimation of monthly variation. It was hoped that from this parallel study a pattern would emerge which would clarify the effect of wind with regard to moisture loss from the leaf. Before this could be proceeded with it was necessary to establish the pattern of organic acid content of leaves on the same, and differing levels of the plant, as had been done for glycanthrone content in section 9.7.1.

In the cases of A.arborescens, A.ferox and A.speciosa, the leaves were taken from the same approximate level on the plant, while in the case of A.africana three leaves were chosen at intervals, encompassing some dozen leaves. The analytical procedure used is that described in section 10.5.8, the same procedure being used for the monthly analyses. The findings of acid content at a level in the plant appear in table 10(v).

Table 10(v) ACID CONTENT AT FIXED LEVEL.

Species.	Ml of N/10 acid per 100g leaf.	Maximum % variation from the mean.
A.arborescens	76.8, 74.0, 70.3	± 5.0%
A.ferox	164.5, 172.0, 158.4	± 4.3%
A.speciosa	64.3, 64.1, 67.3	± 3.0%
A.africana	181.2, 187.3, 168.1	± 5.9%

For the monthly acid content the leaf used was from the same approximate level on the plant, and during a year such a level moved away from the original, but neighbouring leaves were systematically used each month to limit variation. Results of the monthly acid content of the four species appear in table 10(vi), where the date given refers to A.arborescens and A.africana, while the other two species were analysed on the day following. Collection times were 8 a.m. and the leaf was analysed immediately after the sap had been weighed for estimation of glycanthrone content (section 9.6).

Table 10(vi) MONTHLY ACID CONTENT (ml $NaOH^{N}/10$ per 100g leaf)

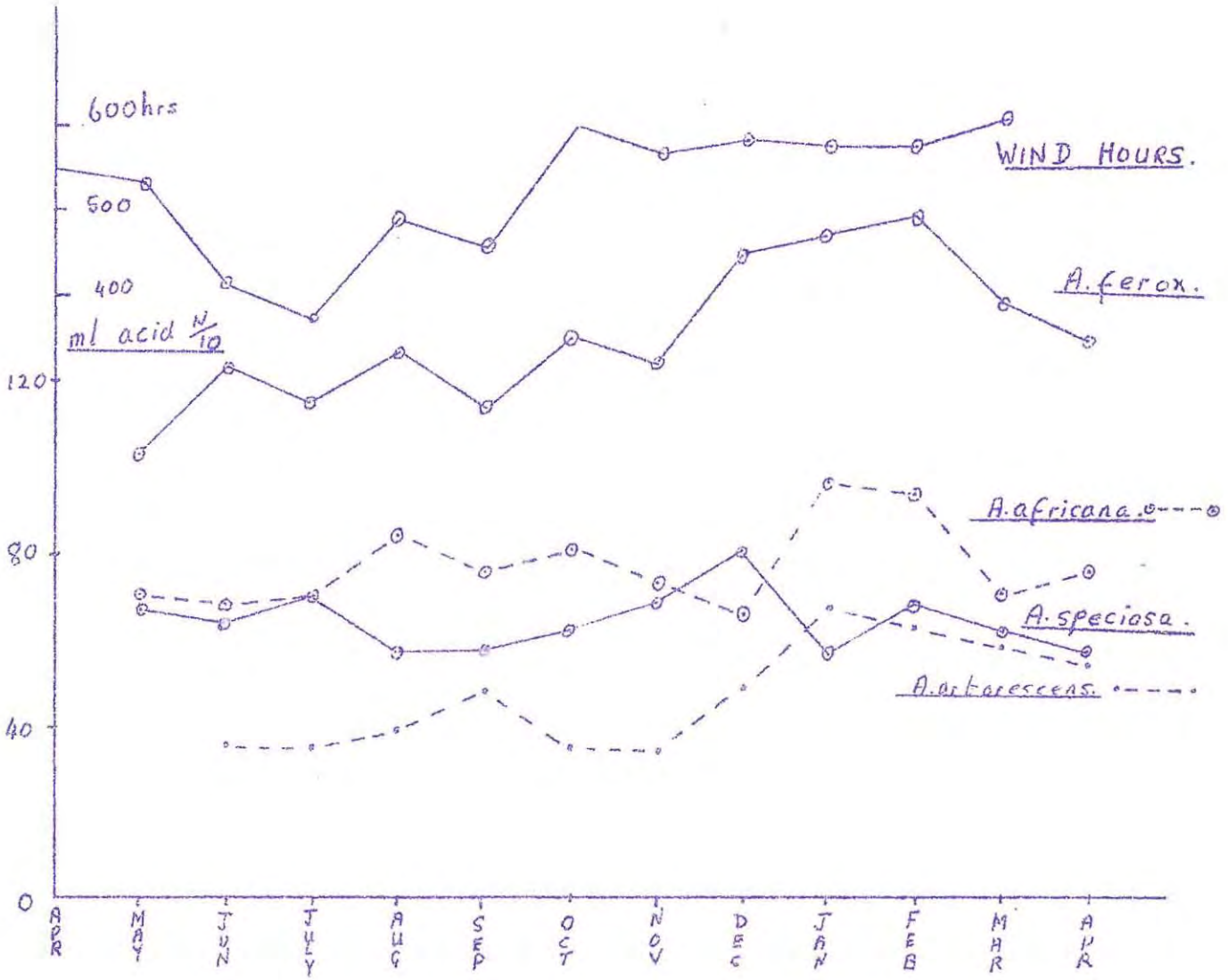
Date.	A.arborescens	A.africana	A.ferox	A.speciosa
May (28/5/65)	-	70.6	102.7	67.5
June (29/6/65)	36.4	67.8	122.9	64.0
July (30/7/65)	35.0	69.1	115.8	70.4
August (1/9/65)	38.6	85.0	127.2	56.8
September (27/9/65)	48.5	76.4	113.8	58.0
October (28/10/65)	34.6	81.3	130.6	62.9
November (24/11/65)	36.4	73.6	124.7	68.9
December (29/12/65)	48.9	70.5	149.6	80.8
January (28/1/66)	68.0	97.0	154.6	57.1
February (25/2/66)	63.4	94.3	158.0	68.5
March (1/4/66)	59.9	70.6	137.5	66.9
April (29/4/66)	54.1	76.6	129.9	58.2

10.6.8.1 DISCUSSION OF RESULTS.

From the results appearing in table 10(vi) (depicted in figure 10(iv)) it is clearly seen that the organic acid content reaches its maximum in the Summer months of December to February, then declines in March and April. (The acid content of A.speciosa for January is inexplicably low.)

figure 10(iv).

Acid content.



This rise in organic acid content from Winter to Summer is in agreement with the findings for the glycanthrones, and much sharper by contrast.

Although a variation in acid content from leaves on the same level is experienced, as shown in table 10(v), the percentage deviation from the mean did not exceed 6% while the difference between any two leaves does not exceed 10%. Reference to table 10(vii) shows that the increase throughout the Summer months is far in excess of this, and hence the intrinsic variation within leaves does not affect the findings.

Table 10(vii) MAXIMUM AND MINIMUM ACID LEVELS RECORDED.

Species.	Lowest figure recorded (A)	Highest figure recorded (B)	$\frac{B}{A}$%
A.arborescens	34.6 ml	68.0 ml	196%
A.africana	67.8 ml	97.0 ml	143%
A.ferox	102.7 ml	158.0 ml	153%
A.africana	56.8 ml	80.8 ml	142%

As with glycanthrones, the acid content appears to bear no relation to the graph of wind hours, and furthermore, plots for each species of glycanthrone and acid content (not reproduced here) showed no overall pattern. Thus it

would appear that metabolism of glycanthrone and organic acid within the leaf proceed independently of one another, the only similarity being the seasonal variation of the two entities, both of which definitely increase in the Summer months.

S U M M A R Y.

Anthracene derivatives enjoy a wide distribution in the plant kingdom, the oxidised forms (anthraquinones) forming the widest group of naturally occurring quinone compounds. The distribution of the non-medicinal and medicinal anthracene derivatives has been reviewed, together with the assay methods used for the determination of the latter type.

Chromatography has been used extensively in this work, hence a short review is included. In table 3(i) and table 3(1b) respectively are listed solvent systems used in the literature and in this work. Spray reagents have been widely used here to identify substances separated chromatographically, and these have been described in sections 3.6.1 to 3.6.4. The sixty-three Aloe species which have been studied have been tabulated in table 3(v), while the description of the process used for making herbarium specimens is found in section 3.7.1. Chemical tests (using acids) on the leaf juice of the Aloe species are listed in table 3(iii).

The distribution of anthracene derivatives in the leaf juice of the species is interesting - aloin has been found in fourteen species, two of which also contained aloinosides.

Isobarbaloin was not present in the sixty-three species examined, but occurred in commercial samples from the West Indies and Zanzibar. With the exception of A.arborescens, the aloin-containing species described here yield sufficient aloetic juice to warrant their use commercially. Homonataloin has been found in sixteen species, but chrysophanol (as an O-glycoside) could be extracted from only one of six species falling in the Series Saponariae, the dried leaf being used.

Non-anthracene derivatives have also been studied. Para-coumaric acid could not be detected in the leaf juice of the fifty-five species described in section 8.7.4, and is either absent, or present in too low a concentration for resolution by thin-layer chromatography. Resins occur widely in the Group A and Group B species described in section 8.7.4, and frequently display a similar pattern, but are conspicuously absent from the species classified as Group C. Electrophoresis of resins in two species is described.

Aloesin, a C-glycosyl chromone discovered very recently, was positively found in nineteen species and occurs possibly in a further four species (section 8.7.12.2). Glucose was found in the seventeen species named in section 6.1.2., while unidentified anthracene derivatives and

sugars (or glycosides) occurred in some of these seventeen species.

Since so little work has been performed on the metabolism of Aloe species, an investigation has been carried out regarding the variations in glycanthrones and organic acids in the leaves of four selected species, which contained the glycanthrones aloin, aloinoside and homonataloin. The fruits, seeds and stem bark and wood have also been examined for anthracene derivatives. Both the glycanthrones and organic acids show a seasonal variation with a maximum content in the Summer months. No correlation between weather factors and monthly variations could be obtained (tables 9(iii) & (iv) and 10(v)). Glycanthrone estimations were performed spectrophotometrically using standard graphs (section 9.6) while organic acids were estimated volumetrically (section 10.5.1), where the extraction procedure is also described. Furthermore, with regard to organic acid content, typical Crassulacean behaviour has been shown both with regard to diurnal/nocturnal variation and with regard to excised leaves stored at different temperatures in the dark (tables 10(iv) & 10(ii) resp.). It has been determined that minimal variation in both total acid and glycanthrone content occurs in leaves situated on the same approximate level on the plant.

O P S O M M I N G.

Antraseen derivate kom wyd versprei voor in die planteryk. Die geoksideerde vorms (antrakinone) vorm die grootste groep van die kinoonverbindings wat in die natuur voorkom. 'n Oorsig is gegee van die nie-medisinale en die medisinale antraseen derivate, tesame met die ontledingsmetodes wat gebruik word vir die bepaling van laasgenoemde tipes.

Daar is op uitgebreide skaal van chromatografie gebruik gemaak in hierdie werk en gevolglik word 'n kort oorsig hiervan ingesluit. 'n Lys van oplossingstelsels wat in die literatuur en in hierdie werk gebruik is, word in tabel 3(i) en tabel 3(1b) onderskeidelik, aangegee. Hier is veelvuldige gebruik gemaak van sproeireagense om verbindings wat chromatografies geskei is, te identifiseer en 'n beskrywing hiervan word gegee in afdeling 3.6.1 tot 3.6.4. Die drie-en-sestig aloïen spesies wat bestudeer is, word aangegee in tabel 3(v); terwyl 'n beskrywing van die proses wat gebruik is om herbaruimmonsters te berei in afdeling 3.7.1 verskyn. 'n Lys van chemiese toetse (deur gebruik te maak van sure) op die blaarsap van aloïen spesies word in tabel 3(iii) aangegee.

Die verspreiding van die antraseen derivate in die blaarsap van die spesies is interessant - aloïen is in veertien spesies gevind, twee waarvan ook aloïenosied bevat het. Isobarbaloïen was nie teenwoordig in die drie-en-sestig spesies wat ondersoek is nie, maar het voorgekom in kommersiële monsters uit die Wes-Indiese eilande en Zanzibar. Met die uitsondering van A.arborescens, het die aloïen-bevattende spesies wat hier beskryf word, genoeg aloëtiensap gelewer om hul kommersiële gebruik te regverdig. Homonataloin is in sestien spesies gevind, maar krisofanol (as 'n O-glikosied) kon slegs uit een van ses spesies, wat binne die Saponariae-reeks val, ge-ekstrakeer word - die gedroogde blare is hier gebruik.

'n Studie is ook gemaak van nie-antraseen derivate. Para-kumaarsuur kon nie gevind word in die blaarsap van die vyf-en-vyftig spesies wat in afdeling 8.7.4 beskryf word nie, en is óf afwesig óf die konsentrasie is te laag vir chromatografiese skeiding. Harse kom veelvuldig voor in die Groep A en Groep B spesies wat in afdeling 8.7.4 beskryf word en vertoon dikwels 'n soortgelyke patroon, maar is opvallend afwesig in die spesies wat as Groep C geklassifiseer word. Elektroforese van harse in twee spesies word beskryf. Aloesin, 'n C-glikosielchromoon wat heel onlangs ontdek is, is positief uitgeken in negentien spesies

en kom moontlik in 'n verdere vier spesies voor (afdeling 8.7.12.2). Glukose is gevind in die sewentien spesies wat in afdeling 6.1.2 genoem word, terwyl nie-geïdentifiseerde antraseen derivate en suikers (of glikosiede) in sommige van hierdie sewentien spesies voorgekom het.

Aangesien so min werk op die gebied van die metabolisme van aloë spesies gedoen is, is 'n ondersoek ingestel na die variasies van glikantrone en organiese sure in die blare van vier uitgesoekte spesies wat die glikantrone, aloïen, aloïenosied en homonataloïen bevat. Die vrugte, sade, bas en hout is ook ondersoek, vir antraseen derivate. Beide die glikantrone en organiese sure toon 'n seisoensvariasie met 'n maksimuminhoud gedurende die somermaande. Geen korrelasie kon gevind word tussen weerfaktore en maandelikse variasies nie (tabelle 9(iii) & (iv) en 10(v)). Glikantroon bepalings is spektrofotometries uitgevoer deur gebruik te maak van standaardgrafieke (afdeling 9.6), terwyl organiese sure volumetries bepaal is (afdeling 10.5.1), waar die ekstrakeerproses ook beskryf word. Met betrekking tot organiese suurinhoud is verder tipiese Crassulacean-gedrag aangetoon beide met betrekking tot dag/nagtelike variasie en tot afgesnyde blare wat by verskillende temperature in die donker weggepak was.

Daar is vasgestel dat 'n minimale variasie in beide totale suurinhoud en glikantrone inhoud voorkom in blare wat op min of meer dieselfde vlak in die plant gelee" is.

B I B L I O G R A P H Y.

1. ADAMANIS F., & PAWLACZYK J., Bull. Soc. Amis. sci. et lettres Poznón C10(1960) 65-69.

2. ALI M.A., & HAYNES L.J., J. Chem Soc. 1(1959) 1033.

3. ASTRUC A., & GIROUX J., Trav. Soc. Pharm. Montpellier 1(1942) 12-15.

4. AUTERHOFF H., & BALL B., Arzneim-Forsch 4(1954) 725-729.

5. AWE W., AUTERHOFF H., & WACHSMUTH-MELM C.L., Arzneim-Forsch 8(1958) 243.

6. AWE W., & KÜMMEL H.J., Arch.Pharm 295(1962)819-822.

7. AWE W., & WACHSMUTH-MELM C.L., Pharmaz Ztg. 102(1957) 1034.

8. BARNES R.A., & HOLFELD W., Chemistry & Industry (1956) 873-4.

9. BAUMGARTNER R., & LEUPIN K., Pharm. Acta. Helv. 34(1959) 296 ex C.A. 54(1960)3849b.

10. BAUMGARTNER R., & LEUPIN K., Pharm. Acta. Helv. 36(1961)445-460 ex C.A. 56(1962) 542g.

11. BELKIN M., FITZGERALD D.B., & COGAN G.W., J.Natl. Cancer Inst. 13(1952) 139-155 ex C.A. 50(1956) 8138d.

12. BELLAART A.C., Pharm. Weekblad 93(1958) 1077-1083.

13. BENBASSAT J., SULMAN F.G., & ZAITSCHEK D.V., Arch. Intern. Pharmacodynamic 120(1954) 152-159 ex C.A. 53(1959) 19130e.

14. BENNET-CLARK T.A., New Phytologist 32(1933) 37-71.

15. BENNET-CLARK T.A., New Phytologist 32(1933) 128.

16. BENNET-CLARK T.A., & WOODRUFF W.M., New Phytologist 34(1935) 77-91.

17. BETTS T.J., & FAIRBAIRN J.W., Planta Medica 12(1964) 64-70.

18. BETTS T.J., FAIRBAIRN J.W., & MITAL V.K., J.Pharm. Pharmacol. 10(1958) 436-441.

19. BEWS J.W., & BAYER A.W., S.African J.Sci. 28(1931) 168-173 ex C.A. 26(1932) 1638.

20. BEZANGER-BEAUQUESNE L., Ann. Pharm. Franc. 20(1962) 44.

21. BHARUCHA F.R., & JOSHI G.V., Sci. & Culture 22(1957) 389-390.

22. BIRCH A.J. & DONOVAN F.W., Austral J.Chem. 8(1955) 523-528.

23. BIRCH A.J. & DONOVAN F.W., Austral J.Chem. 6(1958) 360.

24. BIRKINSHAW J.H., Planta Medica 7(1959) 367-383.

25. BIRKINSHAW & GOURLAY J.Chem. Soc (1963) 716.

26. BOBBITT J.M., Thin Layer Chromatography Reinhold Publ. Corp. N.Y.

27. BÖHME H., & BERTRAM J., Arch. Pharm. 288(1955) 510-516 ex C.A. 50(1956) 8138d.

28. BÖHME H., & KREUTZIG L., Deutsche Apoth-Zeit. 103(1963) 505-508.

28a. BÖHME H., & KREUTZIG L., Arzneim-Forsch. 16(1966)212.

28b. BÖHME H., & KREUTZIG L., Arch.Pharm 297(11)(1964) 681-689.

29. BONNER W., & BONNER J., Amer. J. Botany 35(1948) 113-117.

30. BONNER J., Plant Biochemistry. Academic Press N.Y. 1950.pp140-156 162. 306.

31. BORNTRAGER H., Z.Anal. Chem. 19(1880) 165-167.

32. BOWIE J.H., & COOKE R.G., Austral J.Chem 15(2) (1962) 332-335.

33. BOWIE J.H., COOKE R.G., & WILKEN P.E., Austral J. Chem. 15(2) (1962) 336-341.

34. BRENNER M., NIEDERWIESER A., PATAKI G., & FAHMY A.R., Experientia 18(1962) 101.

35. BRIGGS L.H., & DACRE J.C., J.Chem.Soc. (1948) 564-568.

36. BRIGGS L.H., CRAW N.R., & DACRE J.C., J.Chem.Soc. (1948) 568-570.

37. BRIGGS L.H., & NICHOLLS G.A., J.Chem.Soc. (1949) 1241-1246.

38. BRIGGS L.H., & THOMAS B.R., J.Chem.Soc. (1949) 1246-1249.

39. BRIGGS L.H., NICHOLLS G.A., & PATERSON R.M.L., J.Chem.Soc. (1952) 1718-1720.

40. BRIGGS L.H., & TAYLOR A.R., J.Chem.Soc. (1958) 3298-3299.

41. BRITISH PHARMACEUTICAL Codex 1963., Pharmaceutical Press, London.

42. BRITTAIN R.T., D'ARCY P.F., & GRIMSHAW J.J., J.Pharm.Pharmacol 14(1962) 715-721.

43. BRODASKI T.F., Anal.Chem. 36(6) (1964) 996-999.

44. BRUCE W.G.G., M.Sc.(Pharmacy) thesis, Potchefstroom Univ. 1964., director Prof. Dr. K.W. Gerritsma.

45. BRUNO F., Lavori ist. Botan. Palermo 5(1934) 23-33 ex C.A. 29(1935) 1849.

46. BRYANT F., & OVERELL B.T., Biochem.Biophysica Acta 10(1953) 471.

47. BUCHANAN J.G., DEKKER C.A., & LONG A.G., J.Chem. Soc. (1950) 3162-3167.

48. BUSCH G., & RESENDE F., Portugalia Acta Biol 7(1959) 367 ex C.A. 52(1958) 9318c.

49. CAHN R.S., & SIMONSEN J.L., J.Chem.Soc. (1932) 2573-2582.

50. CAMPBELL J.M., & COOPER R.L., Chemistry & Industry (1955) 64-65.

51. MENDES CONAGIN BRAGANTIA 10(1950) 369 ex C.A. 46(1952) 9171g.

52. COOKE R.G., & JOHNSON B.L., Austral. J. Chem. 16(4) (1963) 695-702.

53. CRELLIN J.K., FAIRBAIRN J.W., FRIEDMANN C.A., & RYAN H.A., J. Pharm.Pharmacol (1961) 639.

54. D'AMICO M.L., Fitoterapia 21(1950) 97, ex C.A. 44(1950) 11036a.

55. DANILOVIC M., & PECI-POPOVIC E., Arkhiv.Za Farmacijii Belgrad 8(1958) 245-251 ex Planta Medica 7(1959) 220.

56. DANILOVIC M., & NAUMOVIC-STEVANOVIC O., J.Chromatog. 19(1965) 613-614.

57. D'ARCY P.F., GRIMSHAW J.J., & FAIRBAIRN J.W., ex J.Pharm.Pharmacol 14(1962) 715.

58. DAS D.B., MITRA M.K., & WAREHAM J.F., J.Indian Chem. Soc. 28(1951) 37-40 ex C.A. 45(1951) 9133b.

59. DENSTON T.C., Textbook of Pharmacognosy, 5th edit. Pitman, London (1950) pp 428 & 470.

60. DÖFF W., Arzneim-Forsch. 3(1953) 627-630 ex C.A. 48(1953) 3569.

61. ERDTMAN H., Chemical Plant Taxonomy, (Edit. T. Swain) Academic Press, London & N.Y. 1963. pp 90-95.

62. FAIRBAIRN J.W., Pharm J. 148(1942) 198.

63. FAIRBAIRN J.W., J.Pharm.Pharmacol 1(1949) 683-692.

64. FAIRBAIRN J.W., The Pharmacology of Plant Phenolics. Academic Press, London (1959) P46.

65. FAIRBAIRN J.W., & MICHAELS I., J.Pharm.Pharmacol. 2(1950) 807-812.

66. FAIRBAIRN J.W., & LOU T., J.Pharm.Pharmacol. 3(1951) 93-103.

67. FAIRBAIRN J.W., & SALEH M.R.I., J.Pharm.Pharmacol. 3(1951) 918-925.

68. FAIRBAIRN J.W., FRIEDMANN C.A., & RYAN H., J.Pharm. Pharmacol 10(1958) 186 T.

69. FAIRBAIRN J.W., & MITAL V.K., J.Pharm.Pharmacol. 10(1958) 217T-222T.

70. FAIRBAIRN J.W., Planta Medica 7(1959) 406-410.

71. FAIRBAIRN J W., & SIMIC S., J.Pharm.Pharmacol. 12(1960) 45T-51T.

72. FAIRBAIRN J.W., FRIEDMANN C.A., & SIMIC S., J.Pharm.Pharmacol. 15(1963) 292T.

73. FAIRBAIRN J.W., B.Pharm.J. (1963) 271-274.

74. FAIRBAIRN J.W., Pharm.Weekblad 100(1965) 1493-1499.

74a. FARNSWORTH N.R., J.Pharm.Sciences 55(3) (1966) 225-277.

75. FERGUSON N.M., & GARDNER J.H., J.Am.Chem.Soc. 72(1950) 2877.

76. FILATOV V.P., & BIBER V.A., Doklady Akad. Nauk S.S.S.R. 62(1948) 259-262 ex C.A. 43(1949) 726f.

77. FLAGG J., Amer.Perfum.Aromat. 74(1959) 27-28 ex C.A. 54(1960) 1808f.

78. FLÜCK H in Chemical Plant Taxonomy (Edit. T. Swain) Academic Press, London & N.Y. (1963) p.167.

79. FRANK & RESHKE Chem.Ber. 193(1960) 347 ex C.A. 54(1960) 12076.

80. FREYTAG A., Pharmazie 9(1954) 705-710 ex C.A. 50(1956) 16897.

81. FRIEDRICH H., Planta Medica 7(1959) 406-410.

82. FURAYA T., J.Chromatog. 18(1965) 152-156.

83. FURAYA T., SHIBATA S., & IIZUKA., J.Chromatog. 21(1966) 116-118.

84. GATENBACK S , Acta-Chim.Scand. 13(1959) 386.

85. GATHERCOAL & WIRTH Textbook of Pharmacognosy 3rd edit. Henry Kimpton, London (1956) p.135.

86. GARDNER J.H., J.Am.Pharm.Assoc. 23(1934) 1177.

87. GARDNER J.H., & JOSEPH L., J.Am.Pharm.Assoc. Sci.Ed. 26(1937) 794-796.

88. GEBHARDT H., Arch.Exptl.Pathol.Pharmakol. 182(1936) 521-526.

89. GERRITSMA K.W., & VAN OUDTSHOORN, M.C.B., Pharm.Weekblad 97(1962) 765-776.

90. GILSON E., Bull.Acad.Roy.Med.Belg. 16(1902) 827.

91. GOLLUB M., & VENNESLAND B., J.Biol.Chem. 169 (1947) 233.

92. GOLSE J., Précis De Matière Médicale. Doin & Co., Paris. p.129.

93. GONNARD P., PELOU A., & SCHMITT H., Ann.Pharm. Franc. 10(1952) 607-610.

94. HAAS P., & HILL T.G., Introduction to the Chemistry of Plant Products, Longmans, Green & Co., London (1913). p.242.

95. HALPAAP H., Manufacturing Chemist (1963) 517.

96. HARDERS C.L., Pharm.Weekblad 84(1949) 250-273.

97. HAUSER F., Pharm.Acta.Helv. 6(1931) 79-85.

98. HAY J.E., & HAYNES L.J., J.Chem.Soc, (1956) 3141.

99. HAYNES L.J., HENDERSON J.I., & TYLER J.M., J.Chem.Soc. (1960) 4879-4885.

100. HEGNAUER R., Pharm. Weekblad 92(1957) 860.

101. HEGNAUER R., Planta Medica 7(1959) 344-366.

102. HENNEBERG M., & HORAK P., Acta.Polon.Pharm. 16(1959) 189 ex Pharm.Weekblad 96(1961) 566.

102a. HILLIS W.E., Austr.J.Chem. 8(2) (1955) 290-292.

103. HODGE W.H., Econ.Botany 7(1953) 99-127.

103a. HOLMAN R.M., & ROBBINS W.W., Textbook of Botany 4th edit. Wiley & Sons., N.Y. 1946.

104. HONNEGGER R., Vierteljahrsschr. naturforsch. Ges. Zurich, Beich 97(1952) 44 ex C.A. 47(1953) 5756d.

105. HÖRHAMMER L., Arch.Pharm. 292(1959) 591.

106. HÖRHAMMER L., WAGNER H., & FÖCKING O., Pharm.Ztg. 104(1959) 1183-1186 ex C.A. 54(1960) 13550g.

107. HÖRHAMMER L., WAGNER H., & LEUE H.J., Dtsch. Apoth.Ztg. 99(1959) 1043.

108. HÖRHAMMER L., WAGNER H., LEUE H.J. & KONIG., Dtsch. Apoth-Ztg. 102(1962) 1278.

109. HÖRHAMMER L., WAGNER H., & BITTNER G., Deutsche Apoth. 14(1962) 148.

110. HÖRHAMMER L., WAGNER H., & BITTNER G., Pharmaz. Ztg. 108(1963) 259.

111. HÖRHAMMER L., WAGNER H., & BITTNER G., Arzneim-Forsch. 13(1963) 537-541.

112. HÖRHAMMER L., WAGNER H., & BITTNER G., Z.Natur-forsch. 19b(1964) 222-226.

113. HÖRHAMMER L., WAGNER H., BITTNER G., & GRAF E., Deutsche Apoth-Ztg. 105(1965) 827-30.

114. IPPEN H., Planta Medica 7(1959) 423-426.

115. JANIAK B., & BOEHMERT H., Arzneim.-Forsch. 12(1962) 431-435 ex C.A. 57(1962) 11309e.

116. KABARA J.J., KABARA G.C., & WOJTALIK R.S., J. Chromatog. 15(1964) 267-269.

117. KAPADIA G.J., & KHORANA M.L., J.Chromatog 6(1961) 537-538.

118. KARAEV A.I., ALIEV R.K., GUSEINOV G.A., & DADASHEV A.G., S.S.R. Ser.Biol. E Sel'skokhoz. Nauk 3(1958) 81-92 ex C.A. 52(1958) 20682f.

119. KAUFMAN E., & LEIVA R., Inform med (Cuba) 11(1947) 34-39 ex C.A. 41(1947) 6671h.

120. KHORANA M.L., & SANGHAVI M.M., Am.J.Pharm.Sci. 53(1964) 110-112.

121. KIENAN G.L , & WELSH L.H., J.Am.Pharm.Assoc. 31(1942) 535 ex C.A. 37(1943) 21369.

122. KINGET R., Pharm.Tijdschr. Belg. 40(1963) 185-191 ex Pharm.Weekblad 100(1965) 852.

123. KREBS H.A., & EGGLESTON L.V., Biochem.J. 34(1940) 1383.

124. KRAUS L., Pharmazie 12(1957) 693-695.

125. KRAUS L., Planta Medica 7(1959) 427-444.

126. LEE & BERGER J.Pharm.Pharmacol. 9(1957) 432.

127. LÉGER E., J.Pharm.Chim. 4(1911) 281-283.

128. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Week-blad 98(1963) 500.

129. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Week-blad 98(1963) 529.

130. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Week-blad 98(1963) 655.

131. LEMLI J , Pharm.Tijdschr.Belg. 40(1963) 149 ex Pharm.Weekblad 100 (1965) 640-641.

132. LEMLI J., Pharm.Weekblad 98(1963) 730.

133. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Weekblad 99(1964) 351.

134. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Weekblad 99(1964) 589-592.

135. LEMLI J., DEQUEKER R., & CUVEELE J., Pharm.Weekblad 99(1964) 613.

136. LEMIEUX R U., & BAUER H.F., Analyt.Chem. 29(1954) 920-921.

137. LEUE H.J., Inaugural Dissertation, München (1962) 146

138. LIDDELL R.W., KING C.G., & BEAL G.D., J.Am.Pharm. Assoc. 31(1942) 161-166.

139. LINCOLN R., Dept.Agric.Mauritius Annual Report 1947 ex C.A. 43(1949) 4345b.

140. LINSKENS H.F., Portugaliae Acta Biol. Ser.A 6(1960) 125-130 ex C.A. 56(1962) 1782h.

141. LISTER R.E., & PRIDE R.R.A., J.Pharm.Pharmacol. 11(1959) 278-282T.

142. LORENZETTI L.J., SALISBURY R., BEAL J.L., & BALDWIN J.N., Am.J. Pharm. Sci. 53(1964) 1287.

143. LOU T.C., J.Pharm.Pharmacol. 1(1949) 673-682.

144. LUKIC P.B., Planta Medica 7(1959) 400-404.

145. MARY N.Y., CHRISTENSEN B.V., & BEAL J., J.Am. Pharm. Assoc. 45(1956) 229-232.

146. McCARTHY T.J., M.Sc.(Pharmacy) thesis, Potchefstroom Univ. (1964), director Prof. Dr. K.W. Gerritsma.

147. McCARTHY T.J. & PRICE C.H., Pharm.Weekblad 100(1965) 761-763.

148. McCARTHY T.J., & PRICE C.H., Pharm.Weekblad 100(1965)912-916.

149. McCARTHY T.J., & VAN OUDTSHOORN M.C.B., Planta Medica 14(1966) 62965.

150. McCARTHY T.J., & PRICE C.H., Planta Medica (1967) to be published.

151. McCARTHY T.J., & PRICE C.H., Planta Medica (1967) to be published.

152. McCORMACK J.R.D., American Cyanamid U.S. no. 2, 987, 449, June 6, 1961 ex. C.A. 56(1962) 3573a.

153. MERCK E., Chromatography. Darmstadt, Germany pp. 80 & 133.

154. METZENBERG & MITCHELL, J.Am.Chem.Soc. 76(1964)4187.

155. MEYER B.S., & ANDERSON D.B., Plant Physiology. Van Nostrand, London, 2ed. (1955) pp. 264-265, 387, 431-3.

156. MÖHRLE H., Deutsche Apoth.Ztg. 102(8) (1962) 227 ex Anal.Ab. 9(9) (1962) 3878.

157. MORITZ ex FRIEDRICH H Planta Medica 7(1959)383-389.

158. MORTON R.A., & EARLAM W.T., J.Chem.Soc. (1941) 159-169.

159. MÜHLEMANN H., Pharm.Acta Helv. 27(1952) 17-26.

160. MÜHLEMANN L.,, & SCHMID T., Pharm.Acta Helv. 30(1955) 350 & 363.

161. MUKERJII B., & KARKUN J.N., Ind.J.Pharm. 12(1950) 236-243 ex C.A. 45(1951) 2628d.

162. MÜLLER K.H., CHRIST B., & KUHN G., Arch.Pharm. 295(1962) 41.

163. NAWA T., Chem.Pharm.Bull. (Tokyo) 8(1960) 566 ex Pharm.Weekblad 96(1961) 713.

164. NIHOUL - GHENNE L., J.Pharm.Belg. 13(1958) 44.

165. OKADO T , Tokuku J. Exptl. Med. 38(1962) 33-44.

166. PARIS R., & DURAND M., Ann.Pharm.Franc 14(1956)755.

167. PARTRIDGE S.M., Biochem. J. 42(1948) 238.

168. PASTUKA G., Z.Anal.Chem. 179(1961) 355.

169. PATAKI G., & KELEMEN J., J.Chromatog. 11(1963) 50-54.

170. PLANT PHYSIOLOGY 2nd Edit. Meyer & Anderson, Van Nostrand, London & N.Y. (1955) P.165.

171. POETHKE W., BEHRENDT H., & MATSCHKE E., Pharm. Zentralh. 102(8) (1963) 492 ex Anal.Ab. 11(1964) 4499.

172. PUCHER G.W., WAKEMAN A.J., & VICKERY H.B., J.Biol.Chem. 119(1937) 523.

173. PUCHER G.W., & VICKERY H.B., Ind.Eng.Chem., Anal.Ed. 13(1941) 244 & 412.

174. RAMAUT J.L., Phytochemistry 1(1962) 259-262.

175. RAMSTAD E., Modern Pharmacognosy. Mc.Graw-Hill, London, N.Y. & Toronto (1959) p.222-229.

176. REYNOLDS G.W., The Aloes of South Africa. The trustees of the Aloes of South Africa Bookfund, Johannesburg 1950.

177. RICKARDS R.W., in Chemistry of Natural Phenolic compounds (Edit. W.D. Ollis) Pergamon Press, London, N Y. & Paris (1961).

178. ROSENTHALER L., Pharm.Acta Helv. 7(1932) 19.

179. ROSILLO J., Laboratory Practice (1964) 210.

180. ROSTOTSKI B.K., Ya A Aleshkina U.S.S.R. 111, 903, May (1958) ex C.A. 52(1958) 17627h.

181. RUGGIERI R., Boll.chim.farm. 96(1957) 491-494 ex C.A. 55(1961) 5874e.

182. RUHLAND W., & WETZEL K., Planta 3(1927) 765.

183. RUHLAND W., & WETZEL K., Planta 7(1929) 503.

184. RUHLAND W., & WOLF J., Ann.Rev.Biochem. 3(1934) 501.

185. SAHA J.C., SAVINI E.C., & KASINATHAN S., Ind.J. Med.Research 49(1961) 130 ex C.A. 55(1961) 14827d.

186. SCHINDLER O., Pharm.Acta Helv. 6(1931) 79.

187. SCHINDLER O., & SEEBECK., Helv.Chim.Acta 29(1946) 31-37.

188. SCHRATZE E., & NIEWOHNER C., Planta Medica 7(1959) 137-170.

189. SCHULTZ O.E., & MAYER G., Arzneim.-Forsch. 6(1956) 334.

190. SEELKOPF C., & TERAN L.R., Arch.Pharm. 293(1960) 636.

191. SEIKEL M.K., & MABRY T.J., Tetrahedron Letters No.16(1965) 1105-1109.

192. SEXTON W.A., Chemical Constitution and Biological Activity 2nd edit. E. & F. Spon Ltd., London. p.203.

193. SHAMATOV N.M., Uchenya Zapiski Moskov Med.Inst. 6(1957) 67 ex C.A. 53(1959) 15345g.

194. SHAMRAI E.F., & FETISOVA ex C.A. 53(1959 6442h.

195. SHELLARD E.J., Laboratory Practice 13(1964)290-294.

196. SHIBATA S., TAKITO M., & TANAKA O., J.Am.Chem. Soc. 72(1950) 2789.

197. SHKOL'NIK R. Ya. Trudy Komissie Anal.Khim. Ahad Nauk. U.S.S.R. ex C.A. 50(1956) 13153b.

198. SIEPER H., LONGO R., & KORTE E., Arch.Pharm. 296(1963) 403.

199. SMALL J., pHand Plants. Balliere, Tindall & Cox, London (1946) pp.39-47, 121-122.

200. SMALL J., Modern Aspects of pH. Balliere, Tindall & Cox, London (1954) pp.27-36, 188-190.

201. SMITH T & H., Pharm.J. 11(1851) 23-25.

202. SODERSTROM T.R., Am.J.Botany 49(1962) 850-855.

203. SPOEHR J., Publ.Carneg.Inst.Washington (1919) 287.

204. STAHL E., Laboratory Practice 13(1964) 496.

205. STAHL E., Proc.Soc.Anal.Chem. 1(1964) 121.

206. STATE PHARMACOPOEIA OF THE U.S.S.R. 8th edit., Vneshtor Giz dat. p.26.

207. STEELE C.C., Introduction to Plant Biochemistry Bell & Sons, London (1963) p.119-120.

208. STEINER Pharm.Acta.Helv. 26(1951) 107 ex C.A. 55(1961) 19133e.

209. STOLL A., KUSSMAUL., & BECKER B., Helv.Chim. Acta 32(1949) 189.

210. STOLL A., BECKER B., & HELFENSTEIN A., Helv.Chim. Acta 293(1960) 636.

211. STRAUB W., & GEBHARDT H., Arch.Exptl. Pathol. Pharmakol. 181(1936) 399-407.

212. STRÖM H., & KIHLSTRÖM T., Medd.Norsk.Farm. Selskap. 10(1948) 67 & 93.

213. STUTZ R.E., & BURRIS R.H., Plant Physiol. 26(1951) 226-243.

214. SUKHORUKOV K., & BOLSHAKOVA N., Compt.Rend. Acad.Sci. U.S.S.R. 53(1946) 471 ex C.A. 41(1947) 38390

215. TEIJGELER C.A., Pharm.Weekblad 97(1962) 43-50.

216. TEIJGELER C.A., Pharm.Weekblad 99(1964) 101-113.

217. THOMSON R.H., Naturally Occurring Quinones. Butterworths, London (1957) p.158.

218. THORPE J.F., & WHITELEY M.A., Thorpe's Dictionary of Applied Chemistry, 4 edit. Longman's, Green & Co., London (1937) p.212.

219. TRANQUILLINI W., in Water Relations of Plants. (Edit.Rutter & Whitehead). Blackwell Scientific Public. Oxford p.156.

220 TREASE, G.E., Textbook of Pharmacognosy 7th edit. Bailliére, Tindall & Cox, London (1957) pp.164-165.

221. TUTIN F., & CLEWER H.W.B., J.Chem.Soc. 99(1911) 946-947.

222. TSCHIRCH & HOFFBAUER Schweiz.Wochenschr. 43(1905) 153-158.

223. TUTIN F., Trans.Chem.Soc. 103(1913) 200.

224. VAN RHEEDE VAN OUDTSHOORN M.C.B., Planta Medica 11(1963) 332-337.

225. VAN RHEEDE VAN OUDTSHOORN M.C.B., Phytochemistry 3(1964) 383-390.

226. VAN RHEEDE VAN OUDTSHOORN M.C.B., & GERRITSMA K.W., Pharm.Weekblad 99(1964) 1425-1430.

227. VAN RHEEDE VAN OUDTSHOORN M.C.B., Dissertation, Potchefstroom Univ.(1965), director Prof. Dr. K.W. Gerritsma.

228. VICKERS C., J.Pharm.Pharmacol. 3(1961) 509.

229. VICKERY H.B., Plant Physiology 27(1952) 9-17.

230. VICKERY H.B., Plant Physiology 27(1952) 231-239.

231. VICKERY H.B., Plant Physiology 31(1956) 455-464.

232. VICKERY H.B., Conn.Agr.Exptl.Stat.Bull (1961) 640.

233. VICKERY H.B., J.Biol.Chem. 238(1963) 2453.

234. WAGNER H., HÖRHAMMER L., LEUE H.J., & KÖNIG H.W., Dtsch. Apoth-Ztg. 102(1962) 1278.

235. WAGNER H., HÖRHAMMER L., & FARKASS L., Zeit. Naturforsch 18b(1) (1936) 89. ex Biol. Abstr. 44(1963) 20991.

236. WANG, LIANG, SHA, LO & CHOU., Acta Pharm.Sinica 10(1963) 720-724.

237. WATT J.M., & BRAYER-BRANDWIJK M.G., Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edit. E. & S. Livingstone, London. p.681.

238. WASICKY R., & HOEHNE W., Univ.São Paulo, ex C.A. 46(1952) 10548e.

239. WHITEHEAD F.H., New Phytologist 61(1962) 59-62.

240. WHITEHEAD F.H., & LUTI R., New Phytologist 61(1962) 56-58.

241. WOLF J., Planta 15(1931) 572.

242. WOLF J., Planta 26(1937) 316.

243. WOLF J., Planta 28(1938) 60.

244. WOLF J., Planta 29(1939) 319.

245. WOLF J., Planta 29(1939) 450.

246. WOOD J.G., CRUIKSHANK D.H., & KUCHEL R.M. Austral.J.Exptl.Biol.Med.Sci. 21(1943) 37.