V A R I A B I L I T Y O F A L O I N

I N C A P E A L O E S.

A Thesis presented for the degree

MASTER OF SCIENCE
(PHARMACY)

at

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by

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LIST OF FIGURES.

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FIGURE 3.

Thin-layer chromatograms of A.ferox juice from:-

1. Oudtshoorn.
2. Mossel Bay.
3. Redhouse.
4. Port Elizabeth.

(FIGURES 4 to 7 inclusive reflect specimens collected in Port Elizabeth).

FIGURE 4.

Thin-layer chromatograms of the juices of the species:-

1. A.ferox.
2. A.speciosa.
3. A.striata.
4. A.arborescens.
5. Aloin.

FIGURE 5.

Thin-layer chromatograms of the juices of the species:-

1. A.africana.
2. A.speciosa.
3. A.arborescens.
4. Aloin.

FIGURE 6.

Thin-layer chromatograms of the juices of the species:-

1. A.saponaria.
2. A.lineata.
3. A.microstigma.
4. Aloin.

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FIGURE 7.

Thin-layer chromatograms of the juices of the species:-

1. A.gracilis.
2. A.humilis.
3. A.tenuior.
4. Aloin.

FIGURE 8.

Thin-layer chromatograms of A.ferox sap or lump from:-

1. Oudtshoorn.
2. Coega.
3. Coega.
4. Mossel Bay.
5. Aloin.

FIGURE 9.

Thin-layer chromatograms of :-

1. A.ferox.
2. Aloin B.P.
3. Aloin amorph (Merck).
4. Aloin crystalline.

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VARIABILITY OF ALOIN IN CAPE ALOES.

INTRODUCTION.

Aloes have been known and used medicinally since antiquity. In the Ebers papyrus of circa 1500 B.C. aloes is mentioned as one of the few of some 800 prescriptions then in use having any therapeutic value (HUTCHINS (1)).

In 1953 HODGE (2) published a comprehensive report on "The Drug Aloes of Commerce, With Special Reference to the Cape Species". He points out that the laxative properties of the bitter resinous (aloe) juice was appreciated by the Greeks at least as early as the fourth century B.C. Hodge compares the current drug production, naming the principal exporters and importers of lump aloe. South Africa, although the principal producer, has to meet the very strong challenge of the islands of the Dutch West Indies - Aruba and Bonaire. His remarks on the "Future Outlook for the Cape Aloe Industry" are most pertinent to this work, and are quoted below:

"As long as such conditions prevail*, little can be accomplished that might bring about a proper development of the (Cape) industry, including such activities as selection of higher yielding strains of A.ferox and extension of its commercial exploitation throughout the natural range of the species. Despite the scarcity of spines on plants in the eastern part of its range, the juice could still be drained satisfactorily by

* Footnote: Unsystematic collection by Coloureds who are poorly paid.

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abandoning the traditional conical stacking, which is dependent on the presence of surface spininess, and by using other methods of draining and collecting the juice. As stated earlier, too, Southern Africa is a land of aloes and there are other species just as abundant as A.ferox. 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. If South Africa were to follow the example of Aruba by manufacturing U.S.P. aloin directly from the fresh juice of aloes, there might be a possibility of growth in this industry."

It is for this reason that an attempt has been made to investigate firstly the influence of the time of collection and the weather on the aloin content of leaves of the same plant, and secondly the geographical influence on aloin content. Furthermore the juice of several aloe species and hybrids growing in the most important collection areas was examined to obtain some idea of their potential compared with those collected at present. To solve the problems of the preparation of amorphous aloin direct from aloe juice some experimental work was done, and finally the problem of drying the juice with the minimum loss of aloin was investigated.

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CHAPTER 1.

In this chapter an outline is given of the various Aloe species found, and of the conflicting evidence of the chemical structure and pharmacologic action of aloe constituents.

Many Aloe species are to be found in the world. More than 200 species are found in Africa alone (RAMSTAD) (3) while some 130 of these occur in Southern Africa (REYNOLDS) (4). Aloe species are to be found in Southern, Northern & Eastern Africa, (including the island of Madagascar), in India and the East and West Indies. The commercial aloe is usually named after its place of origin.

South African types:

Cape and Natal.

East African types:

Socotrine, Zanzibar and Madagascar.

East Indian types:

Jafferabad and Musumbra.

West Indian types:

Curaçao, Barbadoes and Jamaican.

Many of the above aloes are no longer found in commerce, the principal sources being the Cape, Curaçao, and to a lesser degree, Socotra.

Cape aloes comes into the market in olive-black or dusty to dark brown masses frequently covered with a yellowish powder (YOUNGKEN) (5). Its fracture is sharp, exhibiting pieces with a smooth and vitreous broken surface. It has a distinct sour odour and a nauseous, bitter taste.

Curaçao and Socotrine aloes are, in contrast, opaque, the former fracturing to display an uneven waxy surface, the latter a sharp-edged somewhat conchoidal fracture. Both are brownish-black, although Socotrine may be reddish-black.

Collection and Preparation of CAPE ALOES.

The leaves of (principally) Aloe ferox are cut by the workers close to the stem, and stacked in radial bundles of up to two hundred leaves, with the cut ends (exuding the aloetic juice) arranged over a hole in the ground, in which rests a canvas. The thorniness of the A.ferox allows the building of high pyramids of leaves, whereas other species would slip off. The leaves are exhausted after 6 to 8 hours, the canvas is removed, and the juice boiled in large drums. Once the juice is of a suitable consistency the workers cease stirring, remove the drum from the fire and skim off the surface impurities. The viscous mass is poured into 4-gallon tins and allowed to set, when it constitutes Cape aloes.

Although Aloe ferox is the principal aloe used, the workers often use close hybrids of A.ferox with A.africana, A. speciosa, A. arborescens et alia, some of these hybrids being scarcely distinguishable from A. ferox, which itself varies from locality to locality throughout the Cape Province

CHEMICAL CONSTITUENTS OF ALOES.

Much controversy has raged over the chemical constituents of aloes including Cape aloes. In particular there appeared to be much confusion over the nomenclature and formula of the principal constituent aloin. The name barbaloin was given to the glycoside isolated over 100 years ago from Barbadoes aloes (see T. & H. SMITH, Chem. Gaz., (1851) 107).

EVERS and TSCHIRCH (ex(9) stated that Cape or ferox aloes contained barbaloin, although TSCHIRCH first thought that this aloin (which he called cap-aloin or ferox-aloin) differed from barbaloin.

LÉGER (ex(6)) showed that the cap-aloin of Cape aloes, the Socaloin of Socotrine aloes and the barbaloin of Barbadoes aloes were almost identical. The use of the name of the place of origin as prefix has, with the exception of barbaloin, fallen largely into disuse. HARDERS (11) used Curacao aloes, and this name appears to have found general acceptance. However, as many of the commercial types of aloin available contain other aloe by-products, the name amorphous aloin would be more acceptable for these samples, while the name aloin should be reserved for aloin having a definite crystalline structure and melting point. Henceforth this crystalline aloin will be termed aloin.

Various formulae and molecular weights have been attributed to aloin. The formula $C_{16}H_{19}O_7$ was given by TILDEN and supported by JOWETT & POTTER (ex (6)). LÉGER'S formula $C_{21}H_{20}O_9$ was later supported by TILDEN. The formula $C_{16}H_{18}O_7$ is found in SQUIRE'S COMPANION TO THE B.P. 1916 (12), and is said by CAHN and SIMONSEN (13) to be the accepted formula for aloin.

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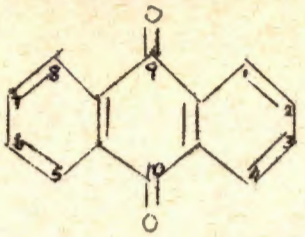
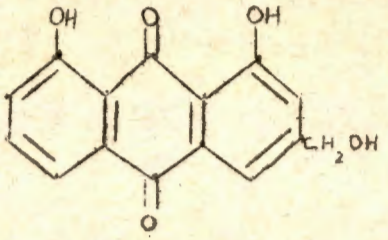
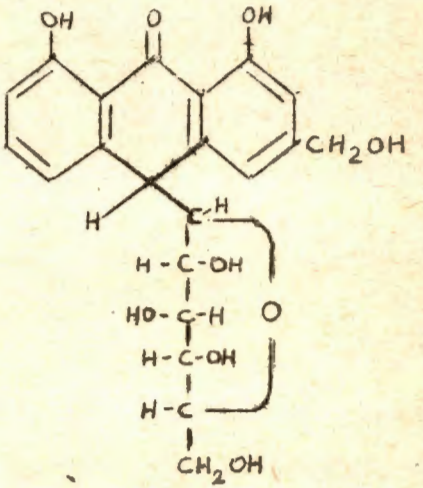


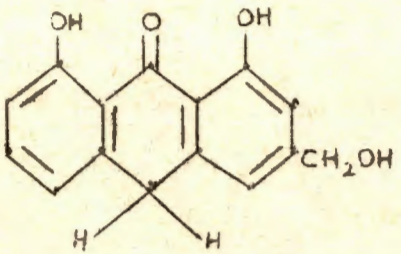
Anthraquinone



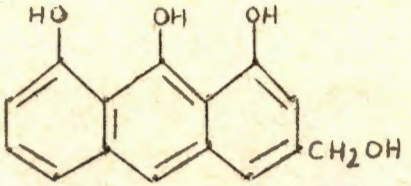
Aloe-emodin



Aloin



Aloe-emodin anthrone



Aloe-emodin anthranol

FIGURE 1

ROSENTHALER (14), in agreement with earlier work of EDER & ZINN (16) and MÜHLEMANN (17), gives the formula as $C_{21}H_{22}O_9$. The latest confirmation of this formula by HAY & HAYNES (18) in 1956 is given in broader detail under Structural Configuration of Aloin.

Apart from the bitter crystalline principle aloin, aloe-emodin (see fig. 1) is also found, but rarely in the fresh juice of Cape aloes. It is, however, formed in relatively large proportion during the boiling of the juice to produce aloe lump. This results in a decrease in the content of aloin. Chromatographically it appears as a red spot just below the front of the solvent system chloroform: ethanol 3:1.

Beta-barbaloin, an amorphous substance obtained by heating aloin at 160° for 3 hours, is said to be present in Cape aloes but absent from the Curaçao variety. Isobarbaloin, a crystalline isomer of barbaloin, is found in quantity only in the Curaçao variety. Resins of variable composition are reported. These are said by RAMSTAD (3) to be condensation products of anthrones and anthranols. YOUNGKEN (5) lists these resins as resinotannols combined with paracumaric acid (p-hydroxy cinnamic acid). The resins of Curacao aloes are said to be barbaloresinotannols combined with cinnamic acid. The purgative activity of these resins is discussed under Pharmacologic Action, together with the other aloe constituents.

Chemical Tests of Aloe Constituents.

The tests that follow are frequently used to differentiate the various commercial varieties of aloes.

Differentiation. The three varieties, Cape, Curaçao and Socotrine aloes, can, apart from their macroscopical appearance described before, be

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distinguished microscopically or chemically.

Microscopically the three species show distinctive crystals of aloin when mounted in a mixture of two parts glycerine and one part alcohol 95% (FAIRBAIRN) (6). According to LISTER & PRIDE (28) crystalline aloin is insoluble in cresol, while amorphous aloin is soluble.

Chemically, when treated with nitric acid, each variety produces a characteristic colour, the Cape aloe passing through reddish-brown to purplish to green, while Curaçao and Socotrine give deep red and brownish-red solutions respectively. Further differentiation is given by the Bornträger Reaction (British Pharm. 1958) (7), where a solution of aloes is shaken with benzene, which is then separated off and made alkaline with ammonia. A cherry red colour indicates Curaçao or Socotrine aloes, while Cape aloes may give a red or brown colour. (Free anthraquinones). Anthranols (see fig. 1) produce a characteristic green fluorescence with borax when the solution is diluted by pouring into water.

The presence of isobarbaloin may be shown by the following tests: (HÉBERT & ELLERY) (8)

Nitrous Acid Test:

To 3 ml of 1-100 aloe solution is added 0.1 g sodium nitrite and a few drops of acetic acid. Curaçao and Cape aloes produce a red colour, which fades rapidly for the latter. Socotrine aloes produce no colour.

KLUNGE'S (Cupraloin) Test:

To 1 ml of the test solution add 4 ml distilled water: add 1 ml of 5% copper sulphate, 0.5 g sodium chloride and 2 ml alcohol. Warm

gently. Both Curaçao and Cape aloes produce a wine-red colour, which fades rapidly for the latter. Socotrine aloes produce no colour.

Structural Configuration of Aloin.

The structural configuration of the glycoside aloin is shown in fig. 1.

MÜHLEMANN (17) by synthesis showed that a C-C bond existed between the aglycone and the sugar, the former being aloe-emodin anthrone (Fig 1), the latter glucose. He gave the formula as 10 (1' 5' anhydroglucosyl) aloe-emodin-9-anthrone. Oxidation of this compound produced aloe-emodin and arabinose. The position of the sugar component in relation to the aglycone gave rise to varying opinions. The sugar was stated by many authors to be a hexose, but opinions differed as to whether the attachment points were C_3 and C_9, or C_{10}. Hydrolysis of aloin produces arabinose, which led earlier workers to the incorrect conclusion that arabinose was attached to the aglycone.

Aloin, although frequently referred to as a glycoside, is more accurately an anhydroglycoside, because the sugar radicle is attached directly to C_{10} of the anthrone molecule, as shown by the synthesis of MÜHLEMANN (17), mentioned above. The anhydroglycosides are very stable to oxidation (RAMSTAD) (3) and this explains the difficulty obtained in breaking this glycoside linkage. To be a true glycoside the sugar must be attached to the aglycone by replacement of the hydrogen atom of an OH radicle. Such an OH radicle exists at C_3 in the form of a CH_2OH radicle. HÖRHAMMER, WAGNER & BITTNER (67) have isolated from East African Aloe species such a compound, which they call Aloinoside B. This substance, isolated in crystalline form, has a melting point of 233°C. Acid hydrolysis thereof

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produces aloin and a sugar, which is as yet unidentified. This sugar appears to be attached O-glycosidally to the oxymethyl side chain of aloin (at C_3).

An apparent isomer of Aloinoside B, called Aloinoside A, is also reported by these authors. Both these compounds, which show on a chromatogram the same colour characteristics of aloin, appear as two separate dots below aloin.

HÖRHAMMER, WAGNER & BITTNER (67) state that the aloinosides can indeed be included in the total estimation of aloin. The Mossel Bay area appears to produce species containing the aloinosides to the extent of circa 6% calculated as aloin, giving a total aloin determination of circa 27%. In contrast to this, the aloes from the Port Elizabeth area (which form the bulk of this writer's work) do not appear to contain these aloinosides, hence HÖRHAMMER and co-workers (67) classify Cape aloes into two groups, namely Type A (containing aloinosides) and Type B (containing no aloinosides). Justification for the inclusion of these aloinosides as "aloin-like substances" is given by the pharmacologic action of Aloinoside B, which was found to be equal to that of aloin, the effective dose for white mice being 300 mgm/Kg.

With the modern analytical tools of infra-red spectroscopy and X-ray crystallography in addition to chemical analysis, HAY & HAYNES (18) confirmed the structural formula for aloin as proposed by MÜHLEMANN (17). The authors found that oxidation of barbaloin with limited amounts of ferric chloride gave merely aloe-emodin and unchanged aloin, which argues for the attachment of the glucose residue to C_{10} in the anthrone nucleus.

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They state further that periodate oxidation of aloin at 0°C under conditions in which the aloe-emodin anthrone is unaffected by the reagents involved, results in a rapid uptake of two mols of oxidant with the formation of formic acid, no further oxidant being consumed during 24 hours. This shows the presence of a - CH(OH).CH(OH).CH(OH) - system and confirms that the glucose residue is present in the pyranose ring form.

This being so, then all the oxygen is accounted for, and the glucose residue cannot be associated with any of the hydroxyl groups in the anthrone nucleus.

Further, the degradation of aloin to aloe-emodin and d-arabinose by treatment with acid for several months as described by LÉGER (see Ann Chim (France) 1916, 6, 318) was not a hydrolysis, but an atmospheric oxidation.

By infra-red studies they show that aloin is not an anthraquinone, since it shows only one band in the infra-red spectrum at 1630 cm-1, whereas aloe-emodin shows two bands in the carbonyl stretching frequency region, one at 1674 cm-1 due to the unassociated carbonyl group, the other at 1624 cm-1 due to the hydrogen bonded carbonyl group: if aloin were an anthraquinone derivative it would similarly be expected to show two bands in that region of the spectrum. X-ray crystallography gave a probable molecular weight of air-dried aloin of 449 ± 12.

HAYNES, HENDERSON & TYLER (19) showed that homonataloin (methyl aloin) showed ultra-violet maxima similar in position to, but differing in intensity from aloin, showing it also to be an anthrone derivative. Mass spectro-photometric molecular weight analysis of 432 is in agreement with the molecular formula $C_{22}H_{24}O_9$ (aloin $C_{21}H_{22}O_9$).

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BIOGENESIS.

Many of the aloe constituents have an anthraquinone nucleus. These so-called anthraquinone drugs constitute a valuable group of medicinals, but little is known of the mode(s) of biogenesis. ROBINSON (ex RAMSTAD (3)) believes that the anthraquinones found in lichens and moulds have as their precursor orsellinic acid. SEXTON (20) mentioning the work done by RAISTRICK says that some hydroxylated anthraquinones are formed in very high yield from glucose by the action of moulds. A noteworthy feature of these mould metabolic anthraquinones is that they bear a carbon constituent in the 3-position (methyl or an oxidation product thereof). Apart from the well-known dihydroxy compounds such as chrysophanic acid, rhein and aloe-emodin from rhubarb, the closely related trihydroxy compounds emodic acid and W-hydroxyemodin have Penicillium cyclopium as their source, while the Helminthosporium species are the source of the tetrahydroxy compounds cynodontin and cetenarin. It may be possible that in higher plants a similar scheme of formation of anthraquinones from sugars is followed.

PHARMACOLOGIC ACTION.

In addition to glycosides, most of the anthraquinone drugs contain resinous substances, which appear to be condensation products of anthraquinones and anthranols. While some pharmacopoeias discard the resinous substance when preparing galenic preparations, others make preferential use of this resin. (RAMSTAD) (3)

The evidence with regard to the pharmacologic action of the resins is conflicting. KIEFER (ex ?) isolated three active resins from Cape aloes,

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and thinks that the purgative action is due to the three resins, and that aloin and emodin play a minor part in producing this effect.

In contrast, TSCHIRCH & HOFFBAUER (21) came to the conclusion that only the resin in aloe is totally inactive or without purging properties, while aloin, aloe-emodin and anthraglucosides have a purging effect.

According to RAMSTAD (3) the resins of aloe require the presence of bile acids in order to act. Aloe enemas are stated to have no stronger effect than water, but the addition of small amounts of bile produces an instantaneous, drastic action. In this connection it is noteworthy that the South African Bantu merely use a teaspoonful of fresh aloe juice in warm water, apparently with complete effect.

Physiological Action.

RAMSTAD (3) states that the laxative action of the anthraquinone drugs (chief of which are aloe, rhubarb, senna and cascara) is considerably higher than might be expected from the anthraglycoside content, there being no direct proportionality between anthraquinone content and activity. However, the several different anthraquinones in the drug act synergistically. Oxidation of the more active anthranol produces corresponding anthraquinones, and due to their special structure the glycosides of aloe and senna do not oxidise readily, hence retaining their action for a very long time. Furthermore, the sugar radicle prevents oxidation of the glycoside in the intestine.

As mentioned before a very special kind of sugar derivative exists in the case of the aloins, (anhydroglucosides), of which several optical isomers are possible e.g. aloin and isobarbaloin, while similarly the

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isomers sennoside A (l-form) and sennoside B (meso form) occur in senna.

An important factor determining purgative action is the length of time that the drug remains in the intestine. In this respect the superiority of the glycosides and acetyl derivatives over the parent substance, and of the natural drug over the synthetic (e.g. tribromaloin), is due to slower absorption of the former (DYSON) (22). According to LEWIS (23) the drugs are absorbed in the small intestine and released at their site of action. the colon.

Structure and Activity.

According to DYSON (22) the number and position of the hydroxyl groups has a powerful influence on the physiological action, frangula emodin (with three hydroxyl groups) having a considerably more powerful action than chrysophanic acid which has two. FAIRBAIRN (24) states that anthraquinones with one hydroxy group are inactive, and grades the activity of these anthracene derivatives as follows:

Highly active - 'anthrone glycosides'

Less active - 'free anthrones'

Much less active - 'free anthraquinones'.

Discussion.

It has been shown that the complexity of the constituents of aloes has given rise to conflicting conclusions from various workers.

The complexity of different principles may account for both the greater difficulty experienced in crystallising aloin from Cape than from

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Curaçao aloes, and for reported differences in biological assays. In part these differences may be due to variations that occur in the same species, and in later chapters it will be shown that variations in aloin content do occur due to weather and season, and on storage and on heating. Furthermore, variations occur in chromatographic appearance of the same species of aloes. Possibly the other constituents of aloes are subject to variation due to climate, soil constituents etc.

Summary of this chapter.

The important commercial aloes have been listed, and the collection and preparation of Cape aloes described. The complex chemistry of aloes has been outlined, and the relationship between chemical structure and pharmacologic activity has been described.

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CHAPTER 2.

In this chapter current methods of analysis of the principle aloe glycoside, aloin, are summarised, and the micro-analytical method of analysis of aloin adopted for this work is detailed.

The methods of analysis applied to the principles found in Aloe fall into two broad groups, namely (a) Biological; and (b) Chemical. In some cases the analysis is of anthraquinone content, in other cases of aloin content.

BIOLOGICAL ASSAYS.

According to BRITTAIN, D'ARCY & GRIMSHAW (25) who investigated the mouse bio-assay method for investigating purgative activity, the method is suitable for senna and its extracts and preparations; it is not so well suited for cascara and rhubarb, and does not demonstrate the purgative activity of aloin (D'ARCY, GRIMSHAW & FAIRBAIRN) (26).

RAMSTAD (3) states that a biological assay is more reliable than chemical evaluations, using the mice faeces technique of FÜHNER. He states that man is about 15 times more sensitive than the mouse to senna, 50 times more sensitive to rhubarb root, 130 times to cascara sagrada and 800 times to aloe.

MÜNCH (quoted from VIEHOVER (27)), commenting on the use of suitable laboratory animals for biological assays, states that in man marked differences occur; that mice are unreliable; that fish are of

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no use; rabbits are too sensitive; cats are good but variations in sensitivity occur, while dogs are more difficult to standardise than cats.

LISTER & PRIDE (28) by using the rat faecal pellet method on barbaloin, aloin B.P. 1953, and amorphous aloin, found a relative potency for each of 100, 82 and 27 respectively.

This latter figure agrees with the range of 20.4% to 32.2% aloin obtained from four samples of amorphous aloin prepared direct from aloe juice.

CHEMICAL ASSAYS.

Several methods of chemical assay have been used to determine the aloin or anthraquinone content of Aloes. A summary of some of these methods appears in table 2 (i), while for more comprehensive reviews reference may be made to VAN OUDTSHOORN (10), HARDERS (11), and HORHAMMER, WAGNER & BITTNER (67).

Table 2 (i).

Method.	Reference.
1. Solvent extraction after clearing with lead acetate	SMITH, JORDAN & DE KAY (34)
2. Polarography	STONE (35)
3. Filter paper electrophoresis	CORE & KIRCH (36)
4(a) Paper chromatography	MARY, CHRISTENSEN & BEAL (37)
4(b) Paper chromatography	AWE, AUTERHOFF & WACKSMUTHMELM (38)
5. Column chromatography	BRODY, VOIGT & MAHER (39)
6. Thin layer chromatography	GERRITSMA & VAN OUDTSHOORN (40)
7(a) Colorimetric (spectrophotometric)	GIBSON & SCHWARTING (41)
7(b) " "	FAIRBAIRN & SIMIC (42)
7(c) " "	MOHRLE (43).

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In addition to these methods, methods are described for the quantitative isolation of aloin. These methods are of interest in the study of the preparation of aloin direct from aloe juice.

The methods to be found by reference to REMINGTON (29), BARROWCLIFFE & CARR (ex (6)), and the U.S.A. DISPENSATORY (30), depend on the acid precipitation of resins from hot aqueous solutions of aloes, and the crystallisation of aloin from the concentrated filtrate.

The precipitation of aloin as the calcium-salt from the resin-freed aqueous extract of aloes is utilised by SCHAEFER (31) and LISTER & PRIDE (28).

The methods of EDER & SCHNEITER (32), and of KONDRACKI (33) utilise solvent extraction of the aloin. In the former case the aloe lump is refluxed with methanol, in the latter case it is macerated in methanol. Extraction with chloroform follows, the resins precipitating on standing, and the solution is filtered. Removal of the solvents by distillation leaves a residue of amorphous aloin.

In the case of Socotrine aloes, digestion of aloe lump in alcohol under a reflux condenser, followed by concentration of the resin-free filtrate, is sufficient to induce crystallisation of aloin (U.S.A. DISPENSATORY (30)).

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ALOIN CONTENT OF ALOES DETERMINED BY THE ABOVE METHODS.

The aloin contents of Cape aloes is given as a very low figure by older references, many of which may be discounted. Spectrophotometric methods give a far better assessment than visual colorimetry used earlier. A summary of aloin content in Cape and Curaçao aloes is given in table 2 (ii).

Table 2 (ii).

Aloin % W/W.		Method.	Reference.
CAPE ALOES.	CURAÇAO ALOES.		
1. 4.04 - 5.32	16.88 - 17.24	TILDEN	44
2. 9	25	Calcium Aloinate	18
3. 10.5 - 14	2 - 31	PARIS & DURAND	10
4. 12 - 15	-	-	28
5. 15 - 17	25 - 29	SCHAEFER	44
6a. 18.5 - 19.2	35.9 - 36.6	GOLDNER (PENTOSE)	11
b. 20.9 - 21.9	38.1 - 39.2	SEEL (PERSULPHATE)	11
c. 20.9 - 22.2	40.2 - 41.4	LÉGER (CHLORINATION)	11
d. -	nearly 40	HARDERS	11
7. up to 20	16 - 18	-	27
8. ca 23	-	SMITH et alia	34
9. 15.16 - 24.6	-	KRAUS	10
10. 19.73 - 27.99	32.61	VAN OUDTSHOORN	10
11. 30.9 (TOTAL ANTHRAQUINONES)	32.2	AUTERHOFF & BALL	10
12. -	15 - 30	SCHAEFER	31

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The fifteen references listed in table 2 (ii) have been arranged in ascending order of the aloin content of Cape aloes. Methods 2, 4 and 5 (calcium aloinate) have been stated to give low results.

EXPERIMENTAL.

Preparation of Aloin.

The sample used was supplied by kind courtesy of Mr. VAN OUDTSHOORN, who used the method of MÜHLEMANN (17).

A brief summary of the method used by VAN OUDTSHOORN (10) is as follows:

A saturated solution of amorphous aloin B.P. in isopropyl alcohol was made with the aid of gentle heat. The fine crystals that separated on cooling were filtered off, using a sintered glass funnel, and dissolved in warm methanol. Crystallisation from methanol was repeated four times, the aloin then being sucked dry on a sintered glass funnel. The sample was dried overnight over phosphorus pentoxide in a vacuum desiccator, then dried in an oven at 60°C for two hours, followed by a period of five hours at 120°C. The melting point of the sample was 142° - 145°C (uncorrected) which is in agreement with the figure obtained by MÜHLEMANN (17). The melting point for aloin given by MARTINDALE (45) is 145°C.

Spectophotometric Analysis of Aloin.

Calibration Curve.

The spectophotometer used was a Unicam SP 600 with one cm quartz glass cells. Seven cells were used, one being reserved for the methanol blank, while each was calibrated and marked. The following concentrations of aloin in 5 ml methanol were used: 20, 40, 60, 80, 109, 120 µg.

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The extinctions were read at 355 nm and 360 nm and these appear in table 2 (iii). (The maximum peak for aloin is given at 354 nm (28) and 355 nm (10)).

Table 2 (iii).

Aloin Concentration (µg).	Extinction.	
	355 nm.	360 nm.
20	0.122	0.122
40	0.244	0.244
60	0.326	0.328
80	0.429	0.428
109	0.572	0.577
120	0.629	0.633

ROGERS (46) demonstrated the importance of narrow slit apertures in spectrophotometric determinations. Consequently it was decided to work at 360 nm, because work at 355 nm entailed a maximum aperture. The graph of the readings at 360 nm was plotted, and appeared to be a straight line in accordance with Beer's Law. This graph was used for all subsequent determinations.

Chromatographic Separation of Aloin.

Thin layer chromatography was used for separation, a modification of VAN OUDTSHOORN'S (10) method being employed for analysis.

Preparation of Plates.

Silica Gel (MERCK) three g was mixed with eight ml distilled water, and spread on plate glass chromatographic plates 5" x $3\frac{1}{2}$" x $^{3}/_{16}$".

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The quantity mentioned is sufficient for three such plates. These were allowed to dry in air. Plates of these specifications were used throughout.

Activation of Plates.

Before use the prepared plates were placed in an oven at 105°C for thirty minutes (STAHL) (47), preferably immediately before use, since on standing the silica gel takes up moisture, with consequent raising of the Rf of aloin. Aloin solutions were applied to the activated plates, which had been cooled by standing on glass.

Application of Solutions.

In all experiments methanol was the solvent for aloin, aloe juice or lump. A micro-pipette (as per GERRITSMA & FREDERICKS) (48) of volume circa 0.005 ml was calibrated accurately.

The sample to be analysed was dissolved and made up to five ml with methanol, and contained between thirty to eighty µg aloin (corresponding roughly to 0.2 - 0.4 g aloe lump or 0.4 - 0.8 g aloe juice). This avoided analytical error at the extremes of the graph (KOLTHOFF & SANDELL) (49). These solutions were placed one cm from the bottom of the plate.

The micro-pipette was placed lightly on the silica gel plate to prevent movement of the powder, and allowed to drain. Both before and after use, and in between applications of the same or different solutions, the micro-pipette was washed three times with methanol, and allowed to drain on to filter paper.

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Development.

The methanol from the aloin solutions was allowed to evaporate, and the plate placed in a solvent tank closed with a glass top. The plates were placed face forward at an angle (i.e. back to back) in the small tank, and allowed to run for forty to fifty minutes (ca. four and a half inches to five inches of the solvent front). The plates were placed that way to ensure easy removal, and no loss of silica by knocking or scraping. The SOLVENT used in all cases was chloroform: ethanol 95% (3 : 1 v/v).

Detection.

Many of the principles of aloe fluoresce brightly under ultra-violet light. Aloin fluoresces orange, in contrast to the blues, greys and browns of other components, and the red of aloe-emodin. It is thus easily identifiable, and once identified, the use of aloin as control is unnecessary.

Quantitative Estimation of Aloin.

Samples of aloe lump or juice were used. Aloe lumps (ca. 0.3g) was finely powdered in a mortar, accurately weighed, dissolved in methanol and made up to 5 ml. Three dots only were applied to each plate to avoid contact on development, using the technique described previously.

After development, the plate was removed and dried in air. The plate was then placed under an ultra-violet lamp and the powder above and below the aloin scraped carefully and completely away, leaving only the three aloin dots joined by silica gel.

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Each aloin dot in turn was then scraped carefully onto a clean sheet of glossy paper and transferred completely to a numbered centrifuge tube, the extra silica gel having no effect on the determination. Methanol 5 ml was pipetted into each centrifuge tube, which was then centrifuged, the supernatant being filtered through Whatman 542 paper direct into the glass cells. These were then examined in the dark under a reading lamp for Tyndall effect or stains on the glass. Triplicate readings were then made in the spectrophotometer at 360 nm, using methanol as blank. These readings were then averaged.

(It had been found that centrifuging alone was insufficient to remove the Tyndall effect from the solution in the cells, hence filtering was resorted to, and further it was ascertained that no aloin was lost by adsorption onto the filter paper).

Reproducibility of Results.

To verify the reproducibility of results by this method, samples of aloe juice were analysed, and to each was added a known amount of crystalline aloin. Results of the analyses appear in table 2 (iv).

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Table 2 (iv).

Wt. of aloe juice.	% Aloin w/w in juice.	Aloin added.	Theoretical Content. (µg)	Actually Found (µg).	Percentage Recovery.
1. 0.6098g	11.3	0.0147g	62.7	62.2	99.2
2. 0.4645g	12.4	0.0142g	80.6	80.0	99.3
3. 0.9790g	9.4(5)	0.0130g	94.9	97.0	102.2
4. 0.3520g	12.4(5)	0.0100g	80.7	82.0	101.6
5. 0.4633g	10.8(3)	0.0198g	84.0	86.2	102.6

These figures reflect an analytical error of circa 3%. This compares favourably with the results obtained by VAN OUDTSHOORN (10). The high figure recorded for samples three to five is due largely to a light blue halo that surrounds the aloin dot in this species. The junction of the two is so intimate that complete removal of the blue is impossible without aloin loss. In some instances this blue halo spreads across and above the aloin (at high Rfs).

In samples one and two the aloin lies just above a brown dot. At low Rfs this and the aloin merge, causing irregular results. The activated plate is thus best when left thirty minutes before development.

Summary of this chapter.

Biological and chemical assay methods used for aloes are discussed and results thereof tabulated. Details of the micro-analytical chemical method used here are given, and the limits of accuracy of this method are stated.

CHAPTER 3.

EXPERIMENTAL.

ALOIN VARIATION IN LEAVES OF THE SAME PLANT.

In this chapter variations in aloin content of the leaves of the same plant are discussed under the headings of

(a) Aloin content in relation to leaf distribution.

(b) Aloin content in relation to the time of day. (diurnal)

(c) Aloin content on successive days.

(d) Aloin content in relation to the month of the year. (seasonal).

(e) Weather factors and aloin variation.

(a) Aloin variation in relation to leaf distribution.

It was decided that before analyses could be undertaken to determine diurnal and seasonal variations of aloin in aloe plants, it was essential to establish whether variations occurred

i) due to the age of the plant

ii) in leaves selected from various levels on the same plant

Due to the difficulty encountered (even by botanists and horticulturists) in attempting to establish the age of the various aloe plants employed, the relative terms "very young plant," "young plant" etc., are accompanied where possible by the approximate height of the plant and/or leaf size.

Two aloe plants from a small park in Port Elizabeth (called Fort Frederick) were studied. The first plant (P.E. i) stood about six feet high, and had a number of withered leaves at the base. The bottom leaf was cut off from those just above the withered leaves, the middle leaf

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was six leaves higher and the top leaf was likewise six leaves higher than the middle leaf, and at the extreme top of the plant.

Three further samples were taken from this same plant at the same levels four days later. Heavy rain had fallen two days prior to this second collection. (In all the cases cited in this chapter, analysis occurred within two days of collection.)

Results of these analyses appear in table 3 (i).

Table 3 (i).

Leaf Collection (Before rain)	Weather	Leaf diameter (Widest part)	Aloin %W/W Fresh sap	Aloin %W/ Dried sa
Top 10a.m. on 25/8/62	Sunny and	2 inches	9.1	20.6
Middle "	clear	$4\frac{1}{2}$ inches	10.4	23.4
Bottom "		5 inches	8.3	19.6
(After rain)				
Top 9.30a.m. on 29/8/62	Sunny with	2 inches	7.9	18.3
Middle "	cold wind &	$4\frac{1}{2}$ inches	9.0	20.5
Bottom "	stratus cloud	$4\frac{1}{2}$ inches	7.3	17.9

From table 3 (i) it will be seen that the middle leaves contained the highest percentage of aloin. It will be shown later that this is not always the case.

In table 3 (ii) the ratio of aloin percentage before and after rain is compared, and it is interesting to note that although the aloin percentage has fallen after rain, the distribution of moisture intake throughout the plant seems remarkably consistent.

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Table 3 (ii).

Leaf	Aloin % W/W	Ratio	Aloin % W/W	Ratio
Top	9.1 / 7.9	1.15	20.6 / 18.3	1.12
Middle	10.4 / 9.0	1.15	23.4 / 20.5	1.14
Bottom	8.3 / 7.3	1.14	19.6 / 17.9	1.09

The second aloe (P.E. ii) stood about six feet high and some twenty feet from the above-mentioned (P.E. i) aloe. On this occasion the 3 leaves were selected from the same level on the plant, two being adjoining leaves, one of which had white scale covering about half of the leaf surface. Results of the analyses appear in table 3 (iii).

Table 3 (iii).

Collection	Weather	Fresh sap Aloin % W/W	Dried sap Aloin % W/W
4 p.m. on 15/8/62	Overcast with cool breeze	9.5	22.3
11 a.m. on 18/8/62	Sunny and hot	10.3	20.3
Ditto (diseased leaf)	ditto	10.3	22.8

(At a later date (14/1/63) it was possible once again to analyse both a healthy and a diseased leaf from the same plant mentioned above. Unfortunately the diseased leaf was two leaves lower, but once again the percentage aloin in the dried juice was higher, viz.,

Healthy leaf 9.1% W/W and 18.3% W/W (fresh and dried sap resp.)
Diseased leaf 9.6% W/W and 21.5% W/W

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Two plants from the village of Redhouse, ten miles from Port Elizabeth were selected for analysis. These were both young plants, one of which (Rii) stood only 18 inches high. In the case of the taller plant (Ri), which stood three feet six inches high, the middle leaf was placed five leaves up from the bottom leaf, and the top leaf taken was six leaves above this. In the case of the very small plant a distance of four leaves was observed in each case. Results of this analysis appear in table 3 (iv).

Table 3 (iv).

Leaf	Collection	Weather	Leaf diameter	Aloin % W/W Fresh sap	Aloin % W/W Dried sap
(Ri)					
Top	3 p.m. on 1/9/62	Hot but	$2\frac{1}{4}$ inches	8.3	19.7
Middle	ditto	overcast	$3\frac{1}{2}$ inches	6.6	14.2
Bottom	ditto		$3\frac{1}{4}$ inches	9.5	19.8
(Rii)					
Top	3.15 p.m. on 1/9/62	Hot but	$1\frac{1}{2}$ inches	7.4	17.3
Middle	ditto	overcast	$2\frac{1}{2}$ inches	6.2	13.5
Bottom	ditto		2 inches	4.5	10.4

Thus the highest percentage of aloin was in the bottom leaf of (Ri) and in the top (youngest) leaf of (Rii).

Summary.

From this it appeared that variation occurred in aloin content in leaves of the same plant, and followed a random distribution. However,

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content variation appeared of a lower order in leaves on approximately the same level in the plant, and hence sampling for the following series of experiments was made, as far as possible, from leaves at the same level.

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b) Diurnal Variation.

The plant selected for this experiment grew in a Municipal Garden in Port Elizabeth. This plant was approximately three feet tall.

Initially, three leaves were collected from a certain level on the plant, to verify the preceding work. This level was obtained by counting downwards seven leaves from the top leaf. Two of the leaves were at the same level, and the third was at the base of the junction of these two. Results of the analyses appear in table 3 (v).

Table 3 (v).

Leaf	Collection Time	Weather	Aloin % W/W Fresh sap	Aloin % W/W Dried sap
1	8 a.m.	Hot & sunny with	14.0	27.6
2	8 a.m.	a light	13.6	26.7
3	8 a.m.	breeze	12.8	25.8

These figures bore out the previous assertion that minimal variation occurs at the same level, but the variation is sufficient to create difficulty in the assessment of aloin percentages when time of collection varies, but the variation is of a small order. Four days later, five further samples were taken at various times of the day from the same plant. These were taken at

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the same approximate level as the previous three samples, and the height of stem denuded by the loss of these eight leaves was about two inches, showing how relatively similar the level was. It is not possible to summarise briefly the weather changes that occurred that day, and they are consequently omitted from table 3 (vi). The weather was as follows:

From 8.30 a.m., when the first sample was taken, the weather was sunny with a cool breeze. These conditions continued, with increasing temperature, until 3 p.m. At 5.30 p.m. the temperature was still high, but the sky was almost completely overcast with rain clouds, and a cool, gusty wind sprang up. Rain fell soon after, and continued intermittently. Although it was showering lightly at 7.15 p.m. when the last sample was taken, the sun shone through just above the horizon.

The results of these analyses appear in table 3 (vi).

Each leaf was between $3\frac{1}{4}$ - $3\frac{3}{4}$ inches broad at its widest point, weighing between 330 and 420 g.

(Table 3 (vi) appears overleaf).

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Table 3 (vi).

Leaf	Collection Time	Temperature	Wind	Aloin % W/W Fresh sap	Aloin % W/W Dried sap
4	8.30 a.m.	22.2°C	Average 13 m.p.h. E.N.E. Gusts 17 m.p.h.	14.6	27.8
5	11.00 a.m.	26.0°C	Average 6 m.p.h. E.N.E. Gusts 10 m.p.h.	14.2	26.1
6	2.00 p.m.	29.3°C	Average 9 m.p.h. Easterly Gusts 13 m.p.h.	15.1	28.3
7	5.00 p.m.	28.4°C	Average 21 m.p.h. S.W. Gusts 32 m.p.h.	12.3	22.2
8	7.15 p.m.	23.0°C	Average 19 m.p.h. W.S.W. Gusts 35 m.p.h.	14.7	27.7

Thus, with the exception of the 5 p.m. sample, the above figures appear relatively constant, bearing in mind that a slight variation occurs even at the same approximate level. There is no explanation for the low percentage of the 5 p.m. sample, but it does coincide with a rapid and severe change in the weather. This, however, may have no bearing whatsoever.

Thus the average for the eight samples is 13.9% W/W in the fresh sap and 26.5% W/W aloin in the dried sap.

The Standard Deviation of the latter figure (26.5%) is $\pm$ 1.9%, giving limits of 24.6% to 28.4%. Since four of the five leaves analysed for the diurnal test fall within these limits, it appears that relatively no significant variation has occurred in aloin content throughout the day.

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c) Variation on Successive Days.

Analyses were performed on a plant (Ford Frederick) on three successive days. Results of these analyses appear in table 3 (vii). Light showers fell on the first day, and it was wondered if this would have any effect on the subsequent days, since heavy rains appear to lower aloin content. However, this appears not to be the case, as will be seen from table 3 (vii). (All 3 samples were collected at 11 a.m., and analysed on the day of collection).

Table 3 (vii).

Date	Weather	Aloin % W/W in	
		Fresh sap	Dried sap
11/6/1963	Showery, cool but windless	10.7	21.6
12/6/1963	Sunny with cold breeze	11.5	21.6
13/6/1963	Sunny and warm, with thin cloud.	10.8	21.3

Thus aloin content does not appear to change radically from day to day over a short period of time. (The leaves, once again, were taken from the same level.)

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d) Seasonal Variation.

A sample was taken each month from the same aloe (Fort Frederick) and to obtain uniformity, the time of collection was 11 a.m. (except September 1962, which was 9 a.m.) The sample was collected in the approximate middle of the month and leaves were taken (as closely as possible) from the same level on the plant.

Results of the analyses of these samples appear in table 3 (viii) while comparisons of monthly aloin content and various weather factors appear in tables 3 (ix) & (x).

Records were kept of the weather at time of collection, but on final analysis, the weather at the actual time of collection seems to have no bearing on the final picture, and details are consequently omitted here. All collection days were sunny and warm, although November to February were hot, humid and overcast. The collection day in April 1963 was the only exception, being cold, windy and overcast.

(See Table 3 (viii) overleaf)

Table 3 (viii).

Month	Collection Date	Aloin % W/W in Fresh sap	Aloin % W/W in Dried sap
July (1962)	16th	8.1	14.8
August	18th	10.3	20.3
September	14th	8.0	19.1
October	13th	10.4	21.9
November	10th	12.5	24.1
December	13th	11.8	22.7
January (1963)	14th	9.1	18.3
February	13th	11.3	23.5
March	13th	10.8	20.8
April	13th	11.3	22.1
May	13th	8.9	16.4
June	13th	10.8	21.6

A graph of these percentages appears in FIGURE 2.

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e) Weather Factors and Aloin Variation.

Figures of average temperatures, pressure, rainfall etc., (supplied by kind courtesy of the Port Elizabeth Meteorological Office) have been divided for convenience into two sets, and appear in tables 3 (ix) & (x). In each table the monthly aloin percentage is repeated for rapid correlation.

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Table 3 (ix).

Month	Aloin % W/W in Fresh sap	Aloin % W/W in Dried sap	Average Temp. °C MAX.	Average Temp. °C MIN	Rainfall (MM)	Average pressure (millibars)	Relative Humidity %
July (1962)	8.1	14.8	20.4	8.7	17.3	21.2	71
August	10.3	20.3	20.2	9.2	91.5	18.8	77
September	8.0	19.1	19.1	10.0	22.9	19.1	76
October	10.4	21.9	20.8	13.3	61.3	no record	78
November	12.5	24.1	22.2	14.5	38.7	15.9	96
December	11.8	22.7	24.9	16.9	31.5	18.2	76
January (1963)	9.1	18.3	25.4	18.2	121.7	14.3	84
February	11.3	23.5	25.1	17.8	5.5	13.9	82
March	10.8	20.8	23.0	15.3	87.6	17.6	86
April	11.3	22.1	21.0	12.7	100.5	17.8	85
May	8.9	16.4	21.9	11.0	58.7	17.6	77
June	10.8	21.6	20.3	8.9	10.4	20.8	78

Table 3 (x).

Month	Aloin % W/W in Fresh sap	Aloin % W/W in Dried sap	Average wind speed (M.P.H.)	Monthly Wind Hours.	Monthly calm Hours.	Evaporation (inches)
June (1962)	-	-	-	585	135	3.78
July	8.1	14.8	8.4	611	133	3.83
August	10.3	20.3	8.7	614	130	4.46
September	8.0	19.1	11.6	645	75	5.41
October	10.4	21.9	11.3	654	90	7.01
November	12.5	24.1	12.2	644	76	8.56
December	11.8	22.7	11.8	640	104	11.44
January(1963)	9.1	18.3	12.3	684	60	9.58
February	11.3	23.5	9.8	574	98	7.75
March	10.8	20.8	9.4	609	135	5.72
April	11.3	22.1	6.9	494	226	4.46
May	8.9	16.4	7.7	562	182	3.59
June	10.8	21.6	7.3	567*	153	2.63

* The month was relatively calm until 20th June, i.e., after analysis of the sam

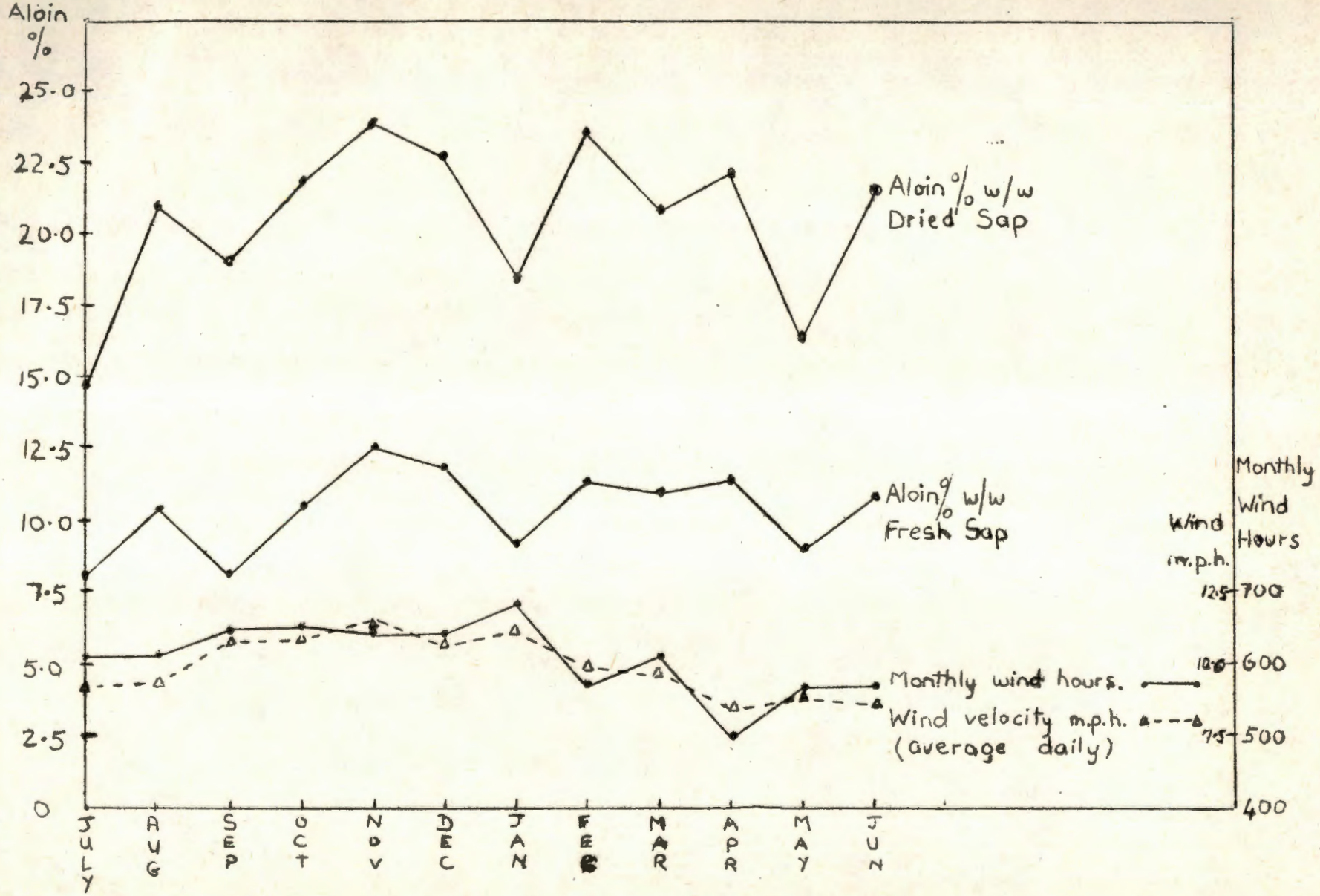
Aloin %
25·0
22·5
20·0
17·5
15·0
12·5
10·0
7·5
5·0
2·5
0
Aloin % w/w Dried Sap
Aloin % w/w Fresh Sap
Monthly wind hours.
Wind velocity m.p.h. (average daily)
Wind m.p.h.
12·5
10·0
7·5
Monthly Wind Hours
700
600
500
400
JULY
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN

FIGURE 2

Discussion.

Temperature: The temperature graph plotted from the figures in table 3 (ix) rises in a curve with a peak in January, whereas the aloin graph (fig 2) is also a curve whose peak is in November, six months after the plant flowers. The two curves are thus similar, but do not coincide. With the exception of January month (see later under Wind) the curves show a close parallel from September to May. Apart from temperature effect, long exposure to direct sunlight causes the aloe leaf to turn red. This is due to the formation of carotin within the chloroplasts. (HAAS & HILL) (50).

Rainfall: No correlation could be obtained between rainfall and aloin percentage. Heavy rain just before analysis can cause an aloin decrease, while light showers have no effect. Daily rainfall figures for the year were kept, and figures from one analytical date to the next were totalled, but still no correlation was apparent. The heavy rainfall that fell in January (121.7 mm) actually fell in the ten days after analysis of the January sample, (which was lower than expected), so could have had no effect on the result.

Relative Humidity: Appears to have no effect. The graph is almost a straight line with a slight peak in November.

Atmospheric Pressure: Appears to have no direct bearing.

Evaporation: This graph rises regularly to December 1962, then falls regularly to June 1963.

Wind: Since wind seems to have a direct bearing on aloin content, the relevant figures have been listed separately in table 3 (x). Under temperature it was mentioned that the graph of monthly aloin content (fig 2) followed

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a curve, rising from July to November, then falling gradually to June. The graph of wind hours plotted against monthly aloin content (fig 2) shows how the fall in aloin for the months of September, January and May in each case coincides with an increase (usually sharp) in the graph of wind hours, and that increases in aloin content frequently coincide with decrease in wind hours.

Wind figures for the day prior to and of analysis were studied for several of the months, and seemed to have no effect. It thus seems a long-term effect, caused possibly by the movement of, or physiological changes in, the plant.

Many authorities state that wind shrinks the leaf, reducing sap flow. In at least three instances this writer has collected samples during windy conditions and found no significant difference in moisture content from the normal, although the sap was slow running in the windy months. WHITEHEAD(15) subjected growing plants to strong winds in a wind tunnel and showed that the stronger the wind, the smaller the leaf area and internode length produced, and the greater the amount of woody and conductive tissues in both stem and leaves. This shrinking of the leaf by wind may effect the aloin-producing cells, situated just under the outer cuticle, and this would account for aloin decrease during windy periods.

Unfortunately little seems to have been recorded on the growth or climatic requirements of aloes, with the exception of the passage on cultivated aloe quoted below (ELIOVSON 51)

Aloes planted in the garden need a certain amount of water to store in the leaves, and also need a period of rest (4 - 6 months) without water.

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Those with summer rain need a dry winter and vice versa. However, aloes are remarkably adaptable, some species growing in regions of 5 inches per annum, others obtaining 100 inches. Aloes need a well-drained soil with compost (i.e. loamy soil, but not manured). Only the roots must be covered or the plant will rot.

Conclusion.

The aloin content of Aloe ferox rises from July to November, falling again from March to June. The low point on the graph coincides with both the flowering period and winter, either of which may have an effect. Wind seems to have a significant effect on aloin production, being inversely proportional to aloin percentage. (Note: the monthly chromatographic picture remained unaltered for the whole year.)

Summary of this Chapter.

Aloin content varies in leaves of the same plant, and follows a random distribution. However, the variation is least in leaves on the same approximate level. Aloin content appears to vary little throughout the day, or on successive days over a short period. It does however increase monthly, the peak being reached in November, and this graph is inversely affected by wind (duration and velocity).

Heavy rain causes the aloin content of the sap to fall. The chromatographic picture of this sample remained unaltered throughout the entire year.

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CHAPTER 4.

GEOGRAPHICAL FACTORS AND ALOIN VARIATION.

Aloin determinations were performed on both aloe juices and commercial lump aloes collected from a large area of the Cape, and the results studied to see if any correlation existed between geographical distribution and aloin content of the samples. Furthermore, altitude of the district of collection was taken into account.

Most of the aloe juices and aloe lump were from a commercial source, * and many of the samples of aloe juice were already many months old when supplied, and hence some aloin loss had occurred. As a rule, the juice is boiled within two to three days of collection. A 44-gallon drum yields on boiling over a wood fire between six and seven 4-gallon paraffin tins of lump aloe, each tin having an average weight of 54 lb nett. These figures apply to juice collected in dry weather when the juice is more viscous. After rain, a 44-gallon drum yields little over five 4-gallon tins. The aloin destruction by this method of boiling is high (see chapter 7).

* Footnote: I am indebted to the late Mr. du Preez of the *Aloe ferox* Company, Port Elizabeth, for many of the commercial lump and juice samples used.

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This chapter embraces samples of commercial lump and/or juice from the following regions:

Riebeeck East
Grahamstown
Alicedale (Groenheuwels)
Sandflats
Kirkwood
Loerie
Coega
Fort Beaufort
Somerset East
Winterhoek - Steytlerville district
Mossel Bay
Oudtshoorn
Lake Mentz.

In addition, freshly collected samples, collected personally from the following places, Port Elizabeth.
Redhouse
Mossel Bay
Oudtshoorn.

were analysed within two days of collection. The latter four samples are described first, results of analyses thereof appearing in table 4 (i).

Port Elizabeth Samples.

These samples were collected for the determination of aloin variation in leaves of the same plant. (See chapter 3 for description of the aloe plant.) Portion of the juice was dried to constant weight at 105°C, and the aloin content of the juice and the dried juice expressed as a percentage weight-inweight. (% W/W). Results are given in table 4 (i).

Redhouse.

These samples, collected at Redhouse, 10 miles from Port Elizabeth,

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were the same as those used for the determination of aloin variation in leaves of the same plant, and description of the plant appears in chapter 3.

Oudtshoorn.

Sample A. This sample was collected from the centre of Oudtshoorn from the same type of A.ferox as found in Port Elizabeth. Chromatographically, a striking similarity between the two samples was found. The juice was extremely watery, and orange brown in colour. This sample contained well above the average of water content, and rain had probably fallen prior to collection.

Sample B. This sample was collected from a very thorny aloe about 20 miles from Oudtshoorn (at Rus en Vrede). The plant may have been a mixed hybrid of A.Marlothii, A.africana and A.ferox, as the chromatogram of the juice showed the double orange dot found in africanahybrids. The juice was fairly viscous, brown, and gave a yellow deposit. Results of aloin determinations appear in table 4 (i).

Mossel Bay.

This plant was thornier than the Port Elizabeth sample, while the juice was golden brown and viscous.

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Table 4 (i).

Geographical Source	Samples	Aloin % W/W in Fresh Juice	Aloin % W/W in Dried Juice
Port Elizabeth	Mixed aloes from 3 (top, middle & bottom leaves)	9.3	21.3
"	Mixed aloes from 2 middle leaves	10.2	25.7
"	Six leaves collected monthly (average)	10.2	20.4
Redhouse	Sample A. Mixed aloes from 3 (top, middle & bottom leaves)	8.1	17.9
"	Sample B. ditto	6.0	13.7
Oudtshoorn	A	4.1	21.5
"	B	4.6	16.9
Mosselbay		13.0	25.0

Commercial Samples.

Analyses of commercial aloe juice. A short description of the juice from each of the commercial regions is given, while results of the analyses appear in table 4 (ii).

Sandflats sap, collected 14/5/1962, analysed 31/7/1962. This sap was brown in colour, and very viscous.

Somerset East sap, collected 11/5/1962, analysed 31/7/1962. This sap was brown in colour, and very viscous.

Riebeeck East sap, collected 21/1/1962, analysed 5/8/1962. This had fermented slightly, was orange brown in colour, and of medium viscosity.

Alicedale sap, collected 22/6/1962, analysed 5/8/1962. This showed no fermentation, was golden in colour, and of medium viscosity.

Coega sap. Coega, some 14 miles from Port Elizabeth, is a region with many small-leafed A.africana, and hybrids occur readily.

Coega sample A, collected circa 20/8/1962, analysed 22/8/1962. This sap was reddish brown in colour, with much aloe-emodin present in the fresh sap.

Sample B, collected 10/9/1962 and analysed 20/9/1962. The sap was orange red in colour, slightly fermented, and of low viscosity. The leaf from which it was collected could have been A.africana or a hybrid thereof. Two aloin dots were found chromatographically, and were so close together that it was decided to analyse them together. The yield was consequently very high.

Sample C, collected 16-19/9/1962, analysed 20/9/1962. The sap was orange brown in colour, and viscous, with no aloe-emodin present.

Loerie sap, collected 9/3/1962, analysed 1/8/1962. This had a heavy yellow deposit. The supernatant sap had fermented considerably, and much gas escaped on opening the bottle.

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Table 4 (ii).

Geographical Source.	Date collected.	Date analysed	Aloin % W/W in Sap	Aloin % W/W in Dried sap
Sandflats	14/5/1962	31/7/1962	7.6	18.4
Somerset East	11/5/1962	31/7/1962	9.7	15.9
Riebeeck East	21/1/1962	5/8/1962	10.9	21.5
Alicedale	22/2/1962	5/8/1962	12.5	24.5
Coega sample A	20/8/1962	22/8/1962	4.5	8.5
" " B	10/9/1962	20/9/1962	13.5	34.7
" " C	19/9/1962	20/9/1962	8.3	15.8
Loerie	9/3/1962	1/8/1962	5.9	-

Analyses of commercial aloe lump.

Aloe lump samples from all regions were in small hemispherical lumps, one to two inches indiameter, externally dark brown to black with shiny surfaces. It should be borne in mind that the lump is obtained by boiling the aloe juice within a few days of collection, hence collection times and results given for the juices can be correlated with results obtained for the lump aloes.

The lump aloes still contain moisture after cooling and setting, and a determination of the residual water content was made (in duplicate) by drying the powdered lump samples at 105°C to constant weight, results of which appear in table 4 (iii).

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Table 4 (iii).

Geographical Source.	Moisture content.
Sandflats	8.0%
Somerset East	8.4%
Alicedale	7.9%
Kirkwood	8.5%
Steytlerville	7.1%
Riebeeck East	7.5%
Coega	8.1%
Grahamstown	7.4%

Average moisture content: 7.8(6)%

A summary of each powdered sample, together with its aloin content appears in table 4 (iv). The chromatograms of some samples differed from the A.ferox as found in Port Elizabeth, these differences being as follows:

Mossel Bay A. This sample showed the double-dot observed before, the upper of which corresponds to the Rf of aloin. Only the upper dot was analysed, and yielded a high percentage.

Mossel Bay B. Much aloe-emodin was present, and an unusual orange-pink dot was observed just above the aloin.

Coega B. This sample also exhibited the double dot, the upper dot corresponding in Rf to aloin. Both dots yielded the same percentage "aloin" (15.6% W/W), giving a total of 31.2% W/W "aloin-like substance."

Riebeeck East and Alicedale. These samples showed a brown dot just below the aloin.

Oudtshoorn. The dot below the aloin was grey-brown, and the dot below this (quoted by some to be resin) was yellow-grey.

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Table 4 (iv).

Region	Collection date	Analysis date	Colour of aloe powder	Aloin % W/W in Lump	Aloin % W/W in Dried Powder.
Riebeeck East	21/1/1962	26/7/1962	Yellow brown	19.7	21.3
Grahamstown	22/2/1962	30/7/1962	light brown	14.1	15.2
Steytlerville	16/3/1962	26/7/1962	olive green to brown	15.8	17.0
Kirkwood	22/3/1962	26/7/1962	reddish brown	16.7	18.2
Somerset East	11/5/1962	30/7/1962	yellowish brown	17.4	19.6
Sandflats	14/5/1962	30/7/1962	brown	13.9	15.1
Alicedale	22/6/1962	26/7/1962	olive brown	19.4	21.0
Coega A	10/7/1962	13/8/1962	dark brown	9.8	10.5
Coega B	10/9/1962	20/9/1962	pale yellow	15.6	-
Coega C	20/9/1962	20/9/1962	brown	14.6	-
Mossel Bay A	-	15/10/1962	orange yellow	26.5	28.9
Mossel Bay B	Nov. 1962	21/11/1962	olive brown	14.5	15.8
Fort Beaufort	Nov. 1962	21/11/1962	light brown	15.2	16.5
Lake Mentz	7/1/1963	27/1/1963	light brown	15.9	-
Oudtshoorn	14/1/1963	27/1/1963	olive brown	18.0	-

(The above are tabulated according to date of collection).

From the above data it would appear firstly that the colour of the powdered drug is not, in itself, indicative of aloin content, although the yellower powders appear to have a higher percentage aloin.

In chapter 3 it is shown that the month of collection can influence the aloin percentage of a sample. Bearing this in mind, and also the fact that some samples lose more aloin on heating than others (see chap. 7), the average aloin content of the fifteen samples above, collected from twelve separate regions, is 16.5% W/W, or circa 17.9% W/W in samples dried at 105°C. Although not pure A.ferox, all samples are sold as Cape aloes.

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HØRHAMMER, WAGNER AND BITTNER (67) made a comparative study of 38 specimens of Cape Aloe, and deduced that Cape aloe can be divided into two types, Type A, centred around Mossel Bay, and Type B, found east and west of Port Elizabeth. The former contain aloinoside, and have a total "aloin" content averaging between 23% - 27%, while the latter contain no aloinoside and average between 11% - 22% aloin. In table 4 (v) the range 9.8% - 19.7% was found for the latter area, while the overall range of 9.8% - 26.5% is in close agreement with that described for various workers in table 2 (ii).

The effect of altitude.

In table 4(v) are listed the altitudes and aloin percentages of the lump aloe samples described before. From this table it would appear that altitude has no effect on the aloin content of A.ferox.

Table 4 (v).

Region	Altitude (in feet)	Aloin % W/W (uncorrected)
Mossel Bay A	0-600	26.5
Riebeeck East	1200-3000	19.7
Alicedale	905	19.4
Oudtshoorn	600-3000	18.0
Somerset East	Town 2304 Region 1200-3000	17.4
Kirkwood	Town 350 Region 0-600	16.7
Lake Mentz	600-3000	15.9
Steytlerville	1200-3000	15.8
Coega B	36	15.6
Fort Beaufort	Town 1395	15.2
Coega C	36	14.6
Mossel Bay B	0-600	14.5
Grahamstown	Town 1745 Region 1200-3000	14.1
Sandflats	0-1200	13.9
Coega A	36	9.8

Thus for altitudes between 1200-3000 feet, the above samples are 19.7 17.4, 15.8 and 14.1 percent respectively, while for 0-600 feet they are 26.5, 16.7, 15.6, 14.6, 14.5 and 9.8 percent respectively. These figures show too large a deviation from the mean to suggest that altitude has any significant influence on aloin content.

In tables 4 (ii) & (iv) figures have been given for samples of commercial aloe juice and lump respectively. In certain instances both juice and the lump prepared therefrom were received from the same region, and a comparison of these appears in table 4 (vi).

Table 4 (vi).

Region	Juice % W/W	Dried Juice % W/W	Lump % W/W	Dried lump % W/W
Riebeeck East	10.9	21.5	19.7	21.3
Alicedale	12.5	24.5	19.4	21.0
Somerset East	9.7	15.9	17.4	19.6
Sandflats	7.5	18.4	13.9	15.1
Coega	4.5	8.5	9.8	10.5

Care must be taken in assessing the above figures, as most samples of sap had stood from between two and six months before being analysed, whereas the lump was boiled immediately after collection. The table gives some measure of the difference in stability of saps from different regions, and the Coega samples decomposed rapidly in the two days between collection and analysis. The Sandflats and Alicedale samples reflect the aloin loss on heating of between 3-4% aloin (i.e. circa 20% relative loss). The Riebeeck East sample showed slight fermentation, while the Somerset East sample has not only lost aloin on standing three months, but also moisture,

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since experience has shown that on drying, the aloin content of aloe sap increases by the order of 1.92 to 2.2 (i.e. contains approximately 50% moisture).

TRIM (63) states that most glycosides are destroyed rapidly when tissue damage occurs. Aloe juice in many instances is relatively stable for weeks or even months, however, with the few exceptions mentioned before. This may, apart from the structure of the aloin itself which confers stability (RAMSTAD (3) be due to the very small tissue damage which occurs, the incision being small in comparison with the large leaf area. VAN OUDTSHOORN(D) showed that a sample containing circa 20% aloin lost only 0.1% aloin over a period of four weeks. The Alicedale sample mentioned in table 4 (vi) lost only 0.1% aloin between the analyses dates 5/8/1962, to 2/12/1962, showing its remarkable stability.

Thus stability of aloe juice can vary markedly, and may possibly be influenced by infection from the outside, weather conditions, etc. Thus for analytical purposes, determinations should be made as soon after collection as possible.

VEGETATION TYPES FOUND IN THE REGIONS FROM WHICH COMMERCIAL ALOE LUMP AND/OR SAP WERE OBTAINED.

An attempt was made to see if vegetation types could be correlated with aloin production for the regions already mentioned. The classification is that of ACOCKS (64), where a comprehensive account of each vegetative region is given. Although certain vegetation areas are common to more than one aloe region, there does not appear to be significant correlation between aloin production and vegetative types. These vegetation types (Veld Types) are given in table 4 (vii).

Table 4 (vii).

Region	Veld Types
Mossel Bay	23, 46, 47
Oudtshoorn	25, 26, 70
Somerset East	38, 60
Steytlerville	25, 26 (31)
Loerie	23
Port Elizabeth	2, 23
Coega	23
Redhouse	23
Alexandria	2
Alicedale	23, 70
Riebeeck East	37, 70
Grahamstown	23, 37, 70
Fort Beaufort	21, 23

From the above it will be seen that Veld Type 23 is common to many regions, whose aloin percentages, however, vary from over 20% to below 10%. Since many aloe species are named for this Type, a summary thereof is given

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later in greater detail than the following Veld Types, which are merely named. (after ACOCKS.)

Table 4 (viii).

Veld type.	Name.
2	Alexandria Forest
21	False Thornveld of Eastern Cape
23	Valley Bushveld
25	Succulent Mountain Scrub
26	Karroid Broken Veld
31	Succulent Karroo
37	False Karroid Broken Veld
38	False Central Lower Karroo
46	Coastal Rheinosterbosveld
47	Coastal Macchia (Fynbos)
70	False Macchia

Veld Type 23 - The Valley Bushveld.

Found in the valleys draining the rivers that drain mostly into the Indian Ocean. These valleys are hot and receive less rain than the intervening ridges, receiving from 20 to 35 inches per annum. Type 23 includes the important aloe regions of the Fish River Scrub (23c), the Addo bush and Sundays River scrub (23d) and the Gouritz River Scrub (23e).

The Fish River scrub area ranges in altitude from 300 to 1500 feet, is very hot and has 13 to 20 inches of rain per annum (Distribution: A.ferox, 1230 per morgan; A.striata, 557 per morgan).

In the Sundays River area tall aloe species are conspicuous, e.g. A.ferox, A.speciosa, A.africana, A.pluridens and A.lineata.

Elevation ranges from 0 to 1500 feet above sea-level, and rainfall from

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10 to 20 inches per annum, spread over the year. (Distribution: A.ferox 855 per morgan, A.speciosa 68 per morgan.)

The Gouritz River scrub variation occurs in the Gouritz, Little Brak and Great Brak river valleys. It is closely related to the Sundays River scrub except that the big scrubby and arborescent Euphorbia species are replaced entirely by tall aloe species (A.ferox, A.speciosa and A. arborescens).

Below Herbertsdale and around Riversdale this veld type may become replaced by groves of A.ferox.

In addition to the aloes named above, A.ferox, A.arborescens, and A.speciosa are named for various regions, as listed in table 4 (ix).

Table 4 (ix).

Species				Region	(ACOCKS) Ref. Page.
A.ferox	A.arborescens	A.speciosa	A.saponaria		
x	x	x	-	Gouritz River-Valley	84
x	-	x	-	Cookhouse to Cradock	85
x	x	-	x	Coastal Rheinoster-bosveld	126
x	-	-	-	Little Karroo	90
-	x	-	-	Eastern Drakens-berg	120
-	x	-	-	Natal	148

x = present.

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SOIL GROUPS OR SUB-GROUPS FOUND IN THE REGIONS FROM WHICH COMMERCIAL ALOE LUMP AND/OR SAP WERE OBTAINED.

The classification is taken from VAN DER MERWE (65). Comparison of the Soil Type and Region is given in table 4 (x).

Table 4 (x).

Region	Soil Type
Mossel Bay	30, 31, 32, 33
Oudtshoorn	30, 37, 38
Somerset East	42
Steytlerville	38
Loerie	29, 30
Port Elizabeth	29
Coega	29
Redhouse	29
Alexandria	28
Alicedale	27, 28
Riebeeck East	27, 28
Grahamstown	27, 28
Fort Beaufort	26, 27

Since Soil Types 27, 28, 29 and 30 are common to many regions, they are given in table 4 (xi).

Table 4 (xi).

27	Portions of Albany, Peddie, King-williamstown, East London, Willow-vale, Elliotdale, etc.	Brown sandy loams and brown loams underlain by gravel and heavy sub-soils, clay loams in vleis. The soils are often shallow.
28	Portions of Peddie, Bathurst, Alexandria, Uitenhage.	Brown sandy soils on heavy sub-soils, gravelly soils and brown loams, not usually deep, resting on rock and secondary formations.
29	Portions of Uitenhage and Port Elizabeth.	Fine sandy loams and loams, some-times with secondary formation; brown to greyish brown heavy loams.
30	Portions of Humansdorp, Knysna, Uniondale, George, Mossel Bay.	Brown fine sandy loams; sandy loams and loams; heavy alluvial soils in vleis.

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Complete description of each of the soil types may be found in VAN DER MERWE, (65) along with soil analyses which unfortunately exclude the rough triangle occupied by the above-mentioned regions. Details are curtailed here, since correlation between aloin production and soil types is difficult without details of the chemical nature of the soils.

As soil composition may well have an effect on aloin production, a short summary of the above appears in table 4 (xii).

Table 4 (xii).

Region	Soil Type
Mossel Bay	Shales, sands, clays & limestone
Port Elizabeth	ditto.
George (near Mossel Bay)	Acid igneous rock
Oudtshoorn	Sands, clays & limestone
Somerset East	Sandstone & shale
Steytlerville	ditto.
Fort Beaufort	ditto.
Grahamstown	ditto, but mainly sandstone
Alexandria	Sand & limestone.

SUMMARY OF THIS CHAPTER.

The aloin content of Cape lump aloes is circa 18% and the moisture content is circa 8%. Altitude has no apprent effect on the aloin content of aloe plants, while insufficient evidence is available to determine the effect of soil on aloin production. The stability of fresh aloe juice varies from region to region, but it is generally stable for one to four months with little aloin loss.

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CHAPTER 5.

ALLIED SPECIES AND HYBRIDS.

In this chapter an account is given of the Aloe species recognised by various pharmacopoeias, and a summary is given of certain species and their local usage. Chromatograms of several species are reproduced, and the quantitative determination of aloin in two species other than A.ferox is given.

There seems to be some diversity of opinion as to which Aloe species form(ed) the aloe of commerce, and the pharmacopoeias of different countries vary in their definition of Cape aloes.

From the work performed it would appear that relatively few aloes contain aloin, but perhaps the main criterion of the value of an Aloe species should not so much be the presence of aloin, but the existence of a purgative action. This may be due in part to the resins of aloe or to as yet unidentified oxymethylanthraquinones. However, until pharmacologic studies of the various aloe species have been made, the aloin content remains one of the most important factors. According to RAMSTAD (3) the U.S.A. consumes about 45,000 Kg of aloin (U.S.P.) annually, this being produced from fresh juice on the island of Aruba.

Apart from aloin, however, lump aloe is still in great demand. In 1952 the U.S.A. imported 413,847 lbs. mostly from South Africa (UNITED STATES DISPENSATORY) (52). However, with the competition from Aruba, which now supplies 90% of the United States annual quota of 350,000 Kg (RAMSTAD (3), South Africa is losing a valuable market for its 500,000 Kg,

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produced annually. As HODGE (2) has stated, South Africa abounds in aloes apart from A.ferox, which not only might yield more aloetic juice, but also a superior drug product. In consequence, several species have been screened in this chapter, results thereof appearing under EXPERIMENTAL WORK.

It is interesting to note the various species of aloe that have been quoted as possible sources of Cape aloes. VIEHOVER (1935) (27) lists the pharmacopoeias at that time accepting A.ferox as the official drug, these being the pharmacopoeias of the United States, Belgium, Britain, France, Germany, Greece, Italy, Mexico, Norway, Spain, Sweden and Switzerland. Official additions to A.ferox were A.africana (U.S.A.) A.spicata (French) and A.spicata and A.arborescens (Spanish Pharmacopoeia). (REYNOLDS (4) doubts the existence of A.spicata, and is of the opinion that A.speciosa is meant.) The French and German pharmacopoeias also recognised "various African species", the British Pharmacopoeia "various Cape aloes".

Certain recognised textbooks of pharmacognosy vary in the type of Aloe used to produce Cape aloes. Thus WALLIS (53) and DENSTON (54) name A.ferox, while YOUNGKEN (5) and TREASE (6) name A.ferox, and its hybrids with A.africana Miller and A.spicata Baker. (speciosa)

Apart from the above, WILSON & GRISFOLD (55) name A.spicata and A.arborescens. The latter is mentioned by WATT & BREYER-BRANDWIJK (9) as a source of Cape aloes, along with A.plicatilis and A.ferox.

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A.arborescens was listed as long ago as 1847 in GRAY'S SUPPLEMENT TO THE PHARMACOPOEIA (1847) (56) along with two that have since fallen into disuse, namely A.commelyni and A.mitriformis. REYNOLDS (4), however, states that the farmers of Mossel Bay area found A.arborescens useless for the purpose of producing Cape Aloes. (This is not in agreement with the writers' findings, as is shown later). The STATE PHARMACOPOEIA OF THE U.S.S.R. 8TH ED. (57) allows the use of A.arborescens in the preparation of galenic products, while the U.S. Dispensatory (25th edition) mentions that it is said to have been cultivated for the production of Barbadoes aloes.

HYBRIDS. With the few exceptions mentioned before ((5) & (6)) it will be seen that most sources state a definite species of Aloe, albeit these differ from country to country. However, some of the hybrids of A.ferox with species such as A.speciosa, A.africana, A.arborescens, (to name but a few) so closely resemble A.ferox that they are unwittingly picked for aloe production. In the writer's opinion the samples of aloe from Coega, Mossel Bay and Oudtshoorn mentioned in Chapter 4 were from hybrids, whose chromatograms showed characteristics of the juice of A.ferox.

The BRITISH PHARMACEUTICAL CODEX 1959 (58) concedes that Cape aloes is from A.ferox and possibly from hybrids of A.ferox with other species. In this it differs from the CODEX 1934 (59) which merely stated A.ferox Miller. The UNITED STATES DISPENSATORY (52) goes further and states "there is little doubt that in many cases a commercial aloe exported from one country is the product of the leaves of several

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species." Thus it would appear that lump aloes as it appears on the market may consist of more than one species, of mixed species, or of hybrids of species.

Local usage of certain South African Species.

Several species of Aloe are used by the African tribes of South Africa as remedies for a number of ailments. The Zulus use a decoction of A.arborescens leaf to assist parturition, while a cold infusion is used as a drench in the treatment of sick calves. It has also been used to treat X-ray burns WATT & BREYER-BRANDWIJK (9). SMITH (60) names A.saponaria as perhaps the best plant for healing inflamed wounds, while it is also useful in severe burns. Its use as a ringworm remedy is understandable when one considers the use of chrysarobin for skin ailments. Recently Recently VAN OUDTSHOORN (68) has shown that A.saponaria contains chrysophanol, a constituent of chrysarobin. A.macracantha Bak, said by REYNOLDS (4) to be, in all probability, A.saponaria Haw, is used as an enema in feverish colds. Apart from its use as a purgative, A.ferox is used in opthalmia, and to rid cattle of ticks, while A.tenuior is used as a tapeworm remedy, the root being used in this case WATT & BREYER-BRANDWIJK (9)

WATT & BREYER-BRANDWIJK (61) have reviewed the literature on aloes extensively. Recently several Aloe species have been screened for antibacterial effect, but have proved negative, or have required a high percentage for inhibiting bacterial growth. Examples of the former are A.saponaria, A.ferox, A.microstigma, A.speciosa, A.tenuior, A.arborescens and A.africana, while A.arborescens, A.saponaria and A.ferox, in high concentration, have inhibited Mycobacterium tuberculosis in vitro.

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In the case of A.ferox the action is ascribed mainly to the resin content. Chromatograms of these species may be found after page 63, . in figures 3 to 9.

EXPERIMENTAL WORK.

Aloe species may conveniently be divided into three broad groups:

(a) Arborescent types, i.e. having an obvious trunk topped by leaves.

(b) Stemless types, i.e. with leaves in a rosette on the ground.

(c) Scrambling types, i.e. having a long thin stem with leaves along the entire stem.

The different species analysed in this chapter appear in table 5 (i), where they are classified under the above headings for convenience. Those marked with a cross are very common and widespread in the Cape.

Table 5 (i).

Arborescent.	Stemless.	Scrambling.
x A.ferox	x A.saponaria	A.ciliaris
x A.africana	x A.striata	A.gracilis
x A.speciosa	A.microstigma	A.tenuior
x A.pluridens	A.humilis (var echinata)	
x A.arborescens	A.variegata	
A.lineata		

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It is not the intention to give a description of each species here, as a very full description of each can be found in REYNOLDS (4). Several species when viewed from a distance appear similar, for example A.ferox and A.africana, although the leaves of the latter are usually recurved. Similarly A.pluridens and A.arborescens appear similar, but the thorns on the leaves are closer together. A.speciosa is generally larger than A.arborescens, but from a distance appears somewhat similar. A.lineata, however, has clear lines under the leaf. A.saponaria, A.variegata and A.microstigma bear whitish spots, while the tiny A.ciliaris, A.gracilis and A.tenuior look similar to the inexperienced collector.

Far greater difficulty is experienced, however, in identifying hybrids, and generally the leaf form is not sufficient, and the plant has to be studied when in flower. REYNOLDS (4) lists the natural hybrids of several species, some of which are reproduced here:

HYBRIDS.

1. A.ferox hybridises with A.africana, A.arborescens, A.broomii, A.microstigma, A.pluridens, A.saponaria, A.speciosa and A.striata.
2. A.africana with A.ferox, A.microstigma, A.pluridens, A.speciosa and A.striata.
3. A.pluridens with A.africana and A.ferox.
4. A.lineata with A.humilis, A.arborescens and A.ferox.
5. A.arborescens forms hybrids with no fewer than 15 species, those of the Cape being A.ferox, A.glauca and A.saponaria. The remainder occur in Natal and the Eastern Transvaal.

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6. A.saponaria hybridises with A.arborescens, A.brevifolia, A.ferox, A.lineata, A.spectabilis and A.striata.

7. A.striata with A.africana, A.ferox, A.humilis (var echinata) & A.saponaria.

It is thus not surprising that chromatograms of lump aloes purported to be A.ferox differed widely. Many of the hybrids which do not resemble A.ferox, but one of the partners of which contains aloin, might well be aloin-containing themselves, and the strong possibility exists that a potential source of aloin is going untapped. Since A.arborescens appears to contain aloin, each of its fifteen hybrids might well do the same.

WATT & BREYER-BRANDWIJK (61) have reviewed the findings regarding the chemistry of certain aloe species, and the pertinent ones are listed in table 5 (ii).

Table 5 (ii).

Aloe species	Chemistry.
A.africana	oxymethylanthraquinones including aloin
A.arborescens	organic acids and purgative principle
A.candelabrum	two aloins
A.ferox	oxymethylanthraquinone derivatives, resin and aloin
A.Marlothii	organic acids and nataloin
A.plicatilis	oxymethylanthraquinone derivatives
A.saponaria	a purgative principle, probably aloin
A.speciosa	aloin
A.spicata	aloin
A.tenuior	aloin

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REYNOLDS (4) states that the validity of the species A.spicata cannot be upheld. A.candelabrum and A.Marlothii have their natural habitat in Natal.

Reference to the chromatograms appearing in figures 3 - 9 will show that in contrast to table 5 (ii), aloin only appears to occur in A.ferox, A.africana and A.arborescens, the criterion being both the Rf of the principle concerned and its colour under ultra violet light when compared with pure aloin run as control. In A.africana a double dot of striking similarity in colour to the aloin run as control has been observed in each case, this effect having been observed in at least two commercial samples of lump aloe sold as Cape aloes. This double dot (which frequently merges into one) appears below aloin, and has been observed both in the large variety of A.africana, which somewhat resembles A.ferox, and in the small variety with the strongly recurved leaf, common around Port Elizabeth and Coega. VAN OUDTSHOORN (68) working on a sample of A.africana grown in Holland, reports the presence of homonataloin in A.africana. Two A.africana samples from Port Elizabeth studied by this writer failed to give the chemical reactions and chromatographic picture described by the former.

Qualitative (Chromatographic) Analysis of Various Aloe Species.

In the chromatograms that follow, no attempt is made to give the exact Rf values of the various constituents of aloe juice. This is due to the fact that the silica gel plates were not machine made, work was not performed in a temperature controlled room, and variations both in activating time and cooling time on removal from the oven causes the Rf of the constituents to vary. Consequently crystalline aloin was run as

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a control in each case, and in every case the chromatographic plates were heated for 30 minutes at 105°C, and placed in the tank 30 - 45 minutes after removal from the oven. The plates were prepared as described in chapter 2. An approximate Rf value can be gauged from the scale drawn on each chromatographic figure. The figures are reproduced from tracings of the actual plates, the outlining of the dots being done under ultra-violet light.

Species Analysed Chromatographically.

A list of the species analysed chromatographically appears in table 5 (i). Of these, all of the Scrambling types and most of the Stemless types (except A.saponaria and A.striata) are far too small to be of any practical value, scarcely enough juice being obtained even for chromatographic purposes.

Of the larger and more succulent species, several yield golden, viscous juice similar to that obtained from A.ferox. The juice of A.speciosa forms an immediate yellow deposit on standing, this deposit changing slowly to reddish-brown. A.striata forms a light yellow deposit after some hours. (Some commercial samples of A.ferox likewise yielded a copious yellow deposit, in particular those from Loerie and Coega. Other commercial samples of A.ferox showed no deposit after standing twelve months.) The juice from A.saponaria and A.africana is yellowish green, while that from A.arborescens is greenish, thin and tenacious (elastic). The exact locality of the above species in the Port Elizabeth region is given by URTON (62).

(NOTE: A.pluridens, A.ciliaris and A.variegata produced no chromatograms, although in one instance, A.ciliaris produced two faint grey dots.)

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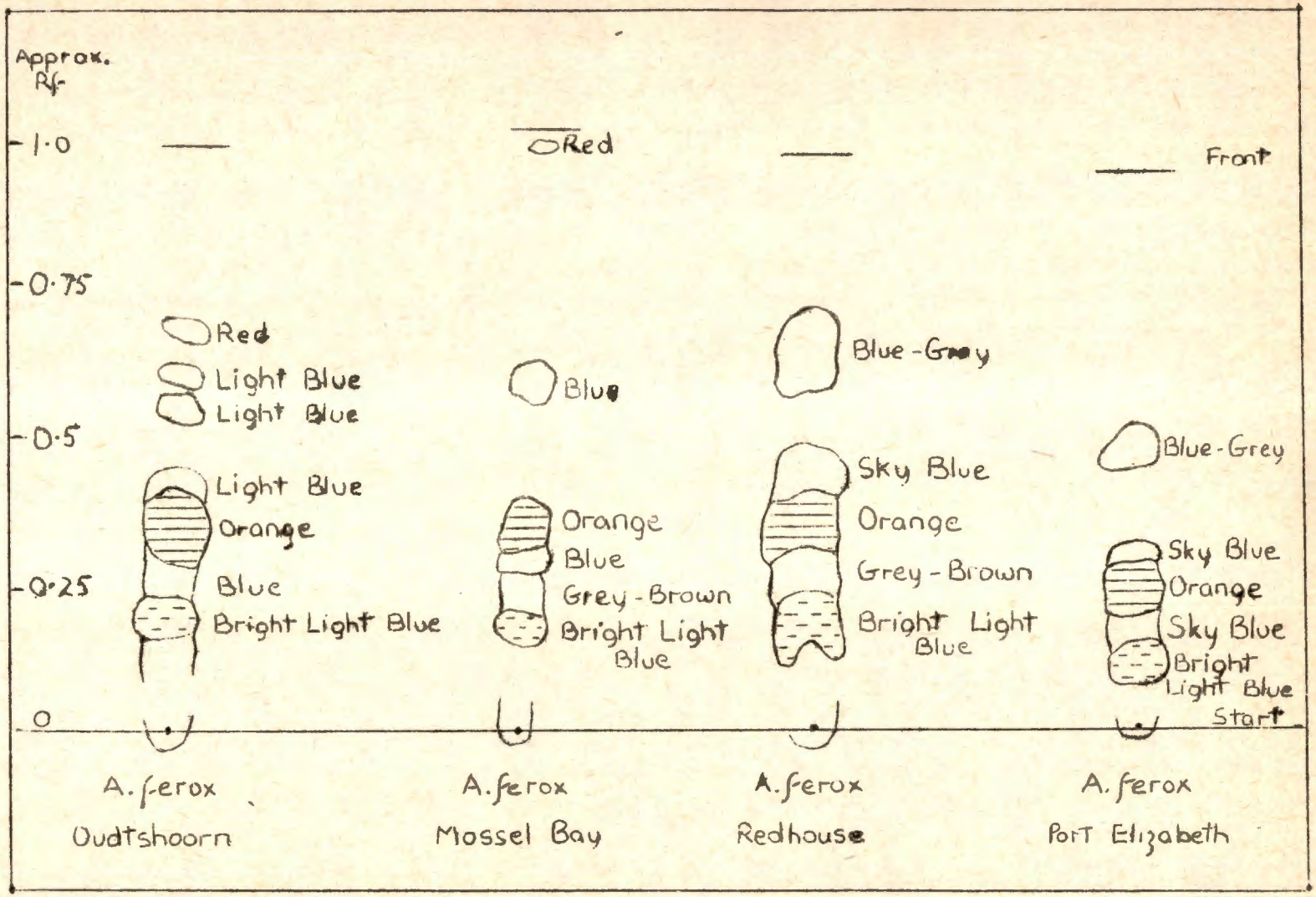

FIGURE 3

(Traced from several plates - hence fronts and Rf's differ)

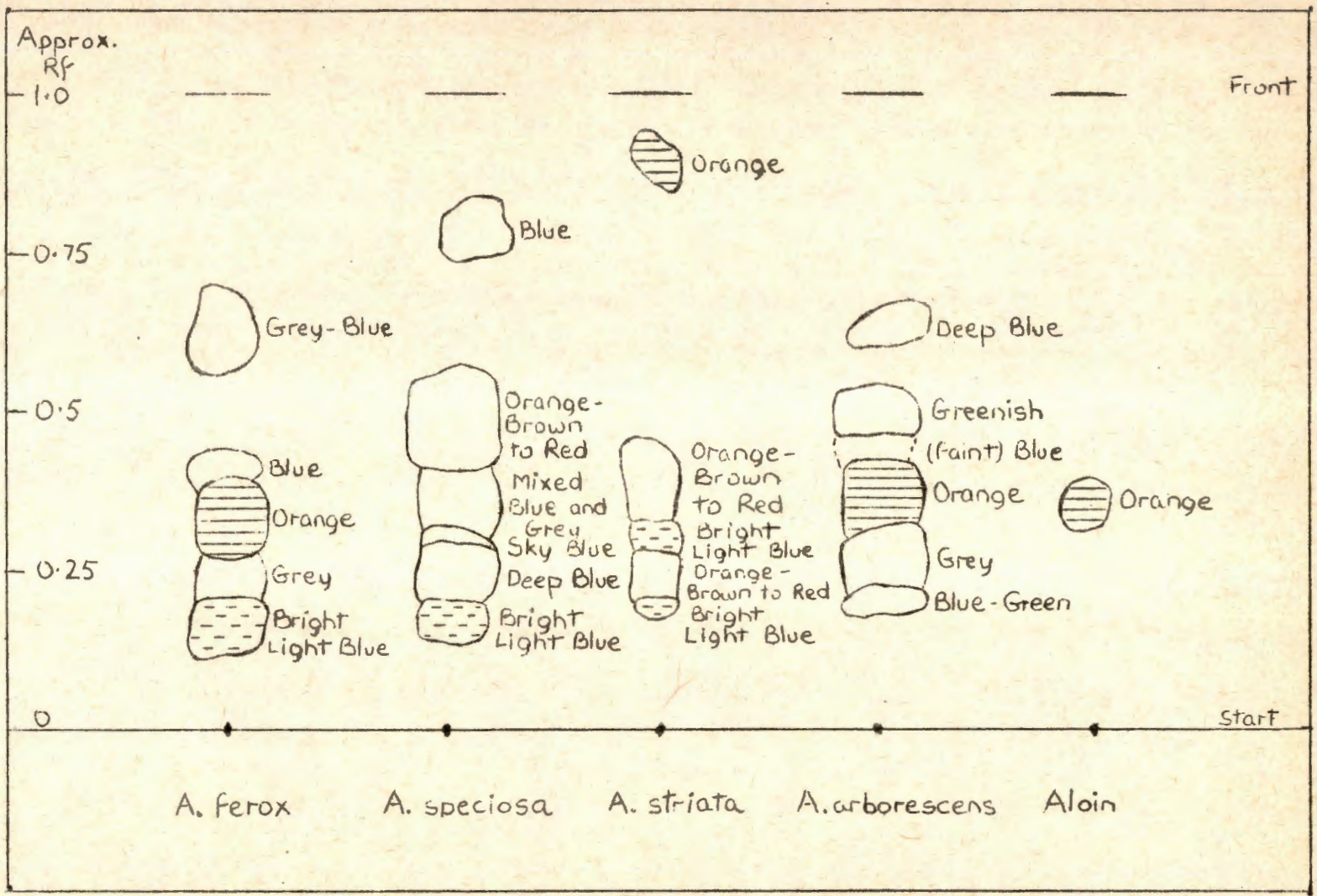
Approx. Rf
1·0
0·75
0·5
0·25
0
Front
Start
Orange
Blue
Grey-Blue
Blue
Orange
Grey
Bright Light Blue
Orange-Brown to Red
Mixed Blue and Grey
Sky Blue
Deep Blue
Bright Light Blue
Orange-Brown to Red
Bright Light Blue
Orange-Brown to Red
Bright Light Blue
Deep Blue
Greenish
(Faint) Blue
Orange
Grey
Blue-Green
Orange
A. ferox
A. speciosa
A. striata
A. arborescens
Aloin

FIGURE 4

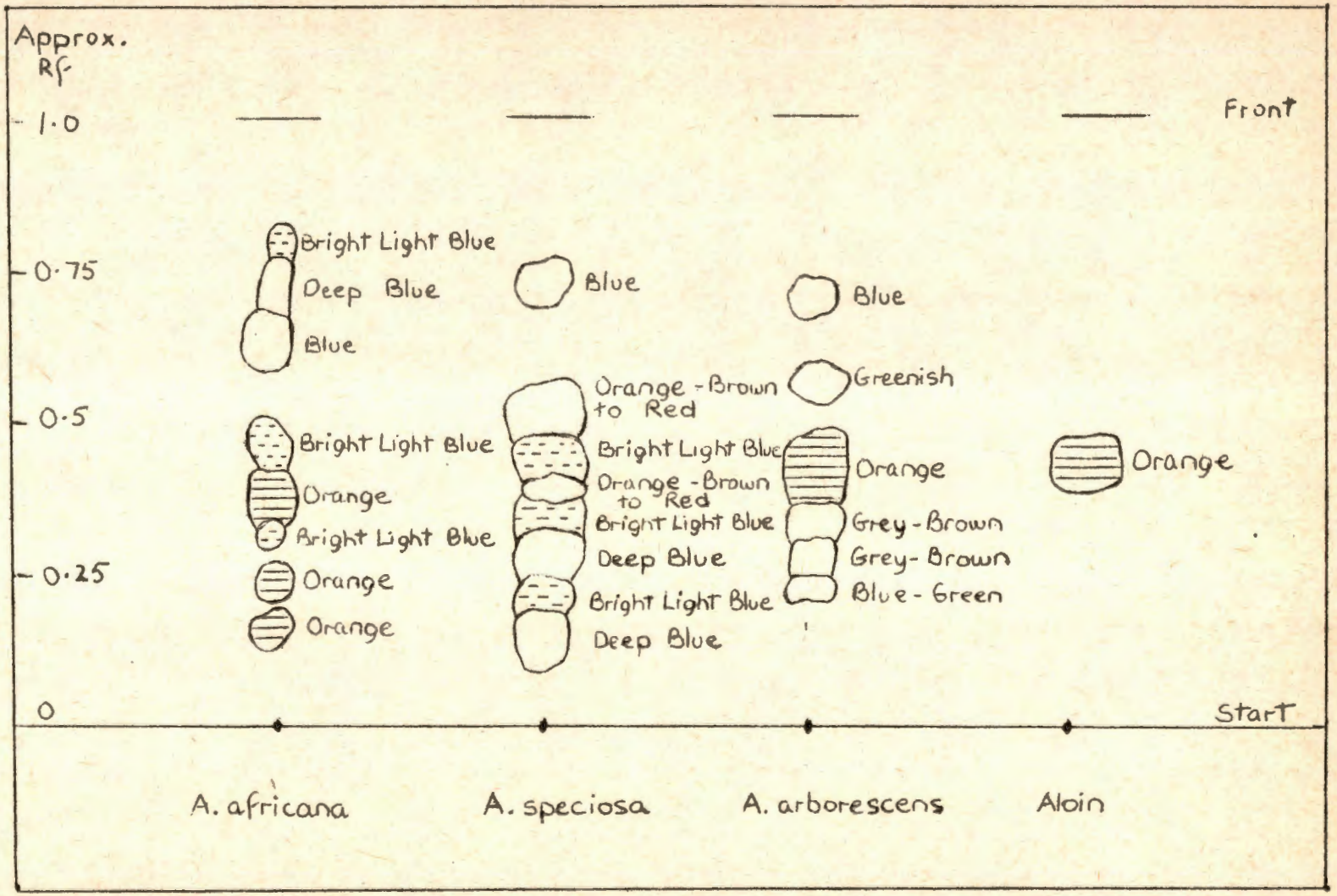

FIGURE 5

(Sap specimens from Port Elizabeth)

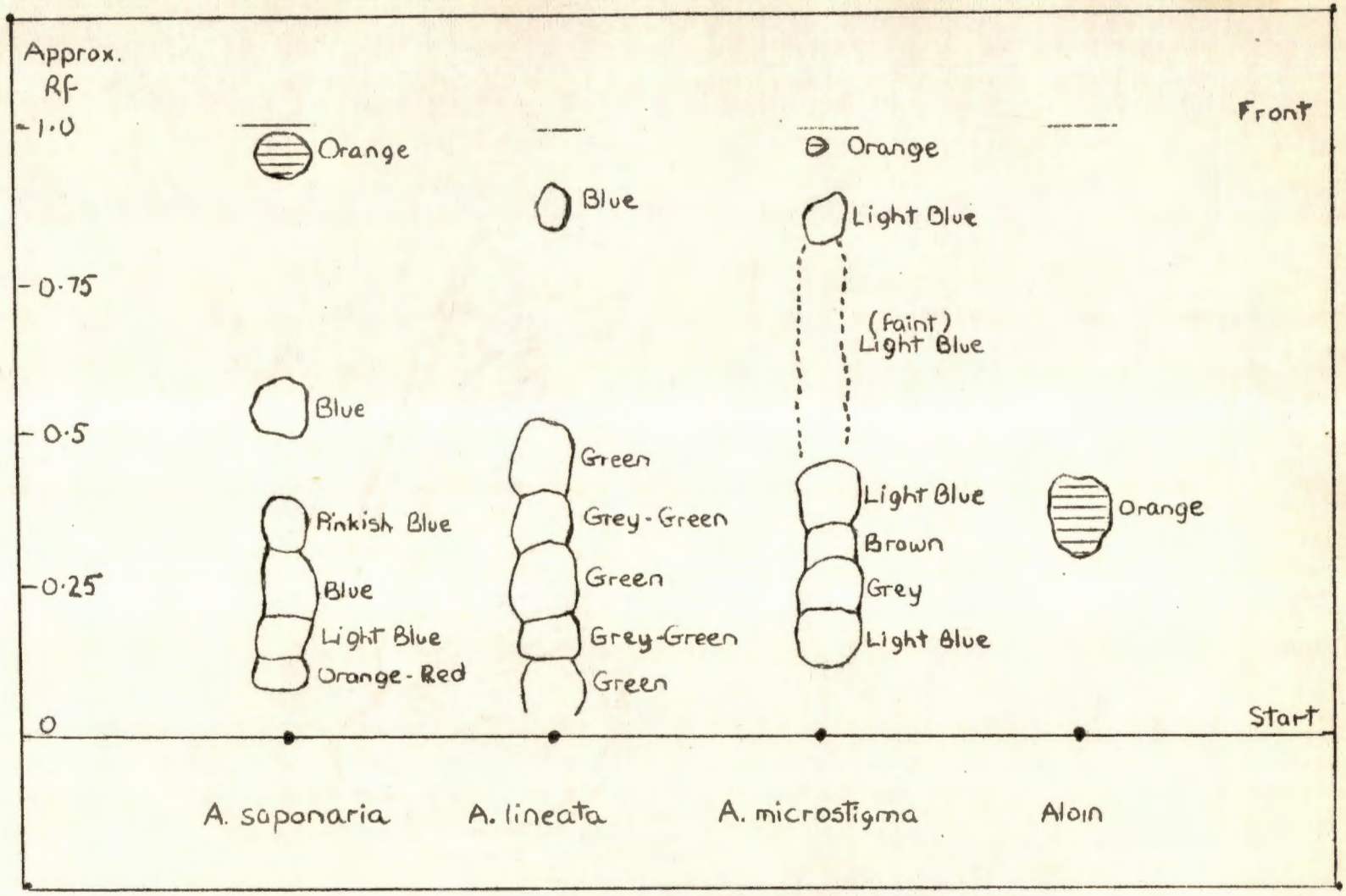
Approx.
Rf
1·0
0·75
0·5
0·25
0
Front
Start
Orange
Blue
Pinkish Blue
Blue
Light Blue
Orange-Red
Blue
Green
Grey-Green
Green
Grey-Green
Green
Orange
Light Blue
(faint)
Light Blue
Light Blue
Brown
Grey
Light Blue
Orange
A. saponaria
A. lineata
A. microstigma
Aloin

FIGURE 6

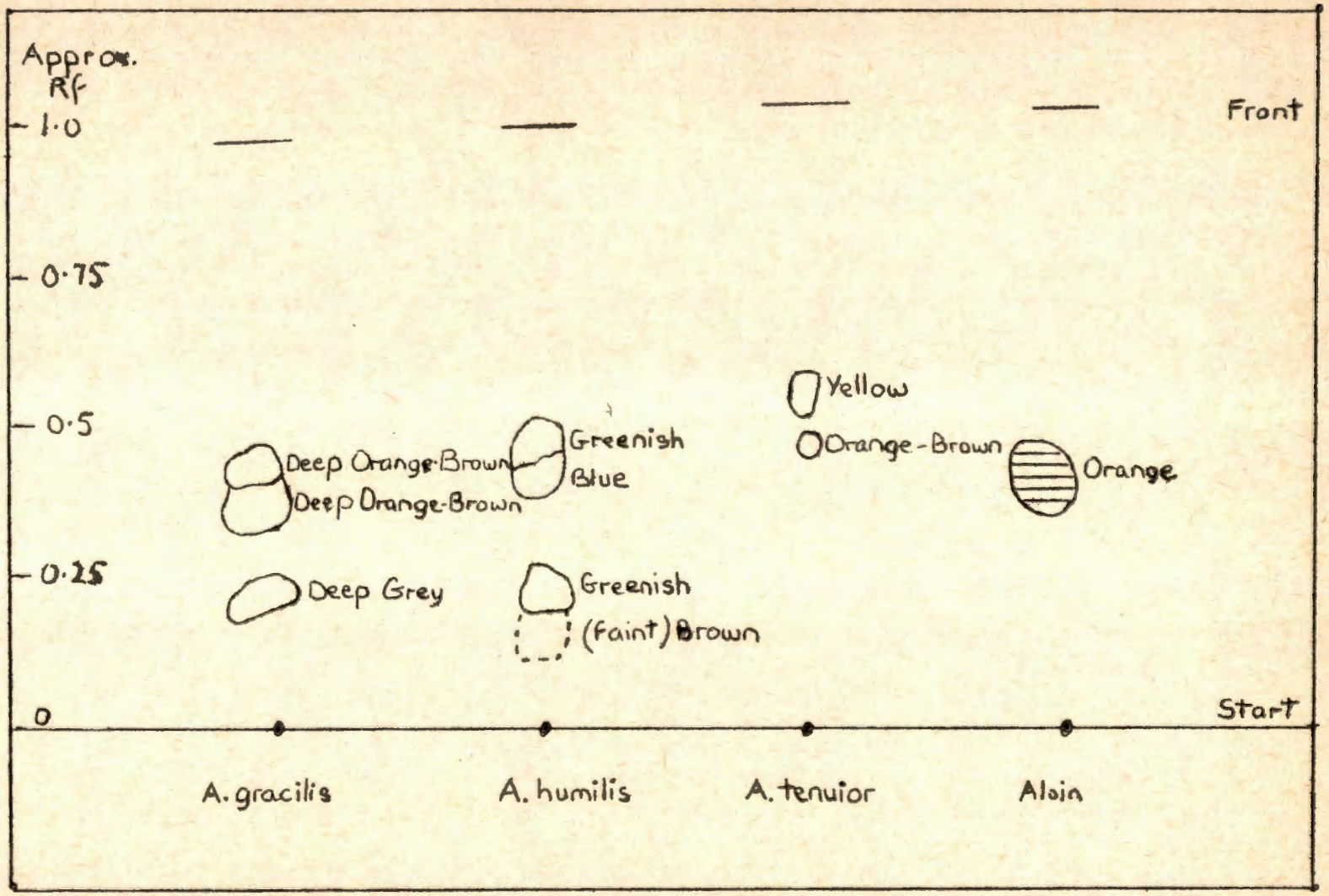
Approx. Rf
1·0
0·75
0·5
0·25
0
Front
Start
Deep Orange-Brown
Deep Orange-Brown
Greenish Blue
Yellow
Orange-Brown
Orange
Deep Grey
Greenish
(Faint) Brown
A. gracilis
A. humilis
A. tenuior
Alain

FIGURE 7

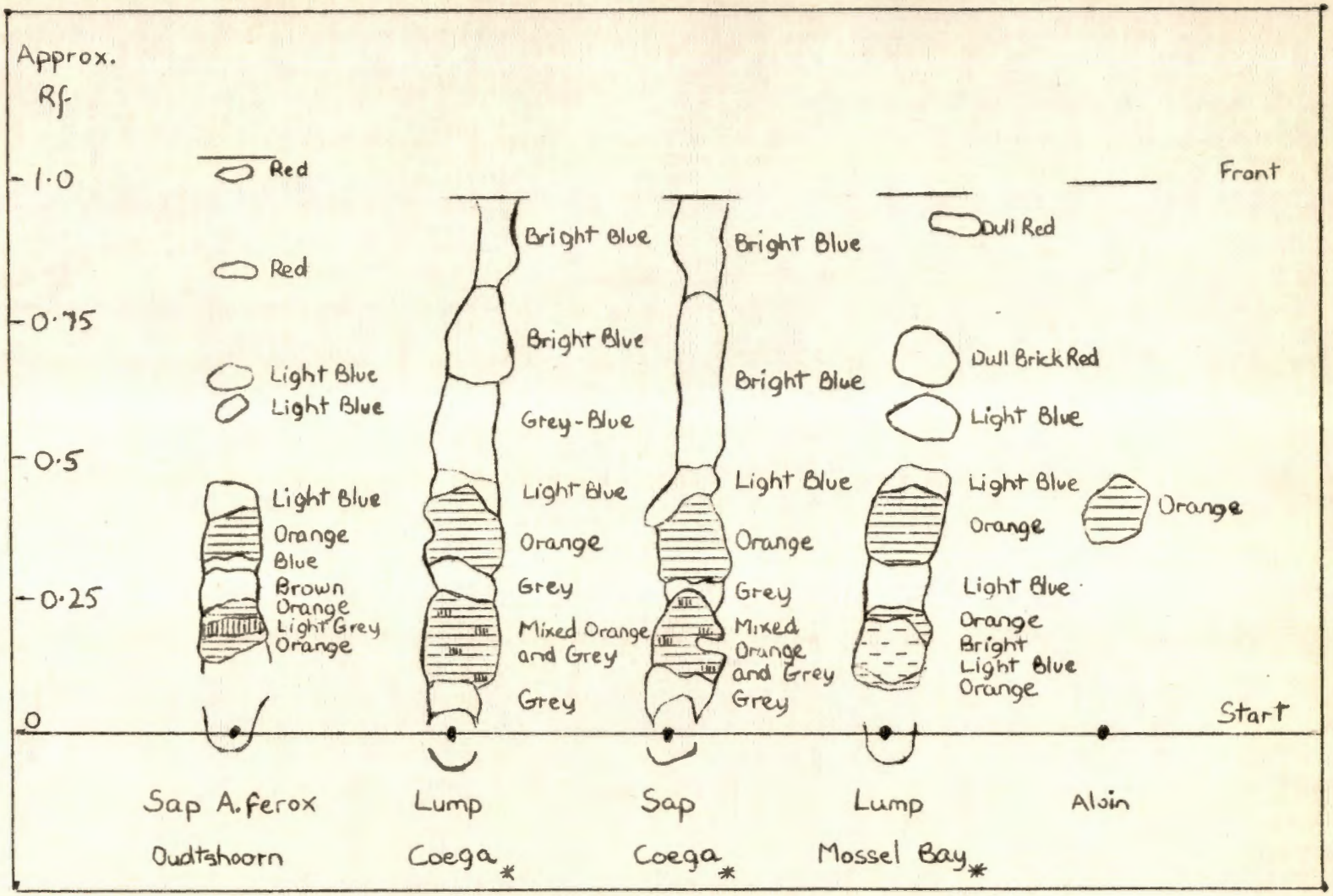

FIGURE 8

*(These samples sold as Cape Aloes)

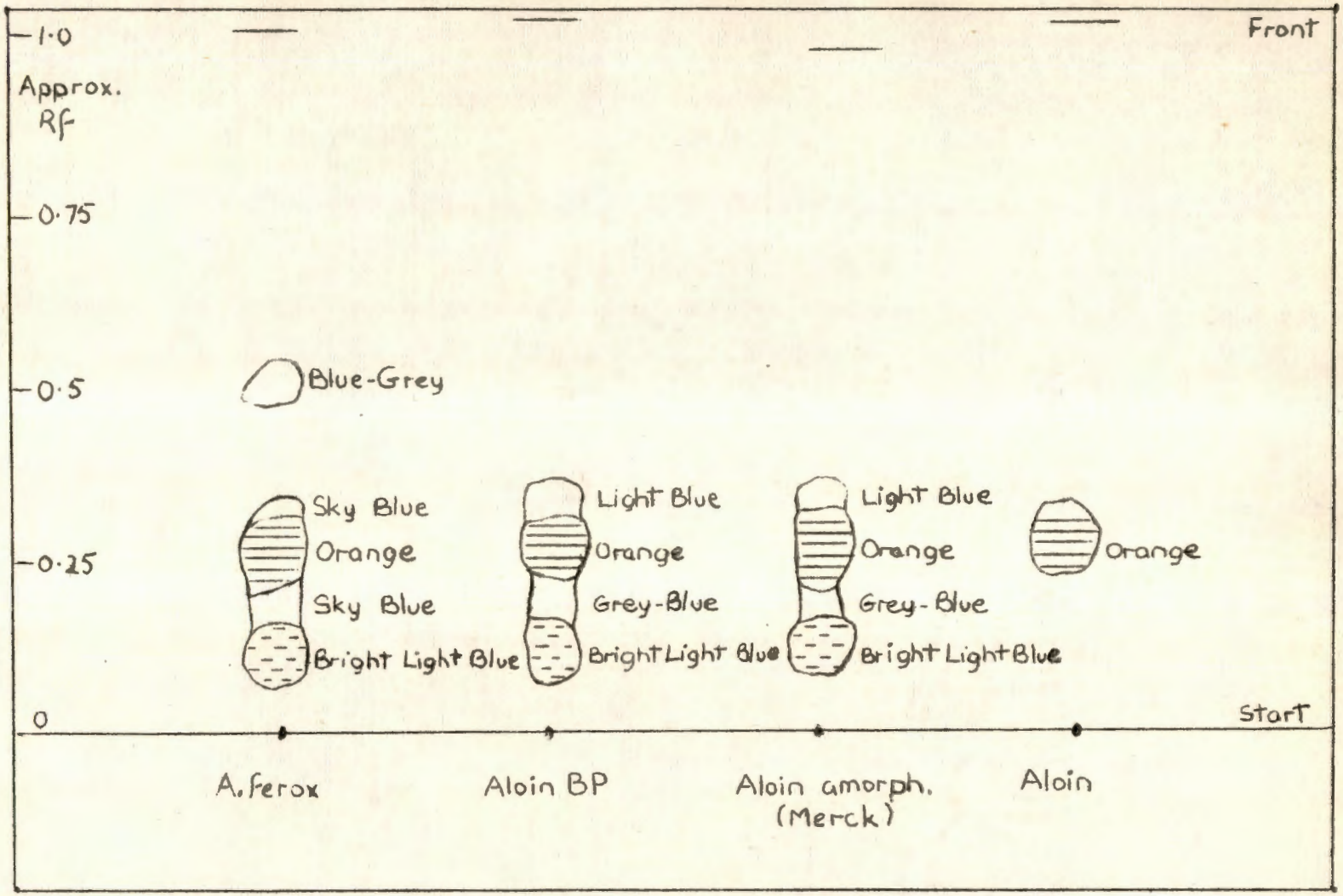
1·0
Front
Approx.
Rf
0·75
0·5
Blue-Grey
0·25
Sky Blue
Orange
Sky Blue
Bright Light Blue
Light Blue
Orange
Grey-Blue
Bright Light Blue
Light Blue
Orange
Grey-Blue
Bright Light Blue
Orange
0
Start
A. ferox
Aloin BP
Aloin amorph. (Merck)
Aloin

FIGURE 9

Quantitative Analysis.

Apart from A.ferox, (analyses of which appear in Chapter 4) A.africana and A.arborescens were quantitatively examined for aloin content on ground of their chromatographic picture, the method used for analysis of A.ferox being applied.

Aloe arborescens.

The first sample was obtained in the centre of Port Elizabeth, and three leaves delivered 4 ml of juice. Results of the analysis appear in table 5 (iii).

Sample two was obtained from the above source, but five months later. In this case 6 leaves delivered circa 4 ml of juice. (The weight of these 6 leaves was 12 oz. By comparison, a 24 oz. A.ferox leaf yielded circa 6 ml. Thus, weight for weight, A.arborescens yields similar volumes of juice.)

For the third sample, leaves from 4 different plants growing in various parts of Port Elizabeth were collected, and the juices pooled. The plants were all in bloom at this time. Results of these analyses appear in table 5 (iii).

Table 5 (iii). Analyses of Aloe arborescens.

Sample.	Collection Date.	Aloin % W/W	
		Fresh juice.	Dried juice.
1	23/7/1962	3.3	24.5
2	14/12/1962	6.2	37.2
3	13/6/1963	4.1	34.9

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It will be seen that the juice of A.arborescens has a far greater moisture content than the juice of A.ferox which has about 50% moisture. and the aloin content of the fresh juice is somewhat lower than that of A.ferox, which is normally circa 9%. However, as the species is the most commonly occurring in South Africa, it seems a likely potential source of aloin or aloe lump for the commercial market.

Aloe africana.

Although far more restricted in its habitat, A.africana appears quite extensively in the Eastern Province, mainly between Humansdorp and Port Alfred. It does, however, form important hybrids, and samples of Cape aloes analysed in chapter 4 appeared to be a hybrid of A.africana by virtue of the 'double dot' effect mentioned before. Should later pharmacologic investigation show these lower dots to be aloinacious, then A.africana would be a most important species. In one instance both the upper (aloin) and lower (merged) dot were analysed and each yielded the same percentage (15.6% W/W - Coega B sample, ex table 4 (iv)). The findings of HÖRHAMMER, WAGNER & BITTNER (67), who reported that a similar 'double dot' effect was observed in samples of Cape aloes from the Mossel Bay area, is of interest. There workers confirmed that these principles, called by them Aloinosides, are pharmacologically active, the aglycone thereof being aloin. The presence of these principles accounts for the constantly high aloin percentages recorded for samples from the Mossel Bay area.

The results of the analysis of the A.africana sample and its three apparent hybrids appear in table 5 (iv), while chromatograms thereof appear in figures 5 and 8.

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Table 5 (iv). Analyses of Aloe africana.

Sample	Source.	Aloin % W/W
A.africana	Port Elizabeth	14.4 (fresh juice)
Cape aloes	Coega B	15.6 (lump)
Cape aloes	Mossel Bay A	26.5 (lump)
Hybrid	Oudtshoorn district	4.1 (fresh juice) 21.5 (dried juice)

Purgative Action of Non-Aloin Containing Species.

Examination of the chromatograms in figures 3 - 9 will show several instances where a substance has an Rf equal or similar to aloin, but which fluoresces a different colour. The colour range is extensive for the various blue colours that occur, making description difficult. Apart from the blues, a greenish-blue is evident in A.arborescens, and has been observed in A.lineata and A.humilis. Orange appears in the aloin-containing species as aloin, while orange dots (not aloin) are observed in A.saponaria, A.microstigma and A.africana. Browns are common, but usually at low Rf values, while orange-red to reddish-brown have been observed. As colours may be named differently by different observers, a classification from the BRITISH COLOUR COUNCIL DICTIONARY (69) is used for frequently occurring colours.

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Bright light blue matches between Alice Blue B.C.C. 43 and Powder Blue B.C.C. 193,

Light blue matches between Mazarine Blue B.C.C. 145 and Salvia Blue B.C.C. 146.

Blue is similar to Smalt B.C.C. 147.

Orange varies between Indian Yellow B.C.C. 6 and Saffron B.C.C. 54.

Reddish brown matches between Rust B.C.C. 58 and Terra Cotta B.C.C. 133.

VAN OUDTSHOORN (10) records the peaks of several dots extracted from eight aloe species and several of these correspond to the two main peaks of aloin, namely at 296 and 355 nm. A pharmacologic survey of the larger species migh show species whose purgative action is equal to or better than that of A.ferox.

SUMMARY AND CONCLUSION.

Several aloes species have been examined chromatographically, and few, apart from A.ferox, A.africana and A.arborescens, appear to contain aloin. The commercial possibilities of A.arborescens and A.africana seem to be overlooked in South Africa, although they find use in certain pharmacopoeias. Only a pharmacologic study will reveal whether the non-aloin containing species have any purgative value, but the simplicity of the chromatographic screening method leads itself to widespread screening of the juice-yielding aloe species throughout South Africa.

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CHAPTER 6.

EXTRACTION OF AMORPHOUS ALOIN FROM ALOETIC JUICE.

In this chapter a summary is given of the various methods employed to extract amorphous aloin from aloe lump, and methods are investigated whereby amorphous aloin may be extracted direct from aloetic juice.

Various methods are recommended for isolating aloin from aloe lump. These include solvent extraction, concentration of aqueous solutions from which the resins have been precipitated by acids, or gravimetric methods where the aloin is precipitated from the resin-freed solution as the calcium salt.

All the methods to extract aloin mentioned above refer to aloe lump. Some of these methods are the following:

Method 1 (a).

Aloe lump is dissolved in boiling water, upon acidification of which the resins commence to precipitate. After some hours the supernatant is decanted or filtered off, and the solution is then concentrated by heating. Upon standing a sufficient length of time, aloin crystalises from the concentrate (REMINGTON (29)).

Method 1 (b).

The preparatory method is as above, but concentration is performed under vacuo (BARROWCLIFFE & CARR - ex (6)).

Method 1 (c).

The method is the same as Method 1 (a), but after filtering off the resins no final concentration is performed, crystallisation occurring from the dilute filtrate (U.S.A. DISPENSATORY (30)).

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Method 2.

Aloe lump is dissolved in boiling water, the resins precipitated by acid, the solution concentrated by heating, and the resins removed by filtration. To the alkalised filtrate is added calcium chloride, and calcium-aloinate commences to precipitate. The precipitate is filtered off after about half-an-hour, and dissolved in a minimum volume of concentrated HCl. After a short while aloin crystallises from this solution, and is dried at a low temperature (SCHAEFER) (31), (LISTER & PRIDE (28).

Method 3.

Powdered aloe lump is refluxed with methanol. Chloroform is then added to the boiling liquid in the flask and set aside overnight for the resins to precipitate. The following morning the solution is filtered, and the filtrate distilled to remove the solvents. The residue produced is then dried at 100°C, and constitutes amorphous aloin (EDER & SCHNEITER) (32).

Method 4.

Powdered aloe lump is macerated in methanol for 2 hours. The product is then heated to 50° - 60°C, and shaken with chloroform, added slowly to the warm liquid. After standing for 30 minutes the solution is filtered, and the filtrate distilled to remove the solvent. The remaining resinous residue is extracted four times with chloroform, and the combined residue from the solvent extraction dried at 100°C (KONDRACKI - Modified LÉGER (33).

Method 5.

Powdered aloe lump is digested with alcohol (95%) for 24 hours, transferred to a water bath, and boiled for two hours. The solution is

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filtered when cool, and set aside to crystallise (U.S.A. DISPENSATORY) (30).

Method 6.

Powdered aloe lump is dissolved in acidulated water and boiled for 15 minutes to precipitate resins. The supernatant liquid is decanted, and the residue is reboiled for 5 minutes with a further volume of acidulated water.

To the mixed filtrates is added 20% lead acetate solution, the product filtered, and H_2S passed through the warm filtrate to remove lead ions. The filtrate is concentrated by heat, extracted with solvents, these being largely removed by distillation. From this final concentrate a thick yellow mass results (SMITH, JORDAN & DE KAY) (34).

Extraction of amorphous aloin direct from aloetic juice.

The literature appears to be confined to descriptions of aloin extraction from aloe lump. RAMSTAD (3) states that aloin is extracted from the fresh juice on the island of Aruba, heavy machinery being used for the process. Unfortunately no details are given.

Experimental Work.

Method 1 (a), was applied to Aloe lump B.P. as a preliminary. 10 g aloe lump in small pieces was added to 98 ml boiling water acidulated with 2 ml concentrated HCl. This was stirred to dissolve, strained through cotton wool, and left for 3 hours to allow the resins to precipitate. After filtration, the aqueous solution was concentrated on a waterbath to 18 ml. This concentrate was set aside for 3 weeks, but no crystallisation occurred, even when crystallin aloin was added.

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The above method was repeated, substituting 15 g aloe juice in 100 ml acidulated boiling water. The concentrate of 25 ml was set aside for a month, but neither refrigeration nor seeding with crystalline aloin could induce crystallisation.

The above method was repeated with the modification that the resins that precipitated on standing were centrifuged down, and the resulting supernatant was filtered. The concentrate from this also failed to yield aloin crystals.

Method 1 (c).

5.0 g aloe juice was weighed, and to it added 40.0 g acidulated boiling water. This was set aside for 24 hours, filtered, and the filtrate set aside for 2 weeks to crystallise. Only a few particles of resin precipitated, despite seeding with pure aloin. TRIM (63) states that highly water-soluble glycosides form supersaturated syrups which may have to stand a very long time before crystallisation occurs.

SCHAEFER'S gravimetric method (Method 2) was then employed as follows: 23 g aloe juice was added to 200 ml acidulated water and concentrated to a volume of ca. 160 ml. This was set aside for 3 hours, strained, and to the filtrate was added 40 ml of ammonia solution (25%) and 15 g $CaCl_2$ in 30 ml water. This was left 30 minutes for the calcium-aloinate to precipitate.

The precipitate was sucked dry on a No. 4 sintered glass filter, dissolved in 2.4 ml concentrated HCl and allowed to crystallise. The first amount of aloin was filtered off after 30 minutes, while more appeared on refrigeration overnight. The amorphous aloin (which showed patches of brown resin) was dried to constant weight at 40°C.

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Repeat of this method using 50 g of the same batch of aloe juice dissolved in 200 ml acidulated water yielded a relatively poorer result. Possibly the original solution was too concentrated, but difficulty was experienced in obtaining a precipitate of calcium-aloinate, and addition of ammonia solution to the filtered solution produced another gelatinous precipitate.

The results appear in table 6 (i).

Table 6 (i).

Weight of juice.	Yield amorph. aloin.	% Purity of amorph. aloin.	% Recovery as crystalline aloin.
23.0 g	0.82 g	46.9%	13.4%
50.0 g	1.12 g	65.8%	11.9%

(The aloe juice contained 12.4% W/W crystalline aloin).

SNYDER (66) states that the above method fails to indicate the aloin content of Cape aloes. It may be questioned if this may not be due to the type of resins found in Cape aloes, because several attempts to crystallise aloin from the concentrated aqueous phase of Method (1), and also to crystallise aloin from amorphous aloin using isopropyl alcohol, resulted in an exceedingly gummy viscous liquid from which crystals would not separate.

Using a variation of the calcium-aloin method, LISTER & PRIDE (28) obtained only a 16% yield of crystalline aloin from a 5 g sample of amorphous aloin, whereas the yield obtained was 91% when aloin B.P. was used as the starting point. They state that this low yield is explicable in terms of the solubility of calcium-aloin. Similarly FAIRBAIRN & SIMIC (42) obtained with this latter method results approximately 30% lower than by their method of conversion to aloe-emodin.

The bulkiness of the gel-like calcium-aloinate precipitate, and the difficulty of drying the precipitate as completely as is required, suggests that this method would not easily lend itself to large-scale production, neither is the yield of a high order.

The method of EDER & SCHNEITER (Method 3), gave the most satisfactory result. This method is designed for aloe lump, and details of the procedure are as follows:

Reflux 1 g aloe powder with 5 ml methanol. To the boiling liquid add 30 ml chloroform slowly, and leave overnight. Filter and distil the filtrate. The residue is air-dried, then dried in an oven for one hour at 100°C. This constitutes amorphous aloin.

Since aloe juice contains approximately 50% moisture, it was decided to attempt two experiments. In the first, approximately 2 g aloe juice (equivalent to 1 g aloe lump) was treated with twice the volumes of methanol and chloroform recommended by EDER & SCHNEITER. In the second, 2 g aloe juice was treated with the volumes recommended for 1 g aloe lump.

Thus in the first method 2 g aloe juice of known aloin content was refluxed for one hour with 10 ml methanol. To this hot liquid was immediately added 60 ml chloroform, and the whole left overnight (17 - 18 hours). The following morning the solution was filtered through medium grade filter paper, most of the resin adhering firmly to the walls of the flask. The filtrate was distilled from a tared flask. The resulting bright yellow residue was dried in air for 30 minutes, and then in the oven at 100°C for one hour.

The same procedure was followed in the second experiment, 2 g aloe juice in this case being refluxed for one hour with 5 ml methanol, and to this 30 ml chloroform being added.

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Results of the experiments, performed in duplicate, appear in table 6 (ii). The yield was calculated as follows:

Yield = $\frac{\text{Weight amorphous aloin x \% crystalline aloin in amorphous aloin}}{\text{Weight of juice x \% crystalline aloin in juice.}}$ ($\frac{\text{crystalline}}{\text{aloin}}$)

Table 6 (ii).

Method	Wt. of juice	% cryst. aloin in juice.	Wt. amorph. aloin yielded	% cryst. aloin in amorph. aloin	Yield cryst.aloin
1st	2.250 g	11.3% W/W	0.981 g	23.8%	ca. 91%
1st	2.019 g	11.3% W/W	0.885 g	20.4%	ca. 79%
2nd	1.997 g	11.3% W/W	0.374 g	30.6%	ca. 50%
2nd	1.992 g	11.3% W/W	0.266 g	32.2%	ca. 38%

From this it will be seen that although the second method gives a purer aloin, the first method produces two to three times the amount of amorphous aloin, with consequently almost twice the yield.

Method 5.

7.23 g aloe juice, to which was added 21.7 ml alcohol (95%), were digested at 40°C for 24 hours in a reflux condenser. The contents of the flask was then boiled for two hours, under reflux, and filtered when cool, there being scarcely any residue of resin. The filtrate, which was dark brown, was set aside for two weeks, and seeding with pure aloin failed to cause any precipitation.

Conclusion.

Most of the previously mentioned methods are unsuitable for extracting amorphous aloin from Cape aloe juice. Precipitation as calcium-aloinate does not offer sufficient yield, but the extraction method of EDER & SCHNEITER produces a relatively good yield, and could be used for small or large scale production.

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CHAPTER 7.

DRYING METHODS AND ALOIN VARIATION.

When aloetic juice is heated aloin is destroyed. In this chapter various drying methods are discussed, and the effect of drying by gas flame, sun drying, oven drying, hot air drying, and rotary drum drying are compared.

When aloetic juice is heated aloin is destroyed, and chromatograms run during the heating process show a progressive increase in, what is suspected to be, aloe emodin content. There is little evidence to show whether the other constituents of aloe juice are affected by the heating process, and the literature on the subject appears scant, although mention is made that rhubarb (torrefactum) is purposely heated to remove its cathartic action, whilst retaining its astringent properties.

In lump aloe not only the aloin but also the non-aloin constituents are claimed to exert a purgative action. TSCHIRCH (ex (9)) claims that the purgative action is due to the three resins found in Cape aloes, and that aloin and aloe emodin play a minor part. RAMSTAD (3) states that while some pharmacopoeias discard the resinous substance when preparing galenic preparations, others make preferential use of the resins.

On these grounds no necessity exists for a close check on the aloin loss due to heating when aloe juice is used merely to produce lump aloe, which will be used as such. When, however, the lump aloe is bought on a basis of its aloin content, and/or is used for the extraction of aloin, an investigation into aloin loss due to heating is warranted.

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Hence to investigate if a practical method could be evolved which would decrease aloin loss on heating and yet remain commercially practicable, aloe juice was heated at various temperatures and in varying layer thicknesses, using the following sources of heat:

1. Wood fires.
2. Gas flame.
3. Sun drying.
4. Electric oven.
5. Hot air.
6. Rotary drum drier.

1. Wood fires.

The present method in use at the Cape is very inexpensive, but aloin loss is relatively high. The juice is boiled in 44 gallon drums over a wood fire and stirred periodically by the collectors (usually Cape coloureds or Bantu). Approximately half the original weight results, namely 6 - 7 four gallon paraffin tins, each weighing about 54 lbs.

Aloin loss by this method.

In table 7 (i) figures are given of aloin loss for aloe juice collected from four different regions. The Mossel Bay sample is quoted from VAN OUDTSHOORN (10) who found that the loss after two hours heating was only about 3%, but at the end of the process was ca. 10% aloin.

Table 7 (i).

Region.	% aloin W/W in dried sap	% aloin W/W after heating	Actual aloin loss %	Relative aloin loss %
Sandflats	18.4	15.1	3.3	17.9
Alicedale	24.5	21.0	3.5	14.3
Fort Beaufort	15.7	14.4	1.3	8.3
Mossel Bay	28.31	18.86	9.45	33.4

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2. Drying by gas flame.

A sample of aloe juice was heated in a metal container over a very weak bunsen flame for 33 minutes. The sample was purposely not stirred frequently during heating, and localised charring occurred, the finished product being a brownish-black friable mass.

Actual aloin loss: 10.5%
Relative aloin loss: 47.9%

3. Sun drying.

Sun drying seems a suitable method in a sunny country like South Africa, and is the method used for the production of the opaque "livery" aloes, such as from Socotrine and Zanzibar (TREASE) (6).

Only one collector could be persuaded to try this method, and his sample of juice was left on canvas for 2 - 3 months. The result is given below:

Aloin % in sun-dried product: 12.0% W/W
Aloin % in boiled product: 9.8% W/W

The sun-dried product was thus 2.2% higher than the lump boiled from the same juice, but unfortunately this juice had fermented so badly that no relative aloin loss could be determined.

Consequently two small-scale experiments were tried to obtain further data. In the first, a 1 cm thick layer was left in the sun for $9\frac{1}{2}$ hours (noon temperature $75^{\circ}F$ in the shade), and in the second a 2 cm thick layer of juice was left in a jar on a sunny window ledge for 1 month. Results appear in table 7 (ii).

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Table 7 (ii).

Time of exposure	% Aloin. Original	% Aloin. After drying	Aloin loss %	Relative loss %
9½ hours	21.9	19.8	2.1	9.5
1 month	22.4	21.1	1.3	5.9

One disadvantage was the amount of dust that accumulated on the (first) exposed sample. This would be even greater in the veld, unless some sort of covering, preferably glass or galvanised iron, could be used. If dried in shallow trays to hasten the process, a large surface area would be required to hold the contents of a 44 gallon drum.

4. Oven drying.

Samples of aloetic juice were placed in uniform glass containers 5 cm deep x 5 cm in diameter. The volumes of juice equivalent to a depth of 1, 2 and 3 cm respectively were circa 20, 40 and 60 ml.

In an oven stirring is virtually impossible, and the thick scum that forms hinders evaporation considerably. Table 7 (iii) indicates the time taken for the juice to solidify.

Table 7 (iii).

Oven temperature.	Depth of juice.	Drying time.
50°C	1 cm (ca. 20 ml)	12 hours
50°C	2 cm (ca. 40 ml)	26 hours
150°C	1 cm	1 hour 33 minutes
150°C	2 cm	2 hours 25 minutes
150°C	3 cm (ca. 60 ml)	3 hours

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Drying was attempted at 50°C to see what the aloin loss would be at low temperatures, but the length of time involved ruled the method out completely.

The analyses of the previously mentioned samples dried at 150°C appear in table 7 (iv).

A different sample of sap was also dried at 150°C (1½ cm deep, for 2 hours) to see if any variation would occur using juice from another region. This sample dried to a friable mass containing no moist slurry, and its aloin loss was very similar to the previous sample dried at the same temperature (see table 7 (iv)).

Both samples of sap were then poured onto glass plates to form a layer 1 - 2 mm thick. The first sample (containing 21.9% aloin) produced a friable mass within 15 minutes at 125°C.

The second sample (containing 24.6% W/W aloin) required 22 minutes at 150°C before drying completely, and the percentage aloin found on analysis appears in the table below.

Table 7 (iv).

Oven temp.	Drying time.	Layer thickness.	Original % Aloin W/W.	Final % Aloin W/W.	Actual loss of aloin %.	Relative loss %.
150°C	1 hour 25 mins.	1 cm	15.7	13.9	1.8	11.4
150°C	2 hours	1½ cm	24.6	21.4	3.2	13.0
150°C	2 hrs. 25 mins.	2 cm	15.7	13.5	2.2	14.0
150°C	22 minutes	1 - 2 mm	24.6	23.6	1.0	4.1
150°C	15 minutes	1 - 2 mm	21.9	20.4	1.5	6.8
125°C	15 minutes	1 - 2 mm	21.9	20.2	1.7	7.7

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From the previous figures it can be seen that drying in thin layers not only reduces the drying time considerably, but causes only half the relative loss of aloin.

An attempt was therefore made to dry the juice in a thin layer by some continuous means, avoiding the use of an oven, and avoiding sun drying.

5. Hot-air drying.

Four ounces of aloetic juice was placed in a tablet-coating pan 18 inches in diameter, and revolved under a blast of hot air (55° - 60°C) for 2 - $2\frac{1}{2}$ hours. One chipping of drying aloe was required during this time to break the surface layer, and analysis of the finished product was as follows:

Original aloin % W/W of dried sap:	21.9%
Aloin % W/W in final product:	21.4%
Aloin loss:	0.5%
Relative loss:	2.2%

6. Drum-drying.

The use of a commercial rotary drum drier could not be obtained, and consequently an improvised apparatus was used.

This consisted of an autoclave (pressure cooker), internally 1 foot high and 1 foot in diameter, mounted sideways on rollers. Movement was fluent on the eight roller wheels, and could be controlled by the handle on the lid.

As a preliminary it was found that about three minutes was required to dry a thin layer of juice smeared on the hot metal whose temperature was between 95° and 100°C.

Measurements of the surface temperatures of the metal, in relation to the pressure, were made using a thermometer bulb on the hot metal surface, and by using compounds of suitable melting points. When removed from the gas burner the temperature dropped fairly rapidly.

Three methods were used, as follows:

Method A.

The autoclave stood upright on the burner. Aloe juice waspoured carefully down the side to form a layer 2 - 3 mm thick. After an appropriate time this layer was scraped off. (Too thick a layer resulted in a pliable scraping, with consequently poor result).

Method B.

The autoclave was laid sideways on rollers and rotated slowly. Aloe juice was applied slowly to the side of the hot rotating drum, and scraped off as before.

Method C.

Aloe juice was placed on a concave tile beneath the drum which dipped into the juice. On rotation a thin layer was picked up by the drum, and scraped off by a stationary blade after a suitable time.

Five tests were done using two different samples of juice, and the results of the analyses appear in table 7 (v).

Table 7 (v).

Method	Contact time	Surface temperature	% Aloin W/W		Aloin loss %	
			Original	Final product	Actual	Relative
A	2-3 minutes	110°C	24.6	25.1	Nil	-
*A	2-3 minutes	110°C	14.4	13.9	0.5	3.5
A	7-8 minutes	110°C	14.4	14.6	Nil	-
B	2-3 minutes	100°C	24.6	24.5	0.1	0.4
C	4-5 minutes	ca. 90°C	24.6	25.1	Nil	-

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In three cases no loss was obtained, maximum recovery being 102%, which is within the limits of experimental error assessed previously in table 2 (iv).

The above figures demonstrate that, provided the layer is suitably thin, and a sharp scraper is used, virtually no aloin loss occurs by this method. Being a continuous process, it is suitable for large-scale application. Perhaps the best type of rotating drum drier would be one fitted with a side-nozzle applicator to prevent contact of the hot drum with a large bulk of the thermolabile juice. A contact time of about 5 minutes seems suitable.

A summary of the various drying methods employed appears in table 7 (vi).

Table 7 (vi).

Heating Method.	Relative Aloin Loss %.
1. Wood fires	8.3 - 17.9 (33.4)
2. Gas flame	47.9 (charred)
3. Sun drying	5.9 - 9.5
4. Electric oven @ 150°C	
(a) thick layer	11.4 - 13.0
(b) thin layer	4.1 - 7.7
5. Hot air	2.2
6. Rotating drum drier	0 - 0.4 (3.5)

Conclusion.

Continuous drying using a rotating drum drier seems eminently suitable for drying aloe juice with a minimum destruction of aloin. According to RAMSTAD (3) South Africa exports 500,000 Kg of lump aloe annually.

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Assuming that Cape aloe averages circa 17% W/W aloïn (see page 46), some 85,000 Kg of contained aloin is exported. Were an approximate 15% not destroyed by boiling on wood fires, (table 7 (vi)) this figure would be circa 100,000 Kg of contained aloin. Thus some 15,000 Kg of aloin (current market price 48 shillings a Kilo) is destroyed annually by the conventional method.)

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S U M M A R Y.

Aloes, a drug known to man from antiquity, has survived the test of time and is still widely used today, despite the modern trend for synthesised (predominantly organic) medicinals.

One of the principal sources of "aloes" is Cape aloes. The chief species comprising the dried extract known as Cape aloes is A.ferox, which has been the subject of most of the investigation in this work.

An outline has been given of the various types of aloes on the commercial market, and the tests employed to differentiate these. The preparation and collection of Cape aloes has been described, and the chemistry discussed. Since in modern times emphasis is on therapeutic action in relation to chemical structure, a note on the pharmacologic and physiologic action has been included.

Aloes (and its principal glycoside aloin) have been the subject of much research, and several assay methods have been evolved. Several of these are reviewed, divided for convenience into Biological assays and Chemical Assays. Of the latter, a micro-analytical method used for all experimental work is described. A description is given of the chromatographic separation by the thin layer technique of the components of Cape aloes, of the localisation of these under ultra-violet light, and of spectrophotometric determination of aloin at 360 nm. The method is suitable for micro quantities (30-60 µg aloin), with limits of accuracy of 99.2% - 102.6%.

Experimental Work.

An investigation was made into the variation that occurred in the aloin content of the individual plant in relation to (a) the time of day,

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(b) the month of the year, and (c) the weather.

Initially, aloin content in relation to leaf distribution was investigated, and it was ascertained that aloin followed a random distribution in leaves of the same plant, with minimum content variation occurring in neighbouring leaves on the same approximate level.

Although little content variation occurred from day to day, a relatively large variation occurred from month to month. The only weather factor appearing to influence the overall seasonal variation was wind, aloin content being inversely proportional to both wind velocity and duration. Apart from this influence, the overall seasonal picture was that aloin content rose in the summer months, and dropped in the winter(flowering) season.

Aloin content in relation to geographical locality was investigated. Both fresh aloe juice and lump aloe from several regions of the Cape (in particular, the Eastern Cape) were investigated, and showed relatively large variation from region to region. The average aloin content of fifteen samples from twelve regions of the Cape (see table 4 (iv)) was circa 18% W/W aloin (range 9.8% - 26.5%). Reference to analyses by other workers (table 2(ii)) shows similar figures (9% - 27.99%). The altitude of each of the collection regions was recorded, and altitude appeared to have no effect on aloin content of the boiled (lump aloes) samples. The average moisture content of lump aloes was circa 8% by weight. With regard to aloe juice, considerable variation was experienced with respect to stability, but most samples were stable for from one to four months with little aloin loss.

Apart from A.ferox, several other aloe species have been employed for the manufacture of Cape aloes. A review has been given of species named by

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several authors as sources of Cape aloes. Thirteen species apart from A.ferox were screened for possible aloin content (table 5 (i)), chromatographs thereof appearing in figures 3-9. A.arborescens and A.africana both appear to contain aloin, and in quantities suitable for commercial usage. Both these species appear to be neglected by South African aloe exporters. Furthermore, A.africana and its hybrids with A.ferox contain two principles chromatographically similar to aloin. These may be glycosides whose aglycone is aloin.

Lump aloe is the commodity usually appearing on the drug markets of Eruope and America. In consequence, aloe juice is boiled to produce the lump aloe, some aloin being destroyed in the process. Aloin is later extracted from the lump. To obviate this heating step and the concommitant aloin loss, it was attempted to extract aloin direct from aloe juice. Several methods were attempted, and these were methods recommended for aloin extraction from the lump aloe. Few yielded aloin in suitable quantities, but a solvent extraction method (table 6 (ii)) produced an amorphous product of good yield, and of a percentage purity comparable to certain commercial samples.

The aloin loss due to heating was then the subject of a more detailed investigation. The conventional Cape method of drying is in large drums over wood fires, and the resulting aloin loss was found to be high, result ng in a poor product for the market. Consequently, other drying methods were

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investigated (table 7 (vi)), most of which produced a better product, but not all of which were economically sound. The best of these methods was that utilising a rotary drum drier, the product (in powder or flakes) having lost extremely little aloin.

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