The Study of the Aloins and Related Compounds

- By -

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INTRODUCTION

In natural products, excluding polysaccharides, sugars are most commonly found as glycosides having the formula $R-\text{O-Grlycosyl}$. These compounds readily undergo hydrolysis when heated with dilute mineral acids. Examples are anthraquinone glycosides, coumarin glycosides and anthocyanins (See "The Plant Glycosides" by R.J. McIlroy, Arnold, London, 1951).

Recent work has led to the discovery of a new series of compounds in which the glycosyl group is not attached through an oxygen atom but directly to the aglycone with a C-C linkage. These compounds belong to the C-glycosyl group.

The first tentative suggestion that a naturally occurring C-glycosyl compound might exist was put forward by Fieser and Fieser ("Textbook of Organic Chemistry", Heath, Boston, 1944) who suggested that the side chain in carminic acid (I, for formulae see page opposite page 5) could possibly be in the form of a sugar linked directly by a C-C linkage. No experimental evidence was quoted to substantiate this suggestion.

The first C-glycosyl compound to which a structure has been definitely assigned is barbaloin.

Barbaloin, $\text{C}_{21}\text{H}_{22}\text{O}_9$, is a lemon-yellow crystalline solid
obtained by careful recrystallisation of commercial aloin
of which it is the principal component. Crystalline barbaloin was first isolated by T. & H. Smith (Chem. Gazz., 1851, 107) but structural investigation did not lead to conclusive results until 1956.

The presence of an anthracene nucleus in barbaloin was demonstrated by Tilden (J. Chem. Soc., 1877, 32, 267) who obtained methyl anthracene on zinc dust distillation. On chromic acid oxidation he isolated a product which he called "aloe xanthin". This was later shown to be a mixture of aloe emodin (II) and rhein (III) by Aesterle and Babel (Arch. Pharm., 1903, 241, 604). These compounds were proved to be derivatives of chrysazin (IV) by Aesterle (Arch. Pharm., 1909, 247, 417, 533; 1911, 250, 305) who converted aloe-emodin into chrysophanic acid (V) by reduction and into rhein by oxidation.

The structures of aloe-emodin and rhein have also been confirmed by synthesis. Mitter and Banerjee (J. Indian Chem. Soc., 1932, 9, 375) converted synthetic rhein, prepared by the method of Eder and Widner, (Helv. Chim. Acta, 1922, 5, 3) to the acid chloride (VI) and thence to the aldehyde (VII). Reduction gave the corresponding alcohol which was shown to be identical with aloe emodin.

In 1916 Leger (Ann. Chim., 1916, 6, 318) found that prolonged acid treatment of barbaloin with ethanolic hydrochloric
acid over a period of six months to a year yielded aloe-emodin and D-arabinose. Leger suggested that barbaloin was an anthraquinone D-arabinoside (VIII).

In 1931, Hauser (Pharm. Acta Helv., 1931, 6, 79) found that treatment with borax solution resulted in the breakdown of barbaloin into aloe-emodin anthrone (IX). As simple alkaline hydrolysis of barbaloin yielded aloe-emodin anthrone, it appeared that barbaloin contained an anthrone or anthranol nucleus and not an anthraquinone nucleus as suggested by Leger. This nucleus, however, could not be bound to the sugar by a simple glycosidic link as it did not undergo hydrolysis under the conditions normally employed for hydrolytic fission of a glycoside.

Permanganate oxidation of the Purdie methylated product of barbaloin gave rhein dimethyl ether (X), (Cahn, Simonsen, J. Chem. Soc., 1932, 2573) proving that the two phenolic groups must be free and not involved in linkage with the sugar residue.

In 1952, Mühleman (Pharm. Acta Helv., 27, 17) succeeded in synthesising barbaloin. On condensing aloe-emodin anthrone with tetra-acetyl-α-D-glucopyranosyl bromide in aqueous acetone containing sodium hydroxide he obtained tetra-acetyl barbaloin. Deacetylation yielded barbaloin identical with the natural product. It would be expected that the synthetic
product would be a glycoside derived from either the anthranol or the aliphatic hydroxyl group; but, on the contrary, Mühlemann believed that the structure of his synthetic product (or barbaloin) was (XI).

Recently Birch and Donovan (Austral. J. Chem., 1955, 8, 523) have put forward work which supported this structure by demonstrating that the ultra-violet absorption spectrum was only consistent with an aloe-emodin anthrone rather than an anthranol system in barbaloin and its derivatives, but they did not interpret their results to give structure (XI).

On reduction with palladium charcoal in acetic acid barbaloin gave a deoxy-barbaloin (Owen, Chem. and Ind., 1956, R 37) which formed a dimethyl ether with diazomethane and differed from barbaloin only in having a methyl group in place of the hydroxymethyl at C(3), as oxidation gave 3-methyl chrysazin (V). Owen also showed that barbaloin took up two moles of sodium metaperiodate with the formation of formic acid but no formaldehyde. This proved the presence of a vicinal triol system not terminating in -CH₂OH and was thus in harmony with the C-glucosyl structure.

Hay and Haynes (J. Chem. Soc., 1956, 3141) studied the infrared spectra of barbaloin, its derivatives and other related compounds, and concluded that barbaloin contained an aloe-emodin anthrone nucleus with the sugar attached at the
6(10).

Ferric chloride oxidation of barbaloin was shown by Cahn and Simonsen (*J. Chem. Soc.*, 1932, 2573) to give aloes-\,emodin. By removing the inorganic materials from the mother liquors of this reaction by ion-exchange technique, Hay and Haynes isolated D-arabinose and later Ali and Haynes (*J. Chem. Soc.*, 1959, 1033) detected D-arabinose and D-glucose.

Smith and Cleve (*J. A.C.S.*, 1955, 77, 3091) recently described a method for the determination of the ring size of glycosides in which the glycoside is oxidised with periodate and the resulting dialdehyde is reduced with sodium borohydride to compounds of the type:

\[
\begin{align*}
\text{OR} & \quad \text{and} \quad \text{OR} \\
\text{CH} & \\
\text{CH}_2\text{OH} & \\
\text{CH}_2\text{OH} & \\
\text{CH}_2 & \\
\end{align*}
\]

These compounds are acetals and so readily hydrolysed with dilute mineral acids to give among other products ethylene glycol and glycerol. This method was modified by Viscontini, Hoch and Karrer (*Helv. Chim. Acta*, 1955, 38, 642) to a micro-method. Using this method Hay and Haynes showed that, on periodate oxidation of barbaloin followed by sodium borohydride.
dride reduction and hydrolysis, neither ethylene glycol nor glycerol could be detected thus showing that the dialcohol was not an acetal. Thus barbaloin was not a glycoside. If the C-glycosyl formula for barbaloin is considered it can be seen that the dialcohol (XIII) (for formulae see page opposite page 10) produced by the reduction of the dialdehyde (XII) would be an ether and so resistant to hydrolysis.

The C-glycosyl structure for barbaloin is now accepted as correct. The second compound that was investigated and found to be a C-glycosyl compound was bergenin.

Bergenin, C_{14}H_{16}O_{9}, is a colourless crystalline polyphenol. Tschitschibabin et al. (Annalen, 1929, 469, 93) showed that it contained a lactone ring, one methoxyl group, and two phenolic hydroxyl groups but no free carboxyl group. Methylation of bergenin with diazomethane gave di-O-methyl bergenin which on permanganate oxidation gave 5:6:7-trimethoxy-isocoumarin-3-carboxylic acid (Synthesised by Haworth, Pindred, Jefferies, J. Chem. Soc., 1954, 3617), 5:6:7-trimethoxy isocoumarin, and 3:4:5-trimethoxy phthalic acid. Alkali fusion of bergenin gave 4-O-methyl gallic acid (XIV). On acetylation bergenin yielded a penta-acetyl derivative which still contained one active hydrogen atom (Zerewitinoff) but could not be further acetylated. From these results Tschitschibabin formulated bergenin as the isocoumarin derivative (XV).
In 1950 Shimokôriyama (Science, Japan, 1950, 20, 576) pointed out that structure (XV) did not explain the resistance to complete acetylation and proposed structure (XVI). The evidence for this appeared to be based entirely on the fact that bergenin readily reduces Fehlings solution.

Hay and Haynes (J. Chem. Soc., 1958, 2231) showed that bergenin had only five free hydroxyl groups by analysis of di-, tri-, and penta-O-methyl bergenin and so only one oxygen atom had to be accounted for. This could only be present as an ether oxygen in the side chain. Periodate oxidation of di-O-methyl bergenin prepared by the action of diazomethane on bergenin showed a rapid uptake of one molecule of reagent. The compound obtained after opening the lactone ring took up two molecules of periodate showing that the two free hydroxyl groups in the side chain must be adjacent to the hydroxyl group involved in the lactone ring formation. Since di-O-methyl bergenin showed no reducing properties structures (XV) and (XVI) must both be wrong as both would give a hetro-hexose side chain on opening the lactone ring in bergenin. So Hay and Haynes put forward structure (XVII) for bergenin. They confirmed this structure by the following synthesis. Condensation of tetra-acetyl-<D-glucopyranosyl bromide with the methyl ester of 4-methoxy gallic acid in the presence of sodium methoxide, followed by
hydrolysis and lactonisation gave bergenin identical with the natural product.

Reduction of the periodate oxidation product of di-O-methyl bergenin with sodium borohydride followed by acidification yielded glycerol which was detected by paper chromatography. In common with barbaloin, bergenin is a C-glycosyl compound but barbaloin when oxidised with periodate and the sodium borohydride reduction product hydrolysed did not give glycerol. Hay and Haynes then studied the conditions required for cleavage of the ether linkage in periodate-oxidised di-O-methyl bergenin. Oxidation of di-O-methyl bergenin with one molecule of periodate must give the dialdehyde (XVIII) which they found to break down slowly at room temperature especially in the presence of excess periodate giving 5:6:7-trimethoxyisocoumarin-3-aldehyde. At room temperature the above reaction was slow but on addition of sodium hydroxide a white flocculent precipitate separated and was identified as the trimethoxyisocoumarinaldehyde. It appeared therefore that the formation of the trimethoxyisocoumarinaldehyde from the dialdehyde (XVIII) was a base catalysed reaction analogous to the alkaline hydrolysis of glycosides of alcohols substituted in the β-position by a negative group. (See Ballou, Adv. Carbohydrate Chem., 1954, 9, 88).
Thus bergenin, since it is synthesised from acetobromoglucose must have structure (XIX). This structure was also confirmed by Posternack and Durr (Helv. Chim. Acta, 1958, 41, 1159).

Interest was then turned to Carminic acid which Fieser and Fieser had postulated as being a C-glycosyl compound.

Carminic acid, isolated from the female scale insect, Dactylopius coccus Costa, crystallises in small red prisms from methanol. The molecular formula $C_{22}H_{22}O_{13}$ was advanced by Liebermann, Höring and Wiedermann (Ber., 1900, 33, 149). In 1920 Dimroth (Ber., 1920, 53, 471) as a result of degradative experiments done by himself and other authors (for review see Thomson, "Naturally Occurring Quinones", Butterworths, London, 1957, 222) put forward structure (XX) for Carminic acid. This formula incorporated a change in empirical formula from $C_{22}H_{22}O_{13}$ to $C_{22}H_{20}O_{13}$. Dimroth pointed out that this was necessary as Miller and Rhöde (Ber., 1897, 30, 1762) showed that carminic acid formed an octaacetyl derivative. As the nucleus accounted for four of the acetyl groups, four must be present in the side chain to which the formula $C_{6}H_{13}O_{5}$ had been assigned. Since the only possible ways to formulate this is either as a saturated univalent hydrocarbon radicle with five hydroxyl groups which is im-
possible or as a chain with an ether link in the middle, e.g. -CHOH-CHOH-CH₂-O-CH₂-CHOH-CH₂OH, which Dimroth considered improbable, he concluded that the formula of the side chain had to be amended to -C₆H₁₁O₅ to allow for a six carbon atom chain with four hydroxyl groups and the fifth oxygen atom present in an ethereal, aldehydic or ketonic form.

This was the stage the work had reached when Ali and Haynes (J. Chem. Soc., 1959, 1033) started to investigate the structure of the C₆H₁₁O₅ side chain. It was known that the side chain contained four hydroxyl groups and that ozonolysis (Miyagawa, Men. Coll. Eng. Kyushu Imp. Univ. (Japan), 1924, 4, 99) gave on reduction a sugar which could not be characterised. On methylation with diazomethane carminic acid gave methyl carminate tetramethyl ether (methylation of the four phenolic hydroxyl groups) which at 0°C consumed 2.1 molecules of periodate with the formation of formic acid. This showed the presence of a >C(OH)-CH(OH)-C(OH)< system in the side chain.

Like barbaloin, carminic acid gave D-arabinose and D glucose on oxidation with ferric chloride. Ozonolysis also gave the same two sugars. These results showed that the side chain in carminic acid was glucopyranosyl. Thus they formulated carminic acid as (XXI).

Excluding the work in this thesis the three compounds
described are the only examples of C-glycosyl compounds to which a structure has been assigned. Another compound, however, which appears to be related to this group of compounds is vitexin.

The yellow pigment, vitexin, along with homovitexin, from New Zealand puriri wood \((Vitex littoralis)\) was first examined by Perkin \((J. Chem. Soc., 1898, 73, 1019)\) who showed that degradation of vitexin with alkali gave phloroglucinol, p-hydroxybenzoic acid and p-hydroxyacetophenone. Perkin also prepared a polyacetate and proposed the empirical formula \(C_{15}H_{14}O_7\) or \(C_{17}H_{16}O_8\) for vitexin. In a later publication \((J. Chem. Soc., 1900, 77, 422)\) he noted that vitexin gave the same degradation products as apigenin and formed a tetranitro derivative which he showed to be tetranitroapigenin. From these results Perkin revised the empirical formula to \(C_{21}H_{20}O_{10}\) and suggested that the compound was a very stable glucoside of apigenin with an abnormal attachment of the sugar residue.

Barger \((J. Chem. Soc., 1906, 89, 121)\) later examined vitexin which he claimed was formed along with a compound homovitexin by the acid hydrolysis of a precursor, saponarin. Barger proposed \(C_{15}H_{14}O_7\) as the empirical formula of vitexin on the basis of molecular weight and acetyl values and drew up structures \(\text{(XXII)}\) and \(\text{(XXIII)}\) as possible structures for
vitexin. In a private communication to Barger, Perkin proposed a third possibility (XXIV) which was later supported by Pefeteri (*J.Chem.Soc.*, 1939, 1635).

Nakaoki (*J.Pharm.Soc. Japan*, 1944, 64, No.11A, 51) prepared an octaacetyl derivative and on fusion with alkali the hydriodic acid reduction product of vitexin gave hexanoic acid whilst on permanganate oxidation vitexin gave 2:4:6-trihydroxyphenylacetic acid. Therefore Nakaoki concluded that vitexin was an apigenin derivative having a 2:3:4:5:6-pentahydroxy-n-hexyl residue attached to the 6- or 8- position.

Evans, McGookin, Jurd, Robertson and Williamson (*J.Chem. Soc.*, 1957, 3510) found from analysis of vitexin and its derivatives that the empirical formula was $\text{C}_{21}\text{H}_{20}\text{O}_{10}$ as previously proposed by Perkin. The compound contained no C-methyl groups and on acetylation with acetic anhydride and sodium acetate formed a heptaacetate whilst acetylation by the pyridine method gave a pentaacetate having a positive ferric chloride reaction. Methylation of the pentaacetate with diazomethane gave tetra-O-acetyltri-O-methylvitexin and with methyl iodide and potassium carbonate it gave tetra-O-acetyltrio-O-methylvitexin. On deacetylation the former gave di-O-methylvitexin and the latter tri-O-methylvitexin.

Hydrolysis of tri-O-methylvitexin with hot aqueous sodium hydroxide gave p-methoxyacetophenone, and p-anisic acid
but with boiling barium hydroxide an additional product, di-O-methylapovitexin, $C_{14}H_{16}O_{7}(O\text{Me})_2$ was obtained. With an excess of aqueous periodic acid di-O-methylapovitexin gave 3-formyl-4:6-di-O-methylphloracetophenone (XXV). The oxidation of tri-O-methylvitexin with lead tetraacetate or warm dilute nitric acid gave 8-formyltri-O-methylapigenin (XXVI).

From these degradations they proposed that vitexin contained a $C_6$-system at the 8-position of the apigenin residue and could be represented as (XXVII).

Since vitexin formed a heptaacetate Evans et al concluded that the side chain contained four hydroxyl groups and since the infrared spectrum showed only one carbonyl peak, the fifth oxygen atom had to be present as an ether linkage. Thus the side chain could be written $-C_6H_7O(OH)_4$.

Oxidation of vitexin with periodic acid at room temperature showed a rapid uptake of 1.5-2 molecules of reagent followed by a gradually slowing oxidation but the results were somewhat variable. The authors interpreted the initial stages as 1:2-glycol splitting but traces of formaldehyde and formic acid were formed with further oxidation. With sodium periodate the results were equally variable. The authors did not regard the small amounts of formaldehyde as significant. A yellow, laevorotatory solid, $C_{21}H_{18}O_{10}$ was
isolated with small amounts of 8-formylapigenin from the oxidation. The composition of this product accorded with the fission of a cyclic 1:2-glycol and accordingly the authors called it dehydrosecovitexin. Improved yields were obtained when one molecular proportion of the oxidising agent was used.

Dehydrosecovitexin reduced Fehlings solution and ammoniacal silver nitrate but showed no aldehydic absorption in the infrared spectrum. It formed a pentaacetate. Unlike vitexin dehydrosecovitexin is sensitive to acids. With a warm solution of 2:4-dinitrophenylhydrazine in dilute sulphuric acid it yielded a precipitate of the bis-2:4-dinitrophenylhydrazone of pyruvaldehyde. With hot methanolic sulphuric acid dehydrosecovitexin gave two optically active crystalline products (A and B) \( \text{C}_{18}\text{H}_{12} \text{O}_6(\text{OME})_2 \) which were readily separated by fractional crystallisation.

The authors pointed out that, since vitexin did not contain a C-methyl group, the pyruvaldehyde produced must be an artifact the most probable source being glyceraldehyde.

\[
\text{CH}_2\text{OH-CH(OH)-CHO} \xrightarrow{\text{H}^+} \text{CH}_3\text{-CO-CHO} + \text{H}_2\text{O}
\]

With methyl iodide and potassium carbonate compounds A and B formed dimethyl ethers, \( \text{C}_{18}\text{H}_{10} \text{O}_4(\text{OME})_4 \), which gave a monop-nitrobenzoate thus showing two phenolic and one alcoholic hydroxyl group. From their ultra-violet spectra compounds
A and B were apigenin derivatives.

To account for the observed reactions the authors put forward formulae (XXVIII) to (XXXIII) (See page opposite page 16). They represented vitexin as (XXVIII) which on uptake of one molecule of periodate gave (XXIX) which they said rearranged spontaneously to the hemi-acetal (XXX) thus accounting for the lack of aldehydic absorption in the infrared. By the action of acids glyceraldehyde and (XXXI) are formed and with methanolic sulphuric acid (XXXII) is produced which cyclises to form (XXXIII) thus accounting for the presence of only two phenolic hydroxyl groups.

This cannot be regarded as a rigid proof as the authors have not accounted for the anomalous results obtained with periodate oxidation or for the formation of formic acid. If a C-glycosyl structure were postulated a series of reactions could be proposed which would also account for the reported facts (See formulae (XXXIV) to (XXXVII) opposite page 24).

With the anomalous periodate uptake and the formation of formic acid, the above work can not be said to prove conclusively the structure of vitexin. It has been found that periodate oxidations carried out at 0° have given much more dependable results and does not result in the oxidation of the phenolic portion of the molecule. If vitexin or trimethyl vitexin were oxidised by this method the true periodate
uptake could be determined and in that way the correct structure for vitexin.

The first part of this thesis describes work carried out on homonataloin which is shown to be another C-glucosyl derivative closely related to barbaloin.
**THE STRUCTURE OF HOMONATALOIN**

**Introduction**

Natal aloes was exported in considerable quantity from Natal as far back as 1870. This aloe was unlike Cape aloes as it was grey-brown and opaque and contained a crystalline principle not found in other sources. Flückiger and Hanbury (Arch.Pharm., 3rd series, IV, 11) reported that the plant from which the drug was isolated was grown in Upper Natal between Pietermaritzburg and the Quathlamba mountains, especially in the Umwat and Mooi River Counties at an elevation of 2,000-4,000 feet. They stated that the plant was a large aloe but could not identify it botanically.

The exports of Natal aloes given by the Blue Book of Natal are:

<table>
<thead>
<tr>
<th>Year</th>
<th>1869</th>
<th>1870</th>
<th>1871</th>
<th>1872</th>
<th>1873</th>
<th>1874</th>
<th>1875</th>
<th>1876</th>
<th>1877</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloes(cwt.)</td>
<td>39</td>
<td>646</td>
<td>372</td>
<td>501</td>
<td>350</td>
<td>-</td>
<td>23</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

The aloes were prepared from the plant by cutting the leaves obliquely into slices and allowing the juices to bleed out. The juices were concentrated in iron pots with stirring to prevent burning and while still hot poured into wooden boxes for export.

On examining Natal aloes in 1871, Flückiger and Hanbury found it to contain a crystalline component much less soluble
in ethanol and water than the ordinary aloin from Barbados aloes. Further examination proved its distinctness from the latter compound and they accordingly named it nataloin. They obtained the pure drug by triturating the crude aloes with an equal weight of ethanol at a temperature not exceeding 48°. This dissolved the amorphous portion from which the crystals were filtered, washed with ethanol and crystallised from methanol giving 16-25% yield.

In 1889 Bainbridge and Morrow (Pharm.J., 1889, 570) described a colour reaction using concentrated sulphuric and concentrated nitric acid by which they could distinguish nataloin from the other aloes and on the basis of this test suggested that Natal Aloes might have been obtained from A. Succotrina. This was contradicted by J.M. Wood in 1890 (Pharm.J., 1890-91, 495) who looked for the plant at Greytown from where it was exported. The average height of the plant was 8-10 feet and he believed it to be A. Ferox. However, Bainbridge (Pharm.J., 1890-91, 899) showed that the plant specimen brought from Natal was not the same as A. Ferox grown in Kew.

XXV, 229) and Tschirch and Klaveness (Arch. Pharm., CCXXXIX,
232) had done some work on nataloin before Leger and assigned
the following properties to nataloin:
1. It crystallised in quadratic plates, nearly insoluble in
water, even when hot, slightly soluble in alcohol, insoluble
in ether.
2. It dissolved in caustic alkalis showing phenolic nature.
3. It gave oxalic and picric acid with nitric acid.
4. It gave no bromide or chlorine derivatives.
5. Alcohol solutions turned red slowly in air, more rapidly
in the presence of bromine fumes.

6. It contained one O-methyl group.

Leger treated a quantity of aloes with twice its weight
of acetone (not alcohol as used by Flückiger). The mixture
was allowed to stand for five to six days with occasional
shaking in which time the material was completely disintegrated,
the crude aloin forming a powder mixed with a black liquid.
The yellow-green powder was filtered off and washed with
acetone. The crude aloin was dissolved in 60% ethanol (100 g.
per litre) and after filtering the solution was allowed to
stand. Crystals were collected the following day and recryst-
allised from methanol. The first few crops were in the
form of crusts adhering to the sides of the flask. After continued evaporations Leger found that the crystals ceased to form crusts but formed small plates which made the mother liquors solidify. The latter fractions were found to be rich in nataloin and those which separated as crusts contained another compound which Leger named homonataloin.

Leger assigned the formula $C_{22}H_{22}O_{10}$ to homonataloin but on examination of his analytical figures (Found: $C$, 58.68, 58.72, 58.67, 58.60; $H$, 5.75, 5.76, 5.68, 5.55%) we found that they agreed better with $C_{22}H_{26}O_{10}$ (Calculated: $C$, 58.7; $H$, 5.77%). He also reported $[\alpha]_{D}^{21} = -112.6$ for $C = 0.5053$ in ethyl acetate and $[\alpha]_{D}^{18} = -149.7$ for $C = 0.9992$ in 60% ethanol.

Acetylation with acetic anhydride and sodium acetate gave a product which Leger claimed was not a simple ester since the naturally laevorotatory homonataloin was converted into a racemic compound. He obtained three different types of pentaacetyl derivative. The first formed octahedral crystals which separated on addition of an equal volume of water to the acetylation mixture. This he claimed was the pentaacetyl derivative of dl homonataloin. On pouring the mother liquors into excess water a reddish precipitate was obtained and was recrystallised from ethanol giving fine needles. This compound he called $\alpha$-pentaacetylhomonataloin to distin-
guish it from an amorphous compound $\beta$-pentaacetylhomonataloin which was left in solution.

A tetrabenzoylhomonataloin was obtained from benzoylation with benzoyl chloride in pyridine and a pentabenzoyl derivative when the former compound was heated for half an hour with excess benzoyl chloride.

Leger interpreted his analytical figures for nataloin (Found: C, 59.33, 59.46; H, 5.78, 5.89) to give $C_{23}H_{24}O_{10}$ but on inspection it was found that they agreed better with $C_{23}H_{23}O_{10}$ (Calculated: C, 59.47; H, 6.08).

As in the case of homonataloin he found three pentaacetyl, a tetrabenzoyl and a pentabenzoyl derivative could be prepared.

Oxidation of both nataloin and homonataloin with sodium peroxide gave an anthraquinone derivative, $C_{16}H_{12}O_{6}$, which was a methoxy derivative of an emodin isomeric with aloe-emodin. Leger called this oxidation product methylnataloemodin.

With concentrated sulphuric acid methylnataloemodin gave a purple colour which changed to green when exposed to nitric acid fumes. It gave oxalic and a little picric acid when oxidised with nitric acid but no nitro derivatives corresponding to tetrabitroaloe-emodin or chrysaminic acid.

On potassium hydroxide fusion, Leger found that methylnataloemodin gave a colourless acid in very small yield. As
the acid melted and sublimed at the same temperatures as α-hydroxymetaphthalic acid Leger assumed that it had that formula.

Methylnataloemodin also gave a diacetyl and a pentabromo derivative.

On treatment with concentrated hydrochloric acid at 170-180° in a sealed tube methylnataloemodin underwent demethylation forming nataloemodin, C₁₅H₁₀O₅·H₂O, m.p. 214-5°.

Nataloemodin formed a triacetyl derivative and so confirmed the demethylation.

On these grounds Leger proposed structure (XXXVIII) (See page opposite page 24) for methylnataloemodin. This structure accounted for the formation of a pentabromo derivative, the transformation of methylnataloemodin to nataloemodin and the formation of α-hydroxymetaphthalic acid.

Leger also isolated D-arabinose by refluxing homonataloin with concentrated hydrochloric acid.

Leger suggested that homonataloin was the condensation product of D-arabinose and methylnataloemodin. If this were the case the formula would be C₂₁H₂₀O₉ with only four hydroxyl groups as he postulated a linkage from the C₅ of the sugar. He thus found it necessary to postulate that in the reaction that formed the methylnataloemodin a group containing the fifth hydroxyl was eliminated. He thus put forward
formula (XXXIX) for homonataloin.

The sugar, he argued, would be joined through the primary alcohol group leaving the aldehydic group free which would explain the reducing properties of homonataloin: the instability of the \(-\text{CH}_2\text{OH}\) group under the influence of \(\text{Na}_2\text{O}_2\) could be explained by the nearness of the \(-\text{OH}\) group.

Nataloin has the same properties as homonataloin and gave the same degradation products and Leger suggested that it differed simply by the replacement of the \(-\text{CH}_2\text{OH}\) group by a \(-\text{CH}_2\text{CH}_2\text{OH}\) group which disappeared under the influence of sodium peroxide.

Since Leger's paper Rosenthaler (Pharm.Acta Helv., 1931, 6, 115) has confirmed Leger's report of finding two aloins in Natal aloes. He, like Leger, separated them by fractional crystallisation from methyl alcohol.

Two notes have also been published on the botanical origin of Natal aloes. R. Marloth (Pharm.Acta Helv., 1928, 3, 10) suggested that the plant might be Aloe Candelabrum Berger and in 1933 Bryant (Pharm.J., 130, 174) suggested that the source was either A. Candelabrum Berger or A. Marlothii Berger.
\[(XXX VIII)\]
\[(XXX IX)\]
\[(XXX IV)\]
\[(XXX V)\]
\[(XXX VII)\]
\[(XXX VI)\]
Botanical Origin of Natal Aloes

Investigation of the behaviour of the juices of different species of aloes by paper chromatography using n-butanol saturated with water as solvent had revealed that each species showed a different pattern when the chromatogram was viewed under ultra-violet light after having been fumed with ammonia. It thus appeared that this might be used as a method to determine the botanical origin of Natal aloes.

An authentic sample of Natal aloes (which had been collected some 50 years ago) was obtained from the museum of the School of Pharmacy of London University. To determine whether the age of the sample had any effect on its composition Cape aloes, of the same age as the Natal aloes obtained from London, and fresh Cape aloes were chromatographed. Both showed the same pattern when viewed under ultra-violet light, showing that the apparent composition of the sample was unaffected by its age.

Parts of the leaves of the following aloe plants were obtained from Inverleith and Kew Botanical Gardens:

<table>
<thead>
<tr>
<th>Inverleith Botanical Gardens</th>
<th>Kew Botanical Gardens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe Candelabrum</td>
<td>Aloe Candelabrum</td>
</tr>
<tr>
<td>Aloe Distans</td>
<td>Aloe Ferox</td>
</tr>
<tr>
<td>Aloe Macracantha</td>
<td>Aloe Vera</td>
</tr>
<tr>
<td>Aloe Plicatilis</td>
<td>Aloe Eru</td>
</tr>
<tr>
<td></td>
<td>Aloe Perryi</td>
</tr>
</tbody>
</table>
The leaves were cut obliquely and the juice allowed to run into petri dishes. Papers were spotted with these juices and with markers of Curacao, Cape and Natal aloes. The papers were run with n-butanol saturated with water, allowed to dry, fumed with ammonia and viewed under ultra-violet light. The \( R_F \) and colours of the different fluorescent spots were noted (See Table 1).

The chromatograms were kept for a year and the colours of the spots which developed on aerial oxidation observed. It was found that barbaloin and all aloes which contained barbaloin had a yellow spot with \( R_F \) 0.62 but that Natal aloes developed a purple spot with \( R_F \) 0.68. Of the different aloes examined only A. Distans and A. Macracantha developed this spot. This suggested that Natal aloes could be obtained from either or both of these species, but the paper chromatographic pattern obtained from A. Distans resembled that from Natal aloes much more closely than that from A. Macracantha.

An attempt was made to isolate homonataloin from a small sample of the juice of A. Distans obtained by Dr. P.C. Spensley and Miss A. Male of the Tropical Products Institute from Kew Botanic Gardens, but this was unsuccessful.
<table>
<thead>
<tr>
<th>Type of Aloe</th>
<th>Colours of fluorescent spots with the following Rp's</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.82  0.68  0.62  0.4  0.34  0.11  0.09  0.07  0.03  0.02</td>
</tr>
<tr>
<td>Barbaloin</td>
<td>Bluish yellow-blue</td>
</tr>
<tr>
<td>Cape Aloes</td>
<td>Bluish yellow-blue Orange-brown</td>
</tr>
<tr>
<td>Curacao Aloes</td>
<td>Bluish yellow-blue Orange-brown</td>
</tr>
<tr>
<td>Homonataloain</td>
<td>Brown</td>
</tr>
<tr>
<td>Natal Aloes</td>
<td>Blue brown</td>
</tr>
<tr>
<td>A. Candelabrum</td>
<td>Blue Orange-brown</td>
</tr>
<tr>
<td>A. Distans</td>
<td>Blue brown</td>
</tr>
<tr>
<td>A. Vera</td>
<td>Orange-brown blue</td>
</tr>
<tr>
<td>A. Plicatilis</td>
<td>Yellow blue</td>
</tr>
<tr>
<td>A. Eru</td>
<td>Bluish yellow-blue Orange-brown</td>
</tr>
<tr>
<td>A. Ferox</td>
<td>Bluish yellow-blue Orange-brown</td>
</tr>
<tr>
<td>A. Perryi</td>
<td>Orange-brown Yellow</td>
</tr>
<tr>
<td>A. Macracantha</td>
<td>Brown</td>
</tr>
</tbody>
</table>
Isolation of Homonataloin

There appears to be some doubt about the number of aloins present in Natal aloes as Leger (Ann. Chim., 1918, 8, 265) described the isolation of two aloins which he called nataloin and homonataloin. These he separated by fractional crystallisation from methyl alcohol. Tschirch and Klaveness (Arch. Pharm., 239, 231) reported, however, that they could only isolate the compound which Leger described as homonataloin. Rosenthaler (Pharm. Acta Helv., 1931, 6, 115) supported Leger in that he reported the isolation of two aloins by fractional crystallisation from methanol.

In our work, after some preliminary experiments, we followed the isolation procedure described by Leger, using as raw material the sample of Natal aloes obtained from the Museum of the London School of Pharmacy. The resinous impurities were removed by treatment with acetone and the resulting impure aloin crystallised from 60% ethanol and recrystallised from methanol. Several crops were obtained and were each recrystallised twice from methanol. The infrared spectra of all these crops, except the last which was brown coloured and contained slow-running impurities when investigated by paper chromatography, were identical and the analytical figures were virtually the same as those obtained by Leger for homonataloin (found: C, 59.1, 58.9; H, 5.87, 5.34. Leger's
figures for homonataloin C, 58.67; H, 5.68%). No trace of the compound nataloin could be seen.

Since barbaloin could be purified very simply using calcium salt precipitation (Cf. Harders, Pharm. Weekblad, 1949, 84, 250, 273) this method was tried for the isolation of homonataloin but no calcium salt precipitated on basifying the solution of the aloe in aqueous calcium chloride solution.

In an attempt to isolate the two aloins chromatography on heavy magnesium carbonate columns was tried. It was found that the aloin could be eluted with 25% ethyl alcohol-ethyl acetate (v/v) but again only one aloin, homonataloin, was obtained.

The structural investigation of homonataloin was undertaken and no further isolation of the other aloin attempted. The reason for the disappearance of the nataloin might be that the compound had decomposed as Leger reported that nataloin was much less stable than homonataloin; but it is possible that nataloin is merely a homonataloin with methanol of crystallisation.

**Structural Investigation of Homonataloin**

From the literature it was obvious that the structure proposed for homonataloin could not account for some of its properties (e.g. its resistance to hydrolysis) and since the structure of the emodin obtained by peroxide oxidation was only
a proposed structure based on very little experimental evidence it was decided to re-examine the structure of homonataloin.

**Analytical Results.**

Three samples of pure homonataloin were analysed. The first sample was dried over phosphoric anhydride in vacuo at room temperature and gave C, 58.9, 59.0; H, 5.75, 5.97; 0, 35.5, 35.3; 0.Me, 7.30, 7.42%. The second sample was dried at 100° and gave C, 59.1, 58.9; H, 5.87, 5.84; 0, 35.6, 35.5; 0.Me, 7.37, 7.10; (C)-Me, 3.65, 3.73%, active hydrogen, 1.55, 1.78. These analyses are in agreement with Leger's figures for homonataloin (C, 58.67; H, 5.68%) and are in agreement with the empirical formula C\(_{22}^{}\)H\(_{26}^{}\)O\(_{10}^{}\) although Leger interpreted them as C\(_{22}^{}\)H\(_{22}^{}\)O\(_{10}^{}\). Assuming this empirical formula the results show one C-methyl group (required, 6.39%), one O-methyl group (required 3.33%) and either 7 or 8 active hydrogen atoms. The third sample was dried at 140° and a loss in weight of 3.83% was observed. Analysis of the dried sample gave C, 61.3, 61.1; H, 5.58, 5.63; 0, 33.3, 33.1%. This analysis agrees with C\(_{22}^{}\)H\(_{24}^{}\)O\(_{9}^{}\) (calculated, C, 61.1; H, 5.59; 0, 33.3%) and the loss in weight agrees with the removal of one molecule of water (required 3.99%). The loss in weight on drying was repeated and a value of 4.01% obtained.

From these analytical figures the best formula for
homonataloin is $C_{22}H_{24}O_9$ with one methoxyl group, one C-methyl group and six active hydrogens but the compound normally exists as the monohydrate $C_{22}H_{24}O_9 \cdot H_2O$.

**Spectral Results.**

**Ultra-violet spectra.**

The ultraviolet spectra of homonataloin and barbaloin show quite a close resemblance as far as the position of the peaks are concerned but the intensities differ considerably (See appendix Figs. 1 and 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>lambda max</th>
<th>Log E</th>
<th>lambda max</th>
<th>Log E</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>4.38</td>
<td>208</td>
<td>4.41</td>
</tr>
<tr>
<td>~250</td>
<td>3.85</td>
<td>~255</td>
<td>3.77</td>
</tr>
<tr>
<td>~273</td>
<td>3.85</td>
<td>270</td>
<td>3.91</td>
</tr>
<tr>
<td>294</td>
<td>4.12</td>
<td>297</td>
<td>3.96</td>
</tr>
<tr>
<td>347</td>
<td>3.85</td>
<td>363</td>
<td>4.05</td>
</tr>
</tbody>
</table>

It thus appeared that homonataloin, like barbaloin, must contain an anthrone nucleus.

**Infrared Spectra**

The presence of an anthrone nucleus was confirmed by the study of the infrared spectrum of homonataloin. The carbonyl stretching frequencies of it and some relevant compounds are
given in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>C = 0 stretching frequencies (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinone</td>
<td>1676</td>
</tr>
<tr>
<td>1-hydroxyanthraquinone</td>
<td>1673, 1636</td>
</tr>
<tr>
<td>Chrysazin</td>
<td>1674, 1625</td>
</tr>
<tr>
<td>Anthrone</td>
<td>1654</td>
</tr>
<tr>
<td>1-hydroxyanthrone</td>
<td>1633</td>
</tr>
<tr>
<td>Dithranol</td>
<td>1631</td>
</tr>
<tr>
<td>Barbaloin</td>
<td>1630</td>
</tr>
<tr>
<td>Homonataloin</td>
<td>1638</td>
</tr>
</tbody>
</table>

Anthraquinone possesses two unassociated carbonyl groups which give rise to the band at 1676 cm⁻¹. This band is also present in 1-hydroxyanthraquinone and chrysazin but in addition there are bands at 1636 and 1625 cm⁻¹ which arise from the hydrogen bonded carbonyl groups. Anthrone contains a single carbonyl peak which shows as a band at 1654 cm⁻¹. This is displaced to 1633 and 1631 cm⁻¹ in 1-hydroxyanthrone and dithranol respectively by the introduction of the hydroxyl groups. The infrared spectrum of homonataloin has only one band in the carbonyl stretching frequency region at 1638 cm⁻¹ thus showing that homonataloin has an anthrone nucleus and that the carbonyl group is associated. The band in homonataloin has not been shifted as far as the one in barbaloin (1630 cm⁻¹).
showing that the carbonyl group is not as heavily hydrogen banded as the one in barbaloin and probably points to the fact which is confirmed later that there is only one α-hydroxy group.

**Periodate Oxidation**

Periodate oxidation of homonataloin at 0° resulted in the consumption of 1.98, 1.97 and 1.92 mols/mol of oxidant, suggesting the presence of a 1:2:3 triol or two diol systems in the molecule. The former was shown to be correct by means of a technique described by Buchanan, Dekker and Long for the detection of formic acid by means of sprays.

**Reduction and attempted hydrolysis oxidation product**

Evidence that homonataloin is not a simple arabinoside of methylnataloemodin as postulated by Leger was obtained by applying to homonataloin a method evolved by Smith and Van Cleve (J.Amer.Chem.Soc., 1955, 77, 3091) to determine the ring structure of naturally occurring glycosides. The periodate oxidised glycoside is reduced with sodium borohydride. The acetal thus obtained is readily hydrolysed with acid to give either ethylene glycol or glycerol depending on the structure of the ring.
A paper chromatographic method is described by Viscontini, Hoch & Karrer (Helv.chim.Acta, 1955, 642) by means of which microgram quantities of ethylene glycol and glycerol can be detected. Using this method no glyceraldehyde or ethylene glycol was obtained with homonataloin thus showing that homonataloin is not a simple glycoside. If a C-glycosyl compound is treated by this method reduction to the di-alcohol results in the formation of an ether which should be stable to acid.

That homonataloin is not a simple glycoside was supported by the fact that it was not hydrolysed by heating with N sulphuric acid for 2 hours at 100\(^\circ\) under which conditions glyco-
sides are known to split (Cf. Gardner, Foster, J.A.C.S., 1936, 58, 597).

Investigation of the Anthrone Nucleus

Ferric chloride oxidation of homonataloin by means of a micro technique gave arabinose and a hydroxyanthraquinone which were detected by means of paper chromatography. Using a macro technique methylnataloemodin was isolated. Recrystallised from ethanol it formed orange needles, m.p. 235-6.

Found, C, 67.9; H, 4.03; (C)-Me, 3.71% which agrees with Leger's empirical formula for methylnataloemodin C_{16}H_{16}O_{5} (Calculated, C, 67.6; H, 4.26%). Assuming this formula the value 3.71% for the C-methyl determination corresponds to 0.70 (C)-methyl groups.

Methylation of this material with diazomethane gave a compound which crystallised in orange needles from methanol m.p. 204-6°. This compound analysed for a dimethoxy compound. Found, O-Me, 20.3%; required for 2 O-Me groups 19.4%. It also shows a value of 7.69% for a C-methyl determination which is equivalent to 1.5 C-methyl groups.

Demethylation of methylnataloemodin with concentrated hydrochloric acid at 170° gave after fractional sublimation a compound m.p. 212-3° in agreement with Leger's melting point for nataloemodin which he obtained in a similar way.

The structural investigation of these three degradation
products will be considered at the same time.

Spectral Results

Ultra-violet spectra

The shape of the three ultraviolet spectra of these degradation products are very similar and resemble closely the shape of \( \alpha \)-hydroxy anthraquinone spectra (Cf. spectrum of aloe-emodin fig. 6 in the appendix).

**TABLE 4**

<table>
<thead>
<tr>
<th>Methylnataloemodin</th>
<th>Methylated Methylnataloemodin</th>
<th>Nataloemodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>log E</td>
<td>( \lambda_{\text{max}} )</td>
</tr>
<tr>
<td>228</td>
<td>4.36</td>
<td>228</td>
</tr>
<tr>
<td>275</td>
<td>4.22</td>
<td>272</td>
</tr>
<tr>
<td>295</td>
<td>4.02</td>
<td>293</td>
</tr>
<tr>
<td>400</td>
<td>3.80</td>
<td>396</td>
</tr>
</tbody>
</table>

Briggs, Nicholls and Patterson (*J. Chem. Soc.*, 1952, 1718) noted that all hydroxy anthraquinones exhibit a broad absorption band due to the quinonoid nucleus. These bands fall into three distinct groups: \( \lambda_{\text{max}} 356-362.5 \) \( \mu \) (log E 3.36-3.81); 407-413.5 \( \mu \) (log E 3.57-3.93) and 427.5-432.5 \( \mu \) (log E 3.92-4.13). Inspection of the formulae of these compounds revealed that the common feature of the first group is that they contain no free \( \alpha \)-hydroxyl groups, in the second that they contain
one free $\alpha$-hydroxyl group and in the third that they contain two such groups.

**TABLE 5**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{max}}$</th>
<th>log E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthragallol-1:2-dimethyl ether</td>
<td>362.5</td>
<td>3.36</td>
</tr>
<tr>
<td>Anthragallol-trimethyl ether</td>
<td>356</td>
<td>3.53</td>
</tr>
<tr>
<td>Anthragallol</td>
<td>413.5</td>
<td>3.72</td>
</tr>
<tr>
<td>Anthragallol-2-methyl ether</td>
<td>407.5</td>
<td>3.57</td>
</tr>
<tr>
<td>Aloe emodin</td>
<td>430</td>
<td>4.02</td>
</tr>
</tbody>
</table>

Although the values of $\lambda_{\text{max}}$ 400 and 396 $\mu m$ are slightly out of the range stated by Briggs et al for anthraquinones with one $\alpha$-hydroxyl group it can be stated with reasonable certainty that Methylnataloemodin and methylated methylnataloemodin fall into that group of compounds and also that nataloemodin contains two $\alpha$-hydroxyl groups. Thus the demethylation of methylnataloemodin involves the formation of an $\alpha$-hydroxyl group thus showing that the methoxyl group in methylnataloemodin and so in homonataloain is in the $\alpha$-position.

**Infrared spectra**

The positioning of the two $\alpha$-hydroxyl groups was done by means of the infrared spectra of the three degradation products. Table 6 shows the peaks exhibited by these compounds.
and other relevant compounds in the carbonyl stretching frequency region.

**Table 6**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( C = 0 ) stretching frequencies (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methynataloemodin</td>
<td>1654, 1638</td>
</tr>
<tr>
<td>Methylated methynataloemodin</td>
<td>1667, 1636</td>
</tr>
<tr>
<td>Natalemodin</td>
<td>1653, 1631</td>
</tr>
<tr>
<td>Alizarin</td>
<td>1660, 1636</td>
</tr>
<tr>
<td>Rhein dimethyl ether</td>
<td>1672, 1649, 1726</td>
</tr>
</tbody>
</table>

Methynataloemodin and methylated methynataloemodin show peaks at 1638 and 1636 respectively corresponding to the carbonyl stretching frequency of an associated carbonyl group which is also observed in homonataloin. In nataloemodin the peak is displaced to a lower frequency showing that the carbonyl group is more heavily hydrogen banded. This points to the fact that the two hydroxyl groups are probably present in the 1:3 positions instead of the 1:4 or 1:5 which are the other possibilities. This conclusion is supported by the fact that a second band is present in the carbonyl stretching frequency in the spectrum of nataloemodin at 1653 cm\(^{-1}\). This is rather low for an unassociated carbonyl frequency as is the second band in methynataloemodin but this is probably due to the effect of a substituent in the \textit{para} position with
respect to the carbonyl group. This effect is also observed in alizarin (1660 cm\(^{-1}\)) and rhein dimethyl ether (1649 cm\(^{-1}\)). If the two \(\alpha\)-hydroxyl in nataloemodin were in the 1:4 or 1:5 positions only one carbonyl peak would be observed. In methylated methylnataloemodin the second peak is at 1667 which is nearer the correct frequency for unassociated carbonyl peaks (1676 cm\(^{-1}\)) thus showing that methylation of a hydroxyl group reduced the effect of that group on the carbonyl group in the \textit{para} position.

**Paper Chromatography**

Shibata, Takito and Tanaka (\textit{J.A.C.S.}, 1950, \textit{72}, 2789) described the paper chromatography of hydroxyanthraquinones using petroleum ether (b.p. 45-70\(^\circ\)) saturated with 97% methanol at 19\(^\circ\) as solvent system. The chromatograms were sprayed with 0.5% methanolic magnesium acetate and heated at 90\(^\circ\) for 5 minutes. Colours developed depending on the position of the hydroxyl groups. Anthraquinones with one hydroxyl group developed no colour with the reagent. 1:3 gives orange-red; 1:4 purple and 1:2 violet. Inspection of their results (see table 7) shows that the \(R_F\)'s of the compounds also are dependent on the position of the substituents. If only \(\alpha\)-hydroxyl groups are present the \(R_F\)'s are between 0.89 and 0.92 but introduction of a \(\beta\)-hydroxyl group roughly halves these \(R_F\) values. Methylation of the \(\beta\)-hydroxyl group in emodin causes
a change of $R_F$ from 0.52 to 0.89.

**TABLE 7**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Position of Substituents</th>
<th>$R_F$</th>
<th>Colour of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysophenol</td>
<td>4:5-dihydroxy-2-methyl</td>
<td>0.92</td>
<td>Orange</td>
</tr>
<tr>
<td>Quinizarin</td>
<td>1:4-dihydroxy</td>
<td>0.89</td>
<td>purple</td>
</tr>
<tr>
<td>Physcionne</td>
<td>4:5-dihydroxy-7-methoxy-2-methyl</td>
<td>0.89</td>
<td>orange</td>
</tr>
<tr>
<td>Emodin</td>
<td>4:5:7-trihydroxy-2-methyl</td>
<td>0.52</td>
<td>pink</td>
</tr>
<tr>
<td>Rubiadin</td>
<td>1:3-dihydroxy-2-methyl</td>
<td>0.49</td>
<td>orange-yellow</td>
</tr>
<tr>
<td>Alizarin</td>
<td>1:2-dihydroxy</td>
<td>0.04</td>
<td>violet</td>
</tr>
</tbody>
</table>

When the three degradation products were investigated by this procedure the results obtained are shown in table 8.

**TABLE 8**

<table>
<thead>
<tr>
<th>Substance</th>
<th>$R_F$</th>
<th>Colour of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>methylnataloemodin</td>
<td>0.27</td>
<td>orange</td>
</tr>
<tr>
<td>methylated methylnataloemodin</td>
<td>0.89</td>
<td>yellow</td>
</tr>
<tr>
<td>nataloemodin</td>
<td>0.10</td>
<td>purple</td>
</tr>
</tbody>
</table>

Since methylation of methylnataloemodin causes a change in $R_F$ from 0.27 to 0.89 this suggests the methylation of a $\beta$-hydroxyl group. The change in colour developed by the reagent
from orange to purple on demethylation shows that either a 1:2- or a 1:4-system has been liberated. Since the 1:4 system is excluded on infrared grounds the only possibility is a 1:2 system. It is therefore possible to place the hydroxyl and methoxyl groups in methynataloemodin as in structure (XL)

![Structure diagram]

Since the analysis showed $C_{16}H_{12}O_5$ with one methoxyl and one C-methyl groups it only remains to place the C-methyl group.

**Methylation of Homonataloin with Diazomethane**

Homonataloin was dissolved in methanol and methylated with diazomethane. Chromatographic investigation showed that the product was homogeneous and that it had a slightly greater $R_F$ than homonataloin. Attempts were made to crystallise this compound from various solvents, the best results were obtained with ethyl acetate but the compound separated as an amorphous solid. This solid was sent for analysis and found to analyse for $C_{24}H_{30}O_{10}$ with three methoxyl groups.

The infrared spectrum of this compound showed only one
band in the carbonyl stretching frequency region at 1666 cm\(^{-1}\) showing that the carbonyl group was now unassociated and so both \(\alpha\)-hydroxyl groups must have been methylated.

**Permanganate oxidation of Methylated Homonataloin**

Permanganate oxidation of methylated homonataloin followed by acidification, continuous extraction with ether and evaporation of the ether extract to dryness gave a yellow solid which on purification by sublimation under high vacuum gave white needles, m.p. 167-9\(^{\circ}\).

Some of this solid was heated with concentrated sulphuric acid and resorcinol at 130\(^{\circ}\) for 5 minutes, dropped into water and the resulting solution made alkaline with sodium hydroxide. A solution was obtained which fluoresced green under ultraviolet light showing that a 1:2-dicarboxylic acid or anhydride was present.

Analysis of the compound gave C, 56.3; H, 4.05; O, 29.11\%. Calculated for C\(_{10}\)H\(_8\)O\(_5\): C, 57.7; H, 3.87\% which gives a value of 29.8\% for two methoxyl groups.

The infrared spectra of the compound showed peaks at 1844 and 1775 cm\(^{-1}\) which suggest that the compound is an acid anhydride. It showed no absorption due to hydroxyl groups and some at 2900 cm\(^{-1}\) due to C-H stretching.

On these grounds we identified the compound as hemipinic anhydride (3:4-dimethoxyphthalic anhydride) which has melting
point 169° (Wegscheider, Monatsh. 3, 351) or 166-7° (Beckett, Wright, J. Chem. Soc., 29, 282), and the correctness of this identification was confirmed by comparison with an authentic sample. From the isolation of this acid it was concluded that the C-methyl group was not on the ring containing the two methoxyl groups in methylated homonataloemodin. It was assumed on biogenetic grounds that the C-methyl group was in a \( \beta \)-position. If it had been on the ring containing the two methoxyl groups a derivative of trimellitic acid would have been formed. Since decarboxylation was considered possible during sublimation at 150° literature was consulted and it was found (Rodd, Chemistry of Carbon Compounds, III B, Elsevier, London, 1956, p.866) that trimellitic acid melted at 238° with the formation of trimellitic anhydride. It was thus argued that if a trimellitic acid derivative had been formed on permanganate oxidation no decarboxylation would have occurred during sublimation at 150°. Hence if the assumptions made were correct the C-methyl group must be in the ring containing one methoxyl group in methylated homonataloin, the 3-position of which is favoured on biogenetic grounds. Methylnataloemodin is therefore formulated as (XL I).

![Formula (XL I)]
Investigation of the Sugar Residue.

Ferric chloride oxidation

Oxidation of homonataloin with aqueous ferric chloride solution gave rise to methylnataloemodin. The mother-liquors from this oxidation were extracted with amyl alcohol until free from methylnataloemodin, inorganic material was then removed using ion-exchange resins and the resulting solutions evaporated to a small volume and then freeze-dried giving a strongly laevorotatory syrup (59.5 mg). Chromatography in three solvent systems (see experimental for details) showed the syrup to be arabinose. The sugar was fully characterised as D-arabinose by the preparation of its benzoyl-hydrazone (mixed melting point). Leger obtained the same sugar by prolonged acid treatment of homonataloin. It is known that homonataloin is stable to acid under conditions which usually effect hydrolysis of a glycoside and it therefore appears likely that Leger's degradation is not a hydrolysis but an aerial oxidation of homonataloin.

Ozonolysis of Homonataloin.

Homonataloin was ozonised for 2 hours in 50% aqueous ethanol, the ethanol was then removed by steam distillation and the ozone passed through the resulting aqueous solution for a further 3 hours. The ozonides were decomposed by steam distillation and the residues, after extraction with ether,
were treated with lead acetate whereby most of the impurities were removed. The excess lead was precipitated with hydrogen sulphide and the resulting almost colourless solution evaporated to dryness. The resulting syrup was dissolved in water and on investigation by paper chromatography with a marker containing arabinose, glucose and xylose in three different solvent systems (for details see experimental) the solution was found to contain glucose and arabinose.

The sugars were separated by chromatography on thick paper cutting the papers at the appropriate positions and eluting with water.

D-arabinose was again characterised by the preparation of its benzoyl hydrazone derivative (mixed melting point).

The other sugar was characterised as D-glucose by preparation of its p-nitroaniline derivative (mixed melting point and rotation).

**Attempted Borax hydrolysis of homonataloin**

Since homonataloin resembled barbaloin so closely the isolation of the anthrone degradation product (Cf. isolation of aloe-emodin anthrone from barbaloin) was attempted. This proved unsuccessful due to the instability of homonataloin in alkaline solutions.

**Structure of Homonataloin**

From the foregoing analysis it is possible to postulate
a structure for homonataloin. The molecular formula is 
\( C_{22}H_{24}O_9 \), with one methoxyl group, one C-methyl group and 
six active hydrogens.

Oxidation with aqueous ferric chloride solution gives
methylnataloemodin (1-methoxy-2:8-dihydroxy-6-methyl anthra-
quinone) thus accounting for the methoxyl group, the C-methyl 
group, and two active hydrogens.

Evidence from the infrared spectrum of homonataloin
however shows that it is a derivative of anthrone. It has
also been observed that homonataloin does not give the colour
reactions characteristic of anthrones (Reaction with p-nitro-
sodimethylaniline), therefore it must be mono- or di- substi-
tuted at \( C_{10} \).

Since homonataloin resembles barbaloin so closely in its
reactions (Cf. resistance to hydrolysis, sugars obtained by
ferric chloride oxidation and ozonolysis, etc.) it can be
postulated that homonataloin like barbaloin must be a C-gly-
cosyl derivative of a substituted anthrone (XLII).
This formula would account for all the following observed properties of homonataloin:

(1) Analytical figures.

(2) Infrared and ultra-violet spectra.

(3) Preparation of the trimethoxyderivative on methylation with diazomethane.

(4) Isolation of methylnataioemodin and D-arabinose on oxidation with ferric chloride.

(5) The isolation of D-arabinose and D-glucose on ozonolysis.

(6) Consumption of two molecules of sodium periodate with the liberation of formic acid.

(7) Resistance to hydrolysis.

(8) Resistance to hydrolysis of the borohydrde reduced periodate oxidation product.
EXPERIMENTAL
METHODS OF ISOLATION OF NATALOIN AND HOMONATALOIN

In the following methods of isolation the Natal aloes used was obtained from the Museum of the London School of Pharmacy.

(A) Chloroform and Methyl Acetate Extraction

Natal aloes (10 g.) was finely powdered, mixed with silver sand (50 g.) and extracted in a Soxhlet extractor for 48 hours with chloroform. The chloroform was cooled to room temperature and the yellow solid which had separated was collected and dried. Yield, 3 g. This solid was chromatographed on paper using n-butanol saturated with water as solvent and was found to contain three components all of which showed as fluorescent spots under U.V. light. (See table below for R_F's).

This material (0.5 g.) was extracted with cold (2 x 5 ml.) and then boiling methyl acetate (2 x 5 ml.) and the residue dissolved in the minimum of ethanol. The four methyl acetate extracts and the ethanol solution were investigated paper chromatographically and the following results obtained:
<table>
<thead>
<tr>
<th></th>
<th>1st Component</th>
<th>2nd Component</th>
<th>3rd Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Cold methyl acetate extraction</td>
<td>+</td>
<td>trace</td>
<td>+</td>
</tr>
<tr>
<td>2nd Cold methyl acetate extraction</td>
<td>trace</td>
<td>trace</td>
<td>+</td>
</tr>
<tr>
<td>1st boiling methyl acetate extraction</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2nd boiling methyl acetate extraction</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol solution</td>
<td>-</td>
<td>bulk</td>
<td>trace</td>
</tr>
</tbody>
</table>

On evaporation and cooling of the ethanol solution yellow plates were obtained. These were recrystallised from methanol.

Melting point = 203-4°

Yield from partially purified mixture = 50 mg.
% yield = 3% of original Natal aloes.

(B) Removal of impurities with acetone.


Natal aloes (78 g.) was powdered and shaken with acetone (156 ml.) for 24 hours on a mechanical shaker. The mixture was filtered giving a red solution and a greenish brown solid. The solid (28 g.) which contained the aloins was heated with 60% ethanol (300 ml.) and the hot solution filtered. The solution was allowed to stand overnight and
yielded a first crop which was recrystallised from 60% ethanol. A first crop (described as 1(a) in the following table) was obtained and on further evaporation a second crop (1(b)) was obtained. Other crops were obtained from the original mother liquor after removal of the first crop by further evaporation and all were recrystallised once from 60% ethanol.

<table>
<thead>
<tr>
<th>Yield</th>
<th>Rotation in ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 g.</td>
<td>([\alpha]_D^{27} = -117.3^\circ, C = 1.108)</td>
</tr>
<tr>
<td>1.6 g.</td>
<td>([\alpha]_D^{28} = -113.0^\circ, C = 1.002)</td>
</tr>
<tr>
<td>0.7 g.</td>
<td>([\alpha]_D^{27} = -116.5^\circ, C = 0.953)</td>
</tr>
<tr>
<td>0.4 g.</td>
<td>([\alpha]_D^{28} = -109.7^\circ, C = 0.976)</td>
</tr>
<tr>
<td>1.2 g.</td>
<td>([\alpha]_D^{28} = -29.5^\circ, C = 1.082)</td>
</tr>
</tbody>
</table>

Crops 1(a), 1(b), 2 and 3 were each recrystallised twice from methanol and crop 4 once from 60% ethanol and infrared spectra of the resulting compounds obtained. Crops 1(a), 1(b), 2 and 3 were found to be identical but crop 4 which was more brown coloured than the others showed basically the same I.R. spectrum with some additional peaks due to impurity.

(C) Calcium Salt Precipitation.

*Cf. C.I. Harders, Pharm. Weekblad, 1949, 84, 250, 273.*

Natal aloes (0.6 g.) was stirred in water (2 ml.) and
hydrochloric acid (0.2 ml. of 2N) added. On heating, an
orange solution was obtained to which a solution of calcium
chloride (0.5 ml. of 50%) was added. The solution was
allowed to stand overnight, heated to 80°C and sodium sul-
phite (20 mg.) added with ammonium hydroxide (0.04 ml. of
20%). No appreciable amount of solid separated (Cf. puri-
fication of barbaloin).

(D) Magnesium Carbonate Column.

Three columns, 9 inches long by 0.75 inch diameter,
were packed with heavy magnesium carbonate as a slurry in
ethyl acetate and washed with that solvent until the columns
were firmly packed.

Three extracts of Natal aloes were prepared as follows:
(1) Natal aloes (0.5 g.) was boiled with ethyl alcohol
(10 ml.). Complete solution was obtained and ethyl acetate
(30 ml.) was added. Solid precipitated and was removed by
filtration. The resulting extract was run on to the column.
(2) Natal aloes (0.5 g.) was boiled with 25% ethyl alcohol-
ethyl acetate (v/v) (40 ml.). The solid that remained
undissolved was removed by filtration and the extract run
on to the column.
(3) Natal aloes (0.5 g.) was boiled with ethyl acetate
(40 ml.). The pale yellow solution which remained after
the removal of the undissolved solid was run on to the column.
The solid which was precipitated in (1) and that which did not dissolve in (2) and (3) were investigated paper chromatographically with n-butanol saturated with water as solvent. A trace of homonataloin was detected in the solid from the third method of extraction but not in the other two.

It had been found previously that homonataloin did not move down the column when eluted with pure ethyl acetate but did when eluted with 25% ethyl alcohol-ethyl acetate. So each of the above columns were eluted with the latter solvent and it was found that a yellow band moved down the column leaving a red band of resinous impurities at the top. 10 ml. fractions were collected from each of the columns and investigated by means of paper chromatography. In each case the bulk of the homonataloin appeared in the first two fractions with only traces in the later fractions mixed with other impurities. On evaporation of the first two fractions crystals separated, which were collected and recrystallised from methyl alcohol yielding pure homonataloin.

No larger scale columns were run as a sufficient store of nataloin had been obtained by the method described by Leger.
ANALYSIS RESULTS

The first sample sent for analysis was obtained by the chloroform methyl acetate method (Cf. p.47). Recrystallised twice from methyl alcohol it formed quadratic plates, m.p., 202-4°; [α]_D^28 = -111.5°, C = 1.121. (Ethanol).

No loss in weight was observed on drying at room temperature over phosphoric anhydride under high vacuum.

Found C, 58.9, 58.9; H, 5.75, 5.97; O, 35.5, 35.3%.
Required for C_{22}H_{26}O_{10}: C, 58.7; H, 5.82; O, 35.5%.

Found -OMe, 7.30, 7.42. Required for one -OMe (assuming C_{22}H_{26}O_{10}) 6.89%.

The second sample was purified by the acetone method (Cf. p.48) and recrystallised from methyl alcohol three times. It had m.p. 202-4° and [α]_D^28 = -112.3°, C = 1.042 (ethanol).

0.89% loss in weight was observed on drying at 100°C over phosphoric anhydride under high vacuum.

Found: C, 59.1, 58.9; H, 5.87, 5.84; O, 35.7, 35.5; -OMe, 7.37, 7.10; (C)-CH₃, 3.65, 3.73; Active H, 1.83, 1.64%.
Required for one (C)-CH₃, 3.33; 7 active H, 1.55; 8 active H, 1.73%.

The third sample was purified in the same way as the second and had m.p. 202-4°.
3.83% loss in weight was observed on drying at 140°C in vacuo over phosphoric anhydride. The results of an analysis carried out on a dried sample were: Found: C, 61.3, 61.1; H, 5.58, 5.63; O, 33.3, 33.1%. Required for C_{22}H_{24}O_9: C, 61.1; H, 5.59; O, 33.3%. Required for one molecule of water, 3.99%.

A repetition of the loss of weight on drying was done in the laboratory with 30 mg. homonataloin and a loss in weight of 4.01% was observed.

From the preceding analyses the best formula for homonataloin is C_{22}H_{24}O_9 with one methoxyl group, one C-methyl group and six active hydrogens. As normally prepared, without rigorous drying, it forms a monohydrate, C_{22}H_{24}O_9·H_2O.

Ultra-violet Spectra.

The ultra-violet spectra of homonataloin and barbaloin in ethanol as solvent showed the following maxima. For spectra see appendix fig. 1 and 2.

<table>
<thead>
<tr>
<th>Homonataloin</th>
<th>Barbaloin</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max.}} )</td>
<td>log E</td>
</tr>
<tr>
<td>222</td>
<td>4.38</td>
</tr>
<tr>
<td>~250</td>
<td>3.85</td>
</tr>
<tr>
<td>~273</td>
<td>3.85</td>
</tr>
<tr>
<td>294</td>
<td>4.12</td>
</tr>
<tr>
<td>347</td>
<td>3.85</td>
</tr>
</tbody>
</table>
Infrared Spectrum

The infrared spectrum of homonataloin determined as a potassium bromide disc showed the following peaks:

3730 (W), 3480 (S), 3220 (S), 2900 (M), 2350 (W), 1638 (S), 1610 (S), 1588 (S), 1487 (S), 1452 (M), 1440 (M), 1378 (S), 1360 (M), 1330 (M), 1294 (S), 1270 (S), 1258 (M), 1215 (S), 1173 (W), 1160 (W), 1147 (W), 1128 (M), 1110 (M), 1087 (S), 1070 (S), 1068 (S), 1040 (M), 1025 (S), 984 (W), 969 (W), 935 (M), 908 (M), 882 (W), 856 (W), 842 (W), 833 (W), 776 (M), 757 (S), 727 (M), 696 (S) cm⁻¹.

(For spectrum see appendix Fig. 1).

Paper Chromatography

The solvent systems examined are summarised in the following table. Whatman No. 1 paper was used throughout.

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Homonataloin Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) n-butanol : water</td>
<td>0.68</td>
</tr>
<tr>
<td>(2) n-butanol : acetic acid : water (4:1:5)</td>
<td>0.69</td>
</tr>
<tr>
<td>(3) n-butanol : pyridine : benzene : water (5:3:1:3)</td>
<td>streaking</td>
</tr>
<tr>
<td>(4) ethyl acetate : pyridine : water (10:4:3)</td>
<td>streaking</td>
</tr>
<tr>
<td>(5) n-butanol : acetic acid : water (2:1:1)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

In all solvent systems containing pyridine very bad
streaking occurred. Acidic solvents were satisfactory as well as neutral ones. The solvent system most commonly used was butanol saturated with water in which homonataloin ran as a single spot with $R_f$ 0.68.
Quantitative Periodate Oxidation

Homonataloin (4-5 mg.) was weighed out accurately and dissolved in 50% ethanol. The solution was cooled to below 5° and standard potassium metaperiodate solution (10 ml., 0.003922M), which had been similarly cooled, added. The solution was kept overnight at 0° during which time the solution turned red. Saturated aqueous borax solution (5 ml.), boric acid (1 g.) and potassium iodide solution (5 ml., 10%) were then added and the liberated iodine titrated with arsenious oxide solution (0.00998N) using 1% starch solution as indicator.

Consumptions of 1.98, 1.97 and 1.92 mols. oxidant/mol. were obtained for homonataloin.

Use of Spray for Detecting 1:2:3 triols

(Cf. Buchanan, Dekker and Long, J.Chem.Soc., 1950, 3162). A few drops of a solution of homonataloin in methyl alcohol were spotted on a filter paper along with a few drops of a similar solution of barbaloin. The paper was allowed to dry, sprayed with 1% aqueous sodium metaperiodate solution and left for seven minutes. It was then sprayed with 10% aqueous ethylene glycol solution, left for ten minutes and sprayed with 5% aqueous potassium iodide solution. No
brown colour due to liberated iodine could be detected in either the case of homonataloin or barbaloin due to the red colour of the oxidation product but on spraying with 1% starch solution a blue spot due to the iodine-starch complex was seen in both cases. Thus it was concluded that homonataloin contained a 1:2:3 triol system.

Reduction and attempted hydrolysis of the periodate oxidation product

Cf. Viscontini, Hock and Karrer, Helv.Chim.Acta, 1955, 642; Hay and Haynes, J.Chem.Soc., 1956, 3145. Sodium metaperiodate (10 mg., 50 M) dissolved in water (2 ml.) was added to a solution of homonataloin (10 mg, 25 M) in ethyl alcohol (2 ml.) and the solution kept at 0° for four hours. The solution turned red. Sodium borohydride (15 mg.) in water (2 ml.) was then added, the solution turned yellow and was allowed to stand overnight at 0°. Hydrochloric acid (2 ml., 2N) was added to 2 ml. of the above solution and the mixture heated at 100° for 15 minutes.

The solution was spotted on a paper chromatogram with spots of ethylene glycol and glycerol to act as markers and allowed to run in ethyl acetate (10)-pyridine (4)-water (3). The papers were set aside to dry and were sprayed with periodate-permanganate reagent; (Cf. Lemieux
and Bauer, Anal. Chem., 1954, 26, 920) 4 parts 2% aqueous sodium metaperiodate and 1 part 1% potassium permanganate in 2% aqueous sodium carbonate solution. Glycerol (RF 0.43) and ethylene glycol (RF 0.54) yielded yellow spots on a red background. Homonataloin gave no spot corresponding to either glycerol or ethylene glycol but gave one with RF 0.13. On running another chromatogram with the solution, glycerol, ethylene glycol and hydrochloric acid it was found that the spot given by the homonataloin hydrolysate (RF 0.13) corresponded to that given by hydrochloric acid (RF 0.13).

Attempted Acid Hydrolysis

Homonataloin (10 mg.) was heated at 100° with N. hydrochloric acid (1 ml.) in a sealed tube for two hours. The solution was investigated by chromatography using two solvents: n-butanol saturated with water and n-butanol (5)-pyridine (3)-water (3)-benzene (1). The two chromatograms were allowed to dry. The first chromatogram was examined under ultra-violet light and only the spot corresponding to unchanged homonataloin could be detected. The second chromatogram was sprayed with saturated aqueous aniline oxalate solution and heated for 10 minutes at 140°. No spots corresponding to sugarlike materials could be detected.
but some interference was encountered due to the presence of hydrochloric acid. The above hydrolysis was repeated using $N$. sulphuric acid and the acid removed with barium carbonate. On examination by paper chromatography with the second solvent again no sugarlike materials could be detected.

**Ferric Chloride Oxidation of Homonataloin**

(1) **Micro Method**

Homonataloin (5 mg.), ferric chloride (25 mg.) and water (0.5 ml.) were heated in a sealed tube at 120° for 6 hours. The homonataloin did not dissolve in the water but reacted with the ferric chloride giving a black precipitate. The reaction mixture was cooled and the black precipitate collected. This precipitate dissolved almost completely in methyl alcohol. The aqueous and methyl alcohol solutions were paper chromatographed in the following two solvent systems: n-butanol (5)-pyridine (3)-water (3)-benzene (1), with arabinose as a marker, and n-butanol saturated with water, with nataloin as marker. The first chromatogram was allowed to dry, sprayed with saturated aqueous aniline oxalate solution and heated in an oven at 140° for 10 minutes. A very faint red spot developed with the same $R_F$ as arabinose but some trouble was encountered due to the presence of ferric chloride and so this could
only be taken as an indication of the presence of arabinose. The second chromatogram was fumed with ammonia and examined under U.V. light. No spot could be detected corresponding to homonataloin but one was present near the solvent front (Rf 0.9) probably due to a hydroxy anthraquinone, (Cf. Aloe Emodin - Rf 0.92, Chrysazin - Rf 0.92) and a spot at the origin probably due to ferric chloride.

(2) Macro Oxidation of Homonataloin


Homonataloin (1.5 g.), ferric chloride (7.5 g.) and water (22.5 ml.) were heated on an oil bath at 115° for 15 minutes and then at 125° for 4 hours. Because of the very slight solubility of homonataloin in water complete solution was not obtained, however, reaction occurred and a dark-brown solid separated. The mixture was cooled and the solid collected, dried and extracted (Soxhlet) into boiling toluene. The toluene extract was extracted with saturated aqueous sodium bicarbonate solution until the bicarbonate layer was no longer coloured. The toluene extract was dried (sodium sulphate) and evaporated to dryness. The solid obtained was crystallised from ethyl alcohol yielding an impure product (70 mg.), m.p. 208-228°. Sublimation was also tried as a method of purification without success. Finally recrystallisation from ethyl alcohol

The dark red filtrate obtained after the removal of the crude emodin was extracted with amyl alcohol (10 x 50 ml.). The yellow aqueous solution obtained was passed through columns of Dowex 50 W(H) until the eluate was free from ferrous ions. The colourless solution thus obtained was passed through columns of Dowex 2 (OH) until all chloride ions had been removed. The neutral solution was concentrated to a small volume and some solid that separated filtered off. The colourless solution was freeze-dried giving a yellow syrup which would not crystallise. The syrup was examined paper chromatographically using the following three solvents: (A) n-butanol (5)-pyridine (3)-water (3)-benzene (1); (B) Ethyl acetate (10)-Pyridine (4)-water (3); and (C) n-butyl alcohol (2)-acetic acid (1)-water (1). A marker containing Xylose, arabinose and glucose was run with the unknown in each solvent and the following results obtained. The sugar spots were detected by means of aniline oxalate.
The crude syrup (58.5 mg.) was strongly laevorotatory. It is clear from the above figures that homonataloin like barbaloin produces the same sugar, arabinose, on ferric chloride oxidation and from the rotation carried out on the crude syrup the sugar appears to be D-arabinose. This was confirmed by the preparation of the benzoyl hydrazone.

**Preparation of Benzoyl Hydrazine**

The method described by Naegeli, Stefanovitch, *Helv. Chim. Acta*, 1928, 11, 609, was found to be unsatisfactory in our hands as only the dibenzoyl compound could be isolated so the following method was used.

Hydrazine hydrate (25 g.) in ethanol (750 ml.) was cooled in a cardice-acetone bath and benzoyl chloride (12 g.) in ether (200 ml.) added dropwise during 3 hours with continuous stirring. The ethanol and ether were removed under vacuum, the residue washed with ether, and recrystallised from water giving benzoyl hydrazine (2.5 g.).
m.p. 111-113°C.

Preparation of the benzoyl hydrazone of arabinose


An ethanolic solution of benzoyl hydrazine (5 ml., 5%) was added to the sugar syrup (53.5 mg.) in water (1 ml.). The mixture was kept at room temperature with occasional shaking for 24 hours and then at 0°C for 48 hours. The derivative which separated was collected and recrystallised twice from ethanol. Yield 27.5 mg., m.p. 203-4°C.

Derivatives were also made from authentic D- and L-arabinose and the following melting points and mixed melting points obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample from ferric chloride oxidation</td>
<td>203-4°C decomp.</td>
</tr>
<tr>
<td>Authentic D-arabinose benzoyl hydrazone</td>
<td>204-5°C</td>
</tr>
<tr>
<td>Authentic L-arabinose benzoyl hydrazone</td>
<td>201-2°C</td>
</tr>
<tr>
<td>Authentic D- plus L-arabinose benzoyl hydrazone (50:50)</td>
<td>196-198°C</td>
</tr>
<tr>
<td>Derivative from sugar obtained by ferric chloride oxidation plus authentic D-arabinose benzoyl hydrazone</td>
<td>203-4°C</td>
</tr>
</tbody>
</table>

(3) Macro oxidation of homonataloin in 50% aqueous ethanol.

Homonataloin (1.5 g.) was dissolved in ethanol (15 ml.) and added to ferric chloride (8.0 g.) in water (15 ml.).
The mixture was refluxed on an oil bath at 115° for 6 hours giving a dark red solution. The mixture was allowed to cool, water (50 ml.) added and the solution evaporated until all the ethanol had been removed. A dark brown precipitate separated, was collected, dried and extracted into boiling toluene (Soxhlet). A similar method as described in (2) page 60 was employed in the purification of the methylnataloemodin. Yield, 58 mg., m.p. 235-6°. No work was done on the isolation of the sugar from this oxidation.
Methylnataloemodin

Analytical Results

The sample sent for analysis was obtained by the ferric chloride oxidation of homonataloin (Cf. p. 60). Recrystallised from ethyl alcohol it formed orange needles m.p. 235-60. No loss in weight was obtained on drying at 100° in vacuum over phosphoric anhydride for 2 hours. Found: C, 67.91; H, 4.03; (C)-Me, 3.71%.

Required for C_{16}H_{12}O_{5}: C, 67.60; H, 4.26%.

Assuming the formula C_{16}H_{12}O_{5} the value of 3.71% for C-methyl determination gives 0.70 (C)-methyl groups.

Chromatography

Methylnataloemodin was found to run at the solvent front with both butanol saturated with water and butanol(4)-acetic acid(1)-water(5). A satisfactory solvent system was found to be petroleum-ether (b.p. 50-70°) saturated with 97% methanol (Cf. Shibata, Takito, Tanaka, J.Amer.Chem.Soc., 1950, 72, 2739). The ascending method was used and methylnataloemodin found to run with R_{f} 0.27.

Ultra-Violet Spectrum

The ultraviolet spectrum of methylnataloemodin in ethanol as solvent showed the following maxima. (For
spectrum see appendix Fig. 3).

<table>
<thead>
<tr>
<th>( \lambda_{\text{max.}} )</th>
<th>log E</th>
</tr>
</thead>
<tbody>
<tr>
<td>228</td>
<td>4.36</td>
</tr>
<tr>
<td>275</td>
<td>4.22</td>
</tr>
<tr>
<td>295</td>
<td>4.02</td>
</tr>
<tr>
<td>400</td>
<td>3.80</td>
</tr>
</tbody>
</table>

**Infrared Spectrum**

The infrared spectrum of methylnataloemodin determined as a potassium bromide disc showed the following peaks:

3630 (W), 3350 (S), 2870 (W), 2340 (M), 1935 (W), 1915 (W), 1860 (W), 1840 (W), 1820 (W), 1800 (W), 1790 (W), 1770 (W), 1755 (W), 1730 (W), 1713 (W), 1700 (W), 1680 (W), 1654 (M), 1638 (S), 1615 (M), 1555 (S), 1535 (W), 1520 (W), 1505 (W), 1495 (W), 1480 (S), 1460 (M), 1447 (M), 1435 (W), 1417 (W), 1365 (M), 1338 (W), 1293 (S), 1274 (S), 1203 (M), 1156 (W), 1133 (W), 1140 (M), 995 (W), 928 (W), 863 (M), 852 (W), 798 (S), 760 (W), 749 (M). (For spectrum see appendix Fig. 2).

**Methylation of Methylnataloemodin with diazomethane**

Methylnataloemodin (47 mg. \( 1.6 \times 10^{-4} \) moles.) was dissolved in 50% methyl alcohol/ether (10 ml.) and Ethereal diazomethane (10 ml., prepared from 0.2 g. p-toluenesul-
phenyl methylmethylnitrosamine by the method of De Boer, Backer, 
Rec.Trav.Chim., 1954, 73, 229). Effervescence took place 
and the solution was left overnight.

The solution was then evaporated to a small volume 
(about 2 ml.) and on cooling orange needles separated. 
These were collected and recrystallised from methanol. 
Yield, 25 mg., m.p. 204-6°.

Chromatography of methylated methylnataloemodin

Methylated methylnataloemodin was found to run at the 
solvent front with butanol saturated with water and with 
butanol(4)-acetic acid(1)-water(5), but was found to run 
as a single spot in petroleum ether (b.p. 47-70°) saturated 
with 97% methanol with Rf 0.89.

Analysis Results for Methylated Methylnataloemodin

The sample sent for analysis was obtained by methyla-
tion of methylnataloemodin (Cf. p.66). Recrystallised 
from methanol it formed orange needles m.p. 204-6°. No 
appreciable loss in weight was observed after heating for 
3 hours under high vacuum over phosphoric anhydride.

Found: C, 69.7; H, 4.82; (C)-Me, 7.69; O-Me, 20.3%.
Required for C\textsubscript{17}H\textsubscript{14}O\textsubscript{5}: C, 68.5; H, 4.69%.
Required for C\textsubscript{18}H\textsubscript{16}O\textsubscript{5}: C, 69.2; H, 5.13%.
Required for 2-0-Methyl groups (assuming C\textsubscript{18}H\textsubscript{16}O\textsubscript{5}), 19.4%. 
Required for 1-C-methyl, 4.67, for 2-C-methyl, 9.34%.

**Ultraviolet Spectrum**

The ultraviolet spectrum of methylated methylnataloemodin in ethanol as solvent showed the following maxima:
(For spectrum see appendix Fig. 4).

<table>
<thead>
<tr>
<th>$\lambda_{\text{max}}$</th>
<th>log E</th>
</tr>
</thead>
<tbody>
<tr>
<td>228</td>
<td>4.34</td>
</tr>
<tr>
<td>272</td>
<td>4.20</td>
</tr>
<tr>
<td>293</td>
<td>3.95</td>
</tr>
<tr>
<td>396</td>
<td>3.76</td>
</tr>
</tbody>
</table>

**Infrared Spectrum**

The infrared spectrum of methylated methylnataloemodin determined as a potassium bromide disc showed the following peaks:

3480 (W), 2370 (M), 2320 (W), 2310 (W), 1667 (M), 1636 (S), 1563 (S), 1537 (W), 1513 (W), 1483 (S), 1450 (M), 1415 (M), 1385 (W), 1365 (S), 1332 (S), 1280 (S), 1265 (S), 1206 (S), 1162 (W), 1140 (W), 1070 (S), 1033 (M), 995 (M), 962 (S), 938 (M), 906 (W), 863 (M), 843 (W), 833 (W), 817 (M), 802 (M), 786 (M), 764 (W), 750 (S), 706 (W), 695 (W), 666 (W). (For spectrum see appendix Fig. 3).
Demethylation of Methylnataloemodin


Methylnataloemodin (10 mg.) was heated in a sealed tube with concentrated hydrochloric acid (0.5 ml.) for 12 hours at 170-180°C. The compound did not dissolve and hardly changed in form. The tube was cooled and opened and the crystals collected. After washing with water the crystals were recrystallised from methyl alcohol giving dark orange needles m.p. 205-225°. The product was then fractionally sublimed and two bands appeared on the tube: nataloemodin (4 mg.) m.p. 212-3°(sublimation temperature 110-75°) and an unknown compound which did not melt below 360° (sublimation temperature 130-150°). (Cf. Leger, loc.cit., nataloemodin m.p. 214-5°).

**Ultraviolet Spectrum**

The ultraviolet spectrum of nataloemodin in ethanol as solvent showed the following peaks. For spectrum see appendix Fig. 5.

<table>
<thead>
<tr>
<th>(\lambda_{\text{max}})</th>
<th>log E</th>
</tr>
</thead>
<tbody>
<tr>
<td>232</td>
<td>4.30</td>
</tr>
<tr>
<td>260</td>
<td>4.30</td>
</tr>
<tr>
<td>~290</td>
<td>4.00</td>
</tr>
<tr>
<td>432</td>
<td>3.89</td>
</tr>
</tbody>
</table>
Infrared Spectrum

The infrared spectrum of nataloemodin (KBr disc) showed the following peaks.
3550(M), 3200(M), 2930(W), 2320(W), 1653(M), 1631(S),
1555(W), 1537(W), 1455(S), 1435(M), 1423(M), 1335(S),
1212(W), 1200(W), 1135(W), 1075(W), 1033(M), 866(M),
822(M), 746(S), 695(W). (for spectrum see appendix Fig.4).

Ozonolysis of Homonataloin

Homonataloin (700 mg.) was dissolved in ethanol (50 ml.) and water (50 ml.) added. The solution was placed in a Drechsel bottle (150 ml.) and cooled to 0° in an ice bath. The bottle was kept partially immersed in the ice bath and a stream of ozonised oxygen from a high tension discharge apparatus (7,500 volts) was passed through the solution until its colour had changed from the original yellow to red and then back to orange-yellow (2 hrs.). The oxidised product was then steam distilled until all the alcohol had been removed (200 ml. distillate collected). The residue which turned reddish brown during the distillation was cooled and ozonised oxygen passed through it for a further 3 hours. The ozonised product was again steam distilled and the distillate (350 ml.) collected.
The residue which turned dark brown during the second steam distillation was extracted with ether (4 x 200 ml.) in a separating funnel. The dark brown aqueous layer was separated and saturated aqueous lead acetate solution (25 ml.) added. A dark brown solid precipitated slowly on standing and after allowing 1 hour for complete precipitation the solid was removed by repeated filtration yielding a clear yellow solution. Hydrogen sulphide was then passed through the heated filtrate which was subsequently heated on a water bath for a few minutes. The precipitated lead sulphide was collected. The pale yellow filtrate was concentrated to 100 ml. and again hydrogen sulphide was passed through it when more lead sulphide precipitated. This was collected and more hydrogen sulphide passed into the solution. Still more lead sulphide precipitated and was filtered off yielding a clear colourless solution which was evaporated to dryness under reduced pressure. A yellow syrup was obtained with a small amount of yellow solid. The syrup and solid were dissolved in water (5 ml.) and the solution used for paper chromatography.

**Chromatography of Sugars obtained from Ozonolysis**

The solution obtained from the ozonolysis of homonataloin was investigated chromatographically using the following three solvent systems, with a marker containing
glucose, arabinose and xylose: A, n-Butanol(5)-Pyridine(3)-
water(3)-benzene(1); B, Ethyl acetate(10)-pyridine(4)-
water(3); and C, n-butanol(2)-acetic acid(1)-water(1).
The results obtained are given in Table. Aniline oxalate
was used as the spraying reagent and the colour given with
that reagent is quoted in brackets.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>R xylose in A</th>
<th>R xylose in B</th>
<th>R xylose in C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>1 (pink)</td>
<td>1 (pink)</td>
<td>1 (pink)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.71 (brown)</td>
<td>0.63 (brown)</td>
<td>0.74 (brown)</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>0.84 (pink)</td>
<td>0.81 (pink)</td>
<td>0.91 (pink)</td>
</tr>
<tr>
<td>Sugars from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozonolysis of homonataloin</td>
<td>(0.70 (brown)</td>
<td>(0.63 (brown)</td>
<td>(0.73 (brown)</td>
</tr>
<tr>
<td></td>
<td>(0.84 (pink)</td>
<td>(0.81 (pink)</td>
<td>(0.90 (pink)</td>
</tr>
</tbody>
</table>

Separation and Purification of the Sugars

The solution containing the sugars was evaporated to
dryness yielding a yellow syrup (230 mg.). Water (2 ml.)
was added and the resulting solution chromatographed on two
sheets of Whatman 3 MM paper using ethyl acetate(10)-pyridine
(4)-water(3) as solvent. The chromatograms were allowed
to dry and narrow strips cut out from the side of each were
sprayed with saturated aqueous aniline oxalate. After heating the paper for 10 minutes at 140° the spots that developed showed that the papers had been overloaded and that no resolution of the sugars had been obtained. The sugars were eluted from the papers with water and the resulting solution evaporated to 2 ml. This solution was chromatographed on three sheets of Whatman No. 3MM paper using butanol(5)-pyridine(3)-water(3)-benzene(1) as solvent. After 48 hours the papers were dried and the positions of the separated sugars determined by cutting a thin strip off each paper chromatogram, spraying this with saturated aqueous aniline oxalated and heating as before. Resolution of the two sugars was obtained and the bands containing the individual sugars were cut from the chromatograms. The paper strips were eluted with water and the two solutions (of arabinose and glucose) thus obtained were evaporated to small volumes (ca. 2 ml.). These concentrates were investigated chromatographically and found to contain only one sugar each.

Preparation of the benzoyl hydrazone derivative of arabinose.

Cf. Hirst, Jones and Woods, J.Chem.Soc., 1947, 1048. The solution containing arabinose was evaporated to dryness giving a pale yellow syrup (120 mg.) which was dissolved
in water (2 ml.) and an ethanolic solution of benzoyl hydrazine (5 ml., 5%) added. The combined solutions were kept at room temperature for 8 hours and then at 0° for 14 hours. The crystalline derivative formed was collected and recrystallised from ethanol. The recrystallised product had m.p. 203-4° with decomposition. Mixed melting point with an authentic specimen of D-arabinose benzoyl hydrazone was 203-4° (Cf. derivative prepared from the arabinose obtained from ferric chloride oxidation page 63).

Preparation of the p-nitroanilide derivative of glucose.

Cf. Weygand, Perkow and Kuhner, Ber., 1951, 84, 594.

The aqueous solution containing glucose was evaporated to dryness yielding an almost colourless syrup (90 mg.) which was mixed with p-nitroaniline (90 mg.) and 2 ml. of a solution of concentrated hydrochloric acid (0.14 ml.) in methanol (200 ml.) added. The mixture was heated on a water bath until the solid dissolved (ca. 10 min.). On cooling yellow crystals were obtained m.p. 182-3° (Cf. literature m.p. 184°). Mixed melting point with an authentic specimen of D-glucose-p-nitroanilide showed no depression. Rotation in 10% aqueous pyridine \([\alpha]_D^{28} = -158°\) \(c = 0.164\). (Cf. literature \([\alpha]_D^{20} = -150\) to \(-215\) (\(c = 0.24\))).
Methylation of Homonataloin with Diazomethane

Homonataloin (50 mg.) was dissolved in methanol (25 ml.) and an ethereal solution of diazomethane (25 ml., from 0.4 g. p-toluenesulphonyl methyl nitrosamide by the method of De Boer, Backer, Rec.Trav.Chim., 1954, 73, 229) added. The solution effervesced and was allowed to stand at room temperature for 24 hours.

The solution was then evaporated to dryness under reduced pressure and various solvents were tried in an attempt to recrystallise the residual solid. Best results were obtained with ethyl acetate but recovery was small and the product was not crystalline.

Since recrystallisation was impossible an ethyl acetate solution of the methylated product was evaporated until solid began to separate, cooled and the solid collected.

Paper chromatography using butanol saturated with water as solvent showed that the substance moved as one spot $R_F$ 0.70. (Cf. homonataloin $R_F$ 0.68).

**Analysis**

The methylated homonataloin obtained above was dissolved in ethyl acetate and treated as above giving a light brown solid m.p. 144-7°. A sample (5 mg.) of this compound was analysed.
Found: C, 60.5; H, 5.90; O-CH₃, 18.6%.
Calculated for C₂₄H₂₈O₉·H₂O: C, 60.2; H, 6.32.
Calculated for 3 O-methyl groups, 19.4%.

Infrared Spectrum of Methylated Homonataloin

The infrared spectrum of methylated homonataloin (Cf. p. 75) done as a potassium bromide disc showed the following peaks:
3400(S), 2900(S), 2850(M), 2330(W), 1666(S), 1610(S),
1580(M), 1535(W), 1487(S), 1454(S), 1413(M), 1361(W),
1320(W), 1306(W), 1270(S), 1155(M), 1087(S), 1053(S),
1011(M), 970(S), 893(W), 838(M), 780(M), 742(W), 723(W),
696(M) (for spectrum see appendix fig. 2).

Attempted Borax Hydrolysis of Homonataloin


Homonataloin (50 mg.) was added to an aqueous solution (10 ml.) of sodium borate (1.0 g.) and phenylhydrazine hydrochloride (200 mg.) and refluxed for two hours in an atmosphere of nitrogen. The dark red solution was acidified with dilute hydrochloric acid and a dark brown
solid separated and was extracted with ether (ca. 100 ml.). Evaporation of the dried (sodium sulphate) ether extract gave a very small quantity of a red oil which could not be purified by recrystallisation from acetic acid (charcoal).

Permanganate Oxidation of Methylated Homonataloin

Gf. Cahn and Simonsen, J. Chem. Soc., 1932, 2573. Homonataloin (250 mg.) was dissolved in methanol (50 ml.) and an ethereal solution of diazomethane (50 ml., prepared from 1.0 g. p-toluenesulphonyl methylnitrosamide) added. The combined solutions were allowed to stand overnight at room temperature and then evaporated to dryness.

A little water was added to the residue and mixed to a paste. A 2.5% aqueous potassium permanganate solution (100 ml.) was added portionwise during 30 minutes. The mixture was heated on a water bath for 3 hours with occasional stirring. The manganese dioxide that separated was removed and the resulting pale yellow solution acidified with dilute hydrochloric acid. No solid separated and the solution was extracted with ether in a continuous extractor. The ether extract was evaporated to dryness giving a yellow solid which on sublimation at 150° under high vacuum gave a pale yellow solid (23 mg.) m.p. 148-160°.
After two further sublimations a white solid (10 mg.) was obtained m.p. 167-169°C.

**Resorcinol Test**


A few mg. of the acid was placed in a micro test tube with a few mg. freshly sublimed resorcinol and concentrated sulphuric acid (5 drops). The crucible was heated at 130°C for 5 mins. The test tube and contents were then dropped into water (10 ml.) and the resulting solution made alkaline with sodium hydroxide. A blank run was also carried.

On examination of the alkaline solutions under ultraviolet light the blank showed no fluorescence whereas the test solution showed a green fluorescence.

**Infrared Spectrum of Acid obtained by permanganate oxidation**

The infrared spectrum of the acid from permanganate oxidation (Cf. p. 77) determined as a potassium bromide disc showed the following peaks:

3450(w), 2900(w), 2320(w), 1944(s), 1775(s), 1737(w),
1715(W), 1700(W), 1685(W), 1660(W), 1650(W), 1640(W),
1620(W), 1600(W), 1585(W), 1560(W), 1530(W), 1505(S),
1455(M), 1435(M), 1395(W), 1360(M), 1291(S), 1256(S),
1203(W), 1165(M), 1150(W), 1057(S), 1016(M), 990(W),
934(M), 906(M), 884(S), 838(M), 752(M), 738(S), 710(W),
697(W), 675(W). (for spectrum see appendix Fig.8).

**Analysis of Acid obtained by permanganate oxidation**

The acid obtained by permanganate oxidation m.p. 167-9\(^\circ\) was sent for analysis.

Found: C, 56.3; H, 4.05; O-CH\(_3\), 29.11%.

Calculated for C\(_{10}\)H\(_8\)O\(_5\): C, 57.7; H, 3.87%.

Calculated for 2 O-methyl groups 29.8%.

The acid was identified as hemipinic anhydride m.p. 169\(^\circ\) by mixed melting point determination and comparison of infrared spectra.
Colour Reactions of Homonataloin and its degradation products


Three drops of p-nitrosodimethyl-aniline in pyridine (0.1% solution) were added to about 1-2 mg. solid. The following colours were observed.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Colour with p-nitrosodimethyl-aniline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homonataloin</td>
<td>No colour change</td>
</tr>
<tr>
<td>Methylnataloemodin</td>
<td>No colour change</td>
</tr>
<tr>
<td>Barbaloïn</td>
<td>No colour change</td>
</tr>
<tr>
<td>Anthrone</td>
<td>Dark red</td>
</tr>
<tr>
<td>Dithranol</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

Histedt's Reaction

Homonataloin (2-3 mg.) was treated with concentrated sulphuric acid (3 drops) in a watch glass. A yellow solution was obtained which turned green when exposed to nitric acid fumes.

Sprays used on a chromatogram of homonataloin

Several spots of a methanolic solution of homonataloin were placed on a paper and chromatographed with
n-butanol saturated with water as solvent. The chromatogram was allowed to dry, cut into strips and sprayed with the following reagents:
1. Diazotised sulphanilic acid.
3. Iodate-iodide.

The following results were obtained:
With spray 1 the homonataloin spot gave an orange colour showing the presence of phenolic hydroxyl groups.

With spray 2 the homonataloin spot showed up as a yellow spot on a red background showing that homonataloin is oxidised with sodium metaperiodate.

With spray 3 no reaction took place showing there were no free carboxyl groups.

Colours on chromatograms due to oxidation by air.

It was found that when a chromatogram was kept for some time (about 3-6 months) the compounds showed up as different colours due to oxidation by air. The following colour changes were observed:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homonataloin</td>
<td>pale yellow → purple</td>
</tr>
<tr>
<td>methylated homonataloin</td>
<td>pale yellow → orange</td>
</tr>
<tr>
<td>barbaloin</td>
<td>pale yellow → bright yellow</td>
</tr>
</tbody>
</table>
This fact can be used as a method of distinguishing homonataloin from barbaloin.

Colours of oxidation products with methanolic magnesium acetate


Nataloemodin, methylnataloemodin and methylated methylnataloemodin were chromatographed using petroleum ether (b.p. 47-70°) saturated with 97% methanol as solvent. The chromatograms were allowed to dry, sprayed with 0.5% methanolic magnesium acetate solution, and heated at 90° for 5 minutes. The colours that developed due to the hydroxy anthraquinones are given in table.

**TABLE**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R_F)</th>
<th>Colour with magnesium acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nataloemodin</td>
<td>0.10</td>
<td>purple</td>
</tr>
<tr>
<td>Methylnataloemodin</td>
<td>0.26</td>
<td>orange</td>
</tr>
<tr>
<td>Methylated methylnataloemodin</td>
<td>0.85</td>
<td>yellow</td>
</tr>
</tbody>
</table>
Fluorescence test for 1:4-anthraquinones.


Nataloemodin (0.5 mg.) was dissolved in glacial acetic acid and examined under ultra-violet light. No fluorescence was observed. Thus nataloemodin is not a 1:4 dihydroxy anthraquinone.
FIG. 4 METHYLNATALOEMODIN

FIG. 5 METHYLATED METHYLNATALOEMODIN

FIG. 6 NATALOEMODIN
FIG. 7 ALOE-EMODIN

FIG. 8 ACID FROM PERMANGANATE OXIDATION
PART II
Introduction

Bitter aloes, the dried juice of *Aloë vera* L. and related plants, is a brown resinous glass-like material. Up to about 25% of this resin is barbaloin, but little is known of the other components.

Recent work by Dopp (*Arzneimittel-Forschung*, 1955, 3, 627; 1955, 5, 391) has shown that crude aloes shows a tuberculostatic effect at dilutions of 1:20,000 and that although barbaloin is tuberculostatic at dilutions of 1:50,000 - 1:100,000, the resin remaining after the removal of aloin from bitter aloes also shows a tuberculostatic effect at dilutions of 1:20,000. Still more recently Rovatti and Brennan (*Ind. Med. Surg.*, 1959, 28, 364) have shown that ointments prepared from aloes are of value in the treatment of thermal burns and it has been suggested that similar ointments are of value in the treatment of radiation 'burns'. This work, together with our general interest in the aloins, pointed to a study of the other components of bitter aloes.

Early work on the separation of aloes into its different components had not given any conclusive results. Various authors gave the following contents of aloes: barbaloin, a small amount of aloë emodin, resins like the
resitannol ester of p-coumaric or cinnamic acid, a little volatile oil and ash 1:5:3 percent. (For analysis see Tschirch, Hoffbauer, Arch.Pharm., 1905, 243, 399; van Italic, Pharm.Weekblad, 1905, 42, 553; Seel, Arch.Pharm., 1919, 257, 212, 229, 254). No work was done to elucidate the structure of the compounds called resitannols.

In 1956 Mary, Christensen and Jack (J.Amer.Pharm.Ass., 1956, 45, 229) in a chromatographic study of aloes detected the presence of aloe-emodin and aloe-emodin anthrone in Cape aloes, Curacao aloes, Socotrine aloes. They prepared chloroform extracts of the drugs and of the materials obtained by hydrolysis with 30% sulphuric acid. These extracts were examined by paper chromatography, using the ascending technique, with petroleum ether (b.p. 65-110°) saturated with 97 percent methyl alcohol at room temperature as the solvent system. The papers were then sprayed with 0.5 percent methanolic magnesium acetate (cf. Shibata and Takido, J.Pharm.Soc. Japan, 72, 2739) to detect hydroxyanthraquinone derivatives. These compounds, however, were only present in very small amounts and were probably degradation products of barbaloin.

In 1954 Anterhoff and Ball (Arzneimittel-Forschung, 4, 725) investigated the constitution of aloes. They found that resin prepared by extraction of crude aloes
with ethyl alcohol and repeated fractional precipitations with acetone and ether gave an alkaline hydrolysis p-coumaric acid, acetic acid and a resinatannol which on acid hydrolysis gave 3.5% arabinose. Oxidation of the resinatannol with nitric acid gave picric acid.

Paper chromatographic studies of different aloes by Awe and Wachsmuth-Melm (Pharm. Ztg., 1957, 102, 1043) showed that Cape aloes, when chromatographed on paper using butanol(4)-acetic acid(1)-water(5) as solvent system, resolved into four spots with $R_F$'s 0.22, 0.5, 0.65 and 0.81. They sprayed the chromatograms with Hirschsohn's reagent (Pharm.Zent.Deutschland, 1901, 42, 63) (Copper-sulphate-hydrogen peroxide), Klunge's Reagent (Schweiz.Wschr. f. Chem. and Pharm., 1882, 20, 497) (Copper sulphate-sodium chloride) and Schoutelen's Reagent (Z.analyt Chem., 1892, 31, 723). The compound $R_F$ 0.22 showed a green fluorescence on the chromatogram with borax but could not be identified. The compound with $R_F$ 0.5 gave a bluish yellow fluorescence only in the presence of borax and they concluded that it was an anthracene derivative. The compound $R_F$ 0.65 was barbaloin and that with $R_F$ 0.81 was probably p-coumaric acid. With Curacao Aloes they found resolution into five bands with $R_F$'s as in Cape aloes except that the band at $R_F$ 0.22 had resolved into
two bands. Again they could not identify the compounds with $R_F$ 0.22 but this time the compound with $R_F$ 0.5 gave a positive reaction with Klunge's Reagent showing it to be iso-barbaloin. They also investigated the behaviour of Natal and Mokka aloes on paper chromatography.

Later, Awe, Anterhoff and Wachsmuth-Melm stated that the first spot ($R_F$ 0.81) was largely but not exclusively $p$-coumaric acid, the second spot ($R_F$ 0.65) was barbaloin and the third spot ($R_F$ 0.5) was a "new" anthracene derivative.

**Discussion**

Chromatography of Curacao and Cape aloes and the dried juice of *A. Candelabrum* using butanol saturated with water as solvent system showed three major components ($R_F$'s 0.82, 0.62, 0.40). In attempts to separate these components (hereafter called first, second, and third component respectively) various methods were employed (for details see experimental) including extraction with solvents, chromatography on different types of column, and counter-current distribution in different solvents.

The method that was found to give the best separation of the third component was counter-current extraction.
(using ethyl acetate as the mobile phase) of an aqueous extract of solid obtained by chloroform extraction of dried A.Candelabrum juice. The third component was held in the first few aqueous phases with some impurity which was slow-running on paper chromatography. This impurity was removed by running the concentrated fractions in a second series of counter-current extractions with n-butanol as the mobile phase. Separation was also achieved by chromatography on a cellulose column using butanol saturated with water as eluant. On one such column all three components were separated but several attempts made to repeat this separation were unsuccessful.

It was found impossible to crystallise either the first or the third component. The third component obtained by the counter-current extraction method was purified by dissolving it in water, charcoaling and freeze drying. A pale brown solid was obtained which was found to be hygroscopic.

Investigation of the structure of the third component (Rf 0.4)

Hydrolysis

The compound was found to be unaffected by 2N sulphuric acid when heated at 100°C for 2 hours thus showing
it was not a glycoside.

**Periodate Oxidation**

The third component was found to be oxidised by sodium metaperiodate as a chromatogram sprayed with periodate-permanganate spray (Lemieux, Bauer, *Anal. Chem.*, 1954, 26, 920) gave a positive reaction. Quantitative oxidation was carried out on the compound obtained from the cellulose column and on that from the counter-current separation. In the first case a value of $4.02 \times 10^{-3}$ was found for the ratio uptake to molecular weight. In the second case a value of $3.2 \times 10^{-3}$ was obtained. The first result gives a molecular weight for the third component of $248 \times n$ if the compound is assumed to consume $n$ molecule of oxidant/mol. The second result gives a molecular weight of $311 \times n$. Work done in collaboration with Dr. J. Tyler has shown that the third component can conveniently be obtained from a butanol extract of aloes by chromatography on Whatman 3MM paper. Analysis of material obtained in this way suggests a molecular formula for the third component of $C_{21}H_{22}O_9.H_2O$, that is, the compound appears to be an isomer of barbaloin with molecular weight 436. The compound forms a crystalline 2,4-dinitrophenylhydrazone (m.p. 175-176°) analysis of which
suggests a molecular formula $C_{21}H_{22}O_8 \left[ E \text{N.N.H.}C_6H_3(NO_2)_2 \right] \cdot H_2O$.

**Ultra-violet spectrum.**

The ultraviolet spectrum of an ethanolic solution of the compound from the column showed a curve which we considered to resemble that given by a $\beta$-hydroxy anthraquinone (Cf. Morton and Earlam, *J. Chem. Soc.*, 1941, 159) but on further purification of the compound it was obvious that some of the peaks were due to impurity. The spectrum of the purest sample obtained from the counter-current separation (Fig. 3 in appendix) showed only three peaks and no conclusions could be drawn from its shape.

**Infrared spectrum**

The infrared spectrum of purified third component (KBr) showed strong hydroxyl absorption, some C-H absorption and peaks at 1720 and 1665 suggesting the presence of two carbonyl groups.

**Ferric Chloride Oxidation**

Since it was thought that this compound might resemble barbaloin it was treated with ferric chloride to see
if it, like barbaloin, gave arabinose (Cf. Hay and Haynes, *J. Chem. Soc.*, 1956, 3141). However on ferric chloride oxidation using a micro technique no arabinose could be detected.

**Acetylation of third component**

In an attempt to isolate the third component as a crystalline solid the acetyl derivative was prepared but this compound also was hygroscopic and could not be crystallised.
Structural investigation of First Component

This work was carried out on material obtained from the cellulose powder column without further purification as the compound could not be crystallised although it separated from an aqueous solution in an amorphous form. The compound coupled with diazotised sulphanilic acid.

Hydrolysis

First component was heated with 2N sulphuric acid for 2 hours at 100° and the solution examined by paper chromatography. It was found that the spot $R_F$ 0.82 had disappeared but no new spot could be detected probably due to the masking effect of the sulphuric acid.

Periodate Uptake

On spraying a chromatogram it was found that the spot $R_F$ 0.82 did not give a positive reaction with periodate-permanganate spray. On quantitative periodate oxidation it was found that there was very small consumption of oxidant but this was probably due to impurity.
Experiments with p-coumaric acid

Since various authors (see introduction) had postulated that this component was p-coumaric acid, authentic p-coumaric acid was prepared. It was found to run with \( R_f \) 0.82 in butanol saturated with water giving a blue fluorescence under ultra-violet light. It gave no periodate uptake and showed an ultra-violet spectrum with peaks at 224 and 304 m\( \mu \).

Ultra-violet spectrum

The ultra-violet spectrum of the partially purified first component showed peaks at 224, 253 and 302 m\( \mu \).

Infrared spectrum

The infrared spectrum determined using a nujol mull showed strong hydroxyl absorption and peaks at 1700 and 1640 due probably to carboxyl absorption. It differed in several respects from that of p-coumaric acid.

From comparison of chromatography and ultra-violet spectra of the first component and p-coumaric acid it could be argued that the two are identical but there is obviously some impurity present. This was shown in the counter-current separation where the first component
resolved partly into two. One of the compounds had a brown fluorescence and was probably aloe-emodin while the other had a blue fluorescence and was probably p-coumaric acid, although it is difficult to explain the reluctance of the material to crystallise if this were so.
Note on the Periodate oxidation of Phenols

Freiger, Smith and Willeford (J. Org. Chem., 1959, 24, 91) have described the periodate oxidation of some phenols and methyl ethers of phenols carried out at room temperature. In the work on aloins it was found that more reliable results were obtained if the oxidation was carried out at 0°C. So the quantitative periodate uptake of catechol, resorcinol and hydroquinone were determined using a micro technique.

The phenol (2-3 mg.) was weighed out accurately and dissolved in water (10 ml.). The solution and the standard periodate solution (ca. 0.004 M) were cooled to between 0 and 5°C. The cooled periodate solution (10 ml.) was then added to the test solution and kept at 0°C for 18 hours. Saturated aqueous borax solution (5 ml.), boric acid (1 g.) and aqueous potassium iodide solution (5 ml. 10%) were then added and the liberated iodine titrated with standard arsenious oxide (0.01025 N) using starch as indicator. The following results were obtained:

<table>
<thead>
<tr>
<th>Compound</th>
<th>periodate uptake (moles per mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol</td>
<td>3.13, 3.00, 3.02</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>2.76, 2.73.</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>1.08, 1.12, 1.14.</td>
</tr>
</tbody>
</table>
In the titration after the oxidation some difficulty was encountered due to the formation of a coloured compound during the oxidation.
EXPERIMENTAL
Chromatography of Crude Aloes

Cape Aloes, Curacao Aloes, the dried juice from Aloe Candelabrum, and barbaloin were examined by paper chromatography using n-butanol saturated with water as the solvent system. The chromatogram was dried and viewed under ultra-violet light. The following fluorescent spots were observed:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour of fluorescent spots &amp; ( R_F ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbaloin</td>
<td>Orange-brown, 0.62.</td>
</tr>
<tr>
<td>Cape Aloes</td>
<td>Bluish-yellow, 0.82; Orange-brown, 0.62; Blue, 0.40.</td>
</tr>
<tr>
<td>Curacao Aloes</td>
<td>Reddish-blue, 0.82; Orange-brown, 0.62; Blue, 0.40.</td>
</tr>
<tr>
<td>Aloe Candelabrum</td>
<td>Blue, 0.82; Orange-brown, 0.62; blue, 0.40</td>
</tr>
</tbody>
</table>

When the chromatograms were fumed with ammonia the fluorescences became much more intense. The spots quoted above are the main spots that appeared on the chromatograms. Other smaller spots appeared which were characteristic of the different aloes.

The chromatogram was sprayed with saturated aqueous aniline oxalate solution, dried and heated in an oven at
140°. No coloured spots appeared. Therefore no free sugars were present in the aloes examined.

Another chromatogram was sprayed with diazotised sulphanilic acid spray. All three spots reacted suggesting that all components contained phenolic hydroxyl groups.

Another chromatogram was sprayed with periodate-permanganate spray (Cf. Lemieux and Bauer, Anal. Chem., 1954, 26, 920). The second and third components were found to react but not the first, showing that the third component contained a diol system.

Preparation of Extracts of Crude Aloes.

1. Benzene

Curacao Aloes (20 g.) was extracted with benzene (200 ml.) in a Soxhlet extractor for 48 hours. The benzene extract was washed with water (25 ml. and 2 x 100 ml.). A paper was spotted with the benzene extract, the first aqueous wash and a marker of barbaloain and run with butanol saturated with water for 16 hours. On drying and examining the chromatogram under ultraviolet light the only spot that could be detected was one with a yellow fluorescence which ran near the solvent front in the
chromatogram of the benzene extract. This did not correspond to any of the three major components and was probably due to natural oils in the aloes.

2. Water

Curacao aloes (20 g.) was stirred with water (500 ml.) at 70° for 2 hours. The extract was allowed to stand overnight and the deep red aqueous solution decanted. The solution was washed with benzene (2 x 100 ml) and ether (200 ml.).

On paper chromatographic examination of the extract with n-butanol saturated with water as solvent, it was found that the extract contained all three major components along with some slow-running resinous material.

3. Methyl Acetate

Curacao aloes (5 g.) was boiled with methyl acetate (100 ml.) for two hours. The extract was allowed to stand and the supernatant solution decanted. The resulting residue was extracted with water (40 ml.) at 70°. Both the methyl acetate and aqueous extracts were examined chromatographically using butanol saturated with water as solvent. All three components were found in both but the methyl acetate solution was the cleaner extract as it contained much less slow-running resinous material.
4. Acetone

Powdered Curacao Aloes (1 g.) was boiled with acetone (10 ml.) for 15 minutes, allowed to cool and the resulting red solution decanted. Paper chromatography showed that all three components were present as well as slow-running impurities.

**Attempted Separation of the three major components in the aqueous extract**

1. **Paper Column**

A stiff slurry of aqueous extract and powdered cellulose was dried and the resulting solid powdered. A column seven inches long by half an inch in diameter was packed with powdered paper and the powder prepared above packed to a depth of half an inch on top. A further half an inch of powdered paper was then added and the column eluted with n-butanol saturated with water. Fractions were collected (6 x 1 ml.; 30 x 5 ml.). Paper chromatographic examination of these fractions showed that no separation of the three major components had resulted although the slow-running impurities had been held back slightly.

A change in solvent to pure butanol was considered but on running a paper spotted with aqueous extract and
a marker of barbaloin a streaked chromatogram was obtained.

A modification of the above method was carried out as follows: A column 0.5 inches in diameter was packed with paper to a length of 12 inches by means of a slurry of powdered paper in n-butanol half saturated with water. Curacao aloes (0.1 g.) was put on to the column as a solution in the same solvent. 1 ml. fractions were collected but on investigation of their contents by means of paper chromatography very little resolution of the major components was obtained although the third component (RF 0.4) was held back slightly on the column.

2. **Counter-current Extraction with Water and methyl acetate**

A five tube counter-current separation of 20 ml. of the aqueous extract using 20 ml. of methyl acetate in each tube was carried out. Examination of the contents of each tube by means of paper chromatography showed that some separation had been obtained as the substance (RF 0.4) was held back in the aqueous phases.

3. **Heavy Magnesium Carbonate Column**

A column 0.5 inch in diameter and 12 inches long was packed with a slurry of heavy magnesium carbonate in butanol half saturated with water. Curacao aloes (0.1 g.) was introduced as a solution in that solvent and eluted with
It. 1 ml. fractions were again collected. It was found that the third component (Rf 0.4 on paper chromatogram) was held back more than it was on the paper column.

4. Alumina Column

A column of similar dimensions was packed with alumina as a slurry. Curacao aloes (0.1 g.) was put on the column as a solution in butanol saturated with water. On washing with various solvents it was found that the compounds adhered too strongly to be eluted.

5. Extraction with different Solvents

Curacao aloes (0.5 g.) was shaken with 5 ml. of each of the following solvents in the order given: benzene, chloroform, ether, ethyl acetate, ethanol. The solutions, except the ethanol in which the bulk of the solid dissolved, were concentrated to about 0.5 ml. and the contents of each determined by means of paper chromatography. The chromatogram showed that chloroform extracted only the fast running component (Rf 0.82) and that none of the other solvents removed any appreciable quantity of any of the major components save ethyl acetate which removed all three.

6. Partition chromatography using chromatographic silica gel.

Two identical columns, 10 inches long by 0.5 inch in
diameter, were prepared by means of a slurry of silica gel saturated with water in dry butanol. Crude aloes (1 g.) was heated with n-butanol saturated with water (10 ml.) at 70° for 2 hours. The solution was cooled, filtered and the residue washed with dry butanol (10 ml.), the washings being added to the solution. The solution was divided into two equal parts and treated as follows:

(1) This portion was put directly on to one of the prepared columns; (2) a further 10 ml. butanol was added and the water removed as the azeotrope by reducing the volume to 10 ml. The resulting solution was filtered on to the second column.

Both columns were run with dry butanol until one band was eluted and then with butanol saturated with water. 1 ml. fractions were collected and spots of each chromatographed with butanol saturated with water as solvent. It appeared that both columns had been overloaded but the third component (R_f 0.4) was held back much more than the two faster running components.

Attempted chloroform extraction of the fast-running component

1. Extraction from aqueous extract

Cape aloes (10 g.) was dissolved in water (100 ml.)
and extracted with chloroform (2 x 100 ml.) by hand shaking. The yellow chloroform extracts were combined, dried (sodium sulphate) and evaporated to dryness. The solid obtained was dissolved in acetic acid in an attempt to purify it by crystallisation. A spot was placed on a paper along with a spot of aqueous extract after extraction with chloroform, and run with butanol saturated with water. The chromatogram showed that only the fast-running component had been removed but some still remained in the extracted aqueous solution. Other attempts were made to crystallise the material from methanol, acetone, butanol and isopropanol without success. Sublimation was also tried unsuccessfully.

2. Continuous extraction of solid aloes

Powdered aloes (5 g.) was mixed with five times its volume of silver sand and extracted (Soxhlet) with chloroform for 24 hours. A yellow solid separated from the chloroform solution during this time. The solid was collected, dried at room temperature and its contents determined by means of paper chromatography. The chromatogram showed that all three components were present with very little slow-running impurity. This method was thus unsatisfactory for isolating the fast-running component but
could be used to prepare a relatively clean extract of the three major components from crude aloes.

3. Use of continuous liquid-liquid extractor

Cape aloes (10 g.) was dissolved in water (100 ml.) and extracted with chloroform in a continuous extractor for 24 hours. The resulting extract was found to contain predominantly the first component with a trace of barbaloain which made further purification very difficult and the method had to be abandoned.

Large scale separation on Heavy Magnesium Carbonate Column.

Cape aloes (1.5 g.) was heated with n-butanol saturated with water (50 ml.) at 70° for 2 hours. The mixture was filtered and the resulting solution introduced on to a column (length 50 cm.) prepared by pouring a slurry of heavy magnesium carbonate in n-butanol saturated with water into a column 25 mm. in diameter.

The column was eluted with butanol saturated with water and 5 ml. fractions collected. Paper chromatography showed that most of the fractions contained all three components but those collected near the end appeared to contain only the third component. Those fractions which
contained only the third component were combined, evaporated to dryness and the residue taken up in water. Extraction of the aqueous solution with methyl acetate, followed by paper chromatographic investigation showed that both the aqueous and methyl acetate extracts contained traces of barbaloin and slow-running impurities.

It was decided to abandon the heavy magnesium carbonate column for two reasons: (1) it would take a very large column to get a working quantity of the third component as the column used was overloaded with 1.5 g. of crude aloes. (2) the purity of the final product was not satisfactory.

5 g. Scale Separation on Silica Gel Column

A column 47 mm. diameter and 32 cm. length containing 500 g. chromatographic silica gel saturated with water was prepared by means of a slurry in butanol saturated with water. Cape aloes (5 g.) was heated with butanol saturated with water (50 ml.) at 70° for 2 hours. The resulting extract was allowed to cool and the clear, dark red supernatant solution collected. Dry butanol (50 ml.) was added and the water removed as the azeotrope by reducing the volume to 50 ml. under reduced pressure. Some
solid which separated was collected and examined by means of paper chromatography. The solid was found to contain only slow-running impurities.

The solution was then run on to the column and 20 ml. fractions collected. The column was washed with dry butanol until one band was eluted and then with butanol saturated with water until the column was almost clean.

**Fractions** (investigated by means of paper chromatography)

First band (eluted with dry butanol)
- Fractions 1-6: first two components present.
- 6-20: barbaloin tailing off

Second band (eluted with BuOH/H₂O)
- 27-39: barbaloin only
- 39-49: both barbaloin and third component

Fractions eluted later were too dilute to examine directly and the following fractions were combined and concentrated to 15 ml.: 39-45, 46-52, 53-58, 59-65, 66-72. These concentrates which will be denoted as (1), (2), (3), (4) and (5) respectively were examined by paper chromatography and found to contain predominantly the unknown third component with traces of barbaloin decreasing from (1) to (5) and also a small amount of slow-running impurity.

This method of separation was unsatisfactory as it did not give pure products. The separation was repeated bringing in the following improvements: (1) The solid ob-
tained from the chloroform extraction of Cape aloes (cf. page 104) was used as it contained only a very small amount of slow-running impurity.

(2) The solid was put on in butanol saturated with water and run with that solvent all the time as it had been found that some water appeared in the fractions eluted with dry butanol and so water was being removed thus reducing the efficiency of the column.

(3) The length of the column was doubled to increase its resolving power.

The solid (5 g.) obtained by extraction with chloroform was dissolved in butanol saturated with water, put on the column and run with the same solvent. 20 ml. fractions were collected and examined by means of paper chromatography. The fractions which contained only the third component were combined and taken down to dryness. Those which contained mainly the third component with traces of barbaloin were combined also and evaporated to 50 ml. The resulting solution was run on a similar column to the above and the fractions which contained only the third component evaporated to dryness and the solid combined with that obtained from the previous column.

Attempts were made to crystallise this solid from different solvents, e.g. methanol, ethanol, isopropyl
alcohol, butanol, ethyl acetate and combinations of these solvents, but without success.

**Experiments with Third Component**

1. **Hydrolysis**
   
   Third component (5 mg.) was heated with hydrochloric acid (1 ml. of 2N) at 100° for 1 hour. On spotting the resulting solution on a paper and running with butanol saturated with water the spot corresponding to the third component was completely masked with that due to hydrochloric acid. When 2N sulphuric acid was used this masking did not occur and it was found that no hydrolysis of the third component had taken place.

2. **Ferric Chloride Oxidation**

   As it was necessary to work on a micro scale a trial run was done on pure barbaloin to see if arabinose and the emodin could be detected.

   Barbaloin (5 mg.), ferric chloride (10 mg.) and water (1 ml.) were heated in a sealed tube for 15 minutes at 115° and then for 6 hours at 125°. Some dark coloured compound separated and was collected. The solid and solution were investigated paper chromatographically using butanol saturated with water and butanol(5)-pyridine(3)-
water(3)-benzene(1) as solvents. The chromatogram that was run with the latter solvent was sprayed with aniline oxalate and the arabinose revealed itself as a pink spot. The other chromatogram showed a fluorescent spot that corresponded to aloe-emodin.

The above experiment was repeated with the third component (5 mg.) but although some solid separated as in the previous experiment no arabinose could be detected.

3. Ultra-violet Spectrum

The ultra-violet spectrum, determined using a methanolic solution of third component showed the following peaks: 245, 254, 267, 297, 337 m.μ.

Attempted isolation of Third Component using Leger's method for isolating Nataloin.


Cape aloes (100 g.) was finely powdered and placed in a flask with acetone (200 ml.). The mixture was stirred for 10 minutes and then shaken from time to time during seven days. The solid which did not dissolve in the acetone was collected, dried over phosphoric anhydride and extracted with ethanol (2 x 10 ml.), methanol (2 x 10 ml.) and the residue dissolved in water (10 ml.). The extracts
were chromatographed using butanol saturated with water as solvent. The first ethanol extraction removed the bulk of the three major components but no enrichment of third component could be seen in the following ones.

**Separation of three components on dry-packed paper column**

1. **Trial run with small column**

   A column 10 inches long by 0.5 inch diameter was packed with dry cellulose powder by adding about one inch of powder, tapping the column on the bench to let the paper settle down evenly and finally pressing the paper down with a glass rod. This was repeated until a column of the required length was obtained. The column was then washed with water and finally with butanol saturated with water until all excess water had been removed.

   Partly purified solid (0.1 g.) obtained by chloroform extraction of Cape aloes was added in butanol saturated with water (1 ml.) and the column run with the same solvent. 1 ml. fractions were collected and their contents examined by means of paper chromatography. Complete resolution of the three components was obtained.

2. **Separation using Large Paper Column**

   A column 45 mm. diameter was packed in a similar way
to that in the previous experiment using 500 g. cellulose powder. It was then washed with water and finally with butanol saturated with water until all excess water had been removed, this being done in a constant temperature room.

The solid (2 g.) from the chloroform extraction of Cape aloes in butanol saturated with water (40 ml.) was introduced on to the column and eluted with the same solvent. The rate of flow was 30 ml. per hour and 10 ml. fractions collected. Alternate fractions were spotted on papers and run with butanol saturated with water. The following results were obtained:

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 60</td>
<td>nil</td>
</tr>
<tr>
<td>77 - 103</td>
<td>first component</td>
</tr>
<tr>
<td>109 - 133</td>
<td>second component (barbaloin)</td>
</tr>
<tr>
<td>129 - 143</td>
<td>compound with blue fluorescence not previously observed.</td>
</tr>
<tr>
<td>147 - 173</td>
<td>third component</td>
</tr>
<tr>
<td>175 - 181</td>
<td>compound with yellow fluorescence (Rp 0.2)</td>
</tr>
<tr>
<td>181 - 206</td>
<td>resinous material</td>
</tr>
</tbody>
</table>

3. Repetition of above experiment using the same column

The column used in the previous experiment was washed
with butanol saturated with water until it was thought that all impurities had been removed. Solid (2 g.) from the chloroform extraction of Cape aloes was again put on to the column as before and eluted with butanol saturated with water. This time no bands separated and although partial resolution of the third component was obtained results were not as satisfactory as before.

4. Repetition of large scale separation using large cellulose column.

Columns were packed the same way as in part (2) of this experiment, washed and equilibrated in a constant temperature room but resolution as obtained in (2) has never occurred again. Partial resolution of third component was always obtained but never complete resolution of the three.

Work done on the fractions from (2).

Ultra-violet spectra

Ultraviolet spectra of the three major components were obtained as follows: 0.2 ml. of a fraction containing only barbaloin was diluted to 100 ml. with ethanol and run against a blank containing the same amount of butanol. 0.3 ml. of a fraction containing only third
component was diluted to 100 ml. and 1 ml. of a fraction containing only first component was diluted to 100 ml. and run similarly. The following peaks were observed in the spectra.

<table>
<thead>
<tr>
<th>1st Component</th>
<th>2nd Component (barbaloin)</th>
<th>3rd Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>220 m(\mu)</td>
<td>215</td>
<td>245</td>
</tr>
<tr>
<td>254</td>
<td>254 ~</td>
<td>253</td>
</tr>
<tr>
<td>266</td>
<td>261</td>
<td>266 ~</td>
</tr>
<tr>
<td>310</td>
<td>269</td>
<td>297</td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>336</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td></td>
</tr>
</tbody>
</table>

Isolation of solid first and third components.

Fractions 77-103 which contained only first component were combined and taken to dryness under vacuum in an atmosphere of nitrogen at 30-40°. A reddish brown oil was obtained which solidified on addition of chloroform. The light brown solid was collected.

Fractions 147-170 which contained only third component were also combined and taken down as above. A light brown solid was obtained. In both cases it was found that the solids were hygroscopic. Several attempts were made to crystallise the first and third components using different solvents. In the case of the first component
the most promising solvent was water but the compound did not come out as a crystalline solid. With the third component the best solvent was ethyl acetate but again the product was amorphous.

**Infrared spectra**

Infrared spectra of the first and third components obtained from the paper column (Cf. p.111) were obtained as nujol mulls. The following peaks were observed:

**First Component**: 3000-3200(S), 2400(W), 1700(M), 1640(S), 1590(S), 1492(W), 1250(W), 1200(W), 1150(S), 1075(S), 1030(M), 910(W), 825(W), 710(W).

**Third Component**: 3200(S), 2900(M), 1710(M), 1640(S), 1590(S), 1492(W), 1325(S), 1233(S), 1217(M), 1154(M), 1080(S), 1025(S), 910(W), 850(W), 710(W).

**Quantitative periodate oxidation of First and Third Components.**

The solid (ca. 5 mg.) was weighed out accurately and dissolved in water (2 ml.). This solution and the standard sodium metaperiodate solution (0.1034 M) were cooled to 0° and the periodate solution (2 ml.) added to the solution to be tested. The mixed solutions were left at 0° overnight. Saturated aqueous borax solution (5 ml.)
boric acid (1 g.) and potassium iodide solution (5 ml., 10%) were added and the liberated iodine titrated with standard arsenite solution (0.04714 N.) using starch solution (1%) as indicator.

The following results were obtained:

With the first component no satisfactory results were obtained as the solid came out of solution and so complete reaction could not occur.

With the third component a ratio of Uptake divided by molecular weight gave a value of 4.02 x 10^-3 which means an uptake of 1 for a compound of molecular weight 248 and an uptake of 2 for a compound of molecular weight 496.

**Acetylation of Third Component.**

To a solution of third component (10 mg.) in acetic anhydride (1 ml.) was added concentrated sulphuric acid (1 drop). The mixture was heated to 90° on a water bath and kept at that temperature for 30 minutes. The solution was then poured into water (50 ml.) and the yellow solid that separated collected. This was taken up in 25% aqueous ethanol and the solid that separated on standing collected, dried in a vacuum desiccator over phosphoric anhydride and investigated by means of paper chromatography using butanol.
saturated with water as solvent. The chromatogram showed only one spot \((R_p 0.8)\) which showed no activity when sprayed with periodate-permanganate spray. The ultraviolet spectrum showed peaks at 294 and 251 m\(\mu\).

**Ultra-violet Spectra**

Ultraviolet spectra of first and third components (See appendix Fig. 1 and 2) in ethanol solution showed the following maxima:

<table>
<thead>
<tr>
<th>First Component</th>
<th>Third Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{max}) (m(\mu))</td>
<td>(\lambda_{max}) (m(\mu))</td>
</tr>
<tr>
<td>244</td>
<td>245</td>
</tr>
<tr>
<td>253</td>
<td>253</td>
</tr>
<tr>
<td>302</td>
<td>296</td>
</tr>
<tr>
<td></td>
<td>(\sim 350)</td>
</tr>
</tbody>
</table>

**Hydrolysis of First and Third Components**

First Component (2 mg.) was heated with sulphuric acid (0.5 ml. 2N.) in a sealed tube at 100\(^\circ\) for 2 hours. A paper was spotted with the resulting solution and run with butanol saturated with water. The spot due to the first component disappeared but no other spot except the one due to sulphuric acid could be detected.
The above hydrolysis was repeated with third component. In this case a spot could be detected corresponding to unchanged third component showing that the compound was not susceptible to acid hydrolysis.

**Ferric Chloride oxidation of First and Third Components**

A solid of first component (5 mg.) and ferric chloride (25 mg.) in water (0.5 ml.) was heated in a sealed tube at 115° for 15 minutes and at 125° for 6 hours. A dark solid separated and was collected. The solid was dissolved in methanol and a paper spotted with both the aqueous and the methanolic solutions and run with butanol(5)-pyridine (3)-water(3)-benzene(1) with a marker of arabinose. No sugar spots could be detected in either the aqueous or methanolic solution after developing with aniline oxalate. The previous oxidation was repeated with third component and again no sugar spots could be detected.

**Counter-current Separation of the three components**

1. **Counter-current separation of A. Candelabrum with ethyl acetate and water.**

   The tubes of a twenty tube Craig counter-current apparatus were filled with water saturated with ethyl acetate to act as lower layer. 50 ml. of ethyl acetate
was placed in the third tube so that it travelled along before the actual counter-current separation thus keeping the aqueous phases saturated. An aqueous solution of Aloe Candelabrum juice (2 g.) was placed in the first tube and 33 transfers carried out with successive 50 ml. volumes of ethyl acetate. The ethyl acetate fractions were concentrated to 5 ml. and their contents investigated by means of paper chromatography. The aqueous phases were similarly examined. The following results were obtained (fraction 1 in the ethyl acetate fraction is the first taken across the aqueous)

Ethyl acetate fractions 1 - 5 fast moving impurity probably volatile oil.
5-13 first component
11-23 barbaloin

Aqueous fractions.

Third component held back with slow-running impurities in the first three aqueous fractions.

It was found on examination of the chromatograms that the first component was not pure but resolved partly into two. One compound had a brown fluorescence and so was probably aloe- emodin and the other which ran with a slightly slower Rf had a blue fluorescence.
2. Attempted Separation of the two compounds in the first component

In an attempt to separate the two compounds, the fractions for the previous experiment which contained them (5-13) were combined, taken to dryness and the residue dissolved in water (50 ml.). The solution was used as the first fraction of a counter-current and 25 transfers were done with ethyl acetate. On investigation by chromatography it was found that no resolution of the two compounds was obtained.

3. Counter-current of A. Candelabrum purified by chloroform extraction

A solution in water saturated with ethyl acetate (50 ml.) of the solid (2 g.) obtained by extraction of A. Candelabrum with chloroform was used as the first fraction of a counter-current extraction in which 40 ethyl acetate fractions were transferred completely across 40 aqueous fractions.

The following results were obtained:

Ethyl acetate fractions

4 - 22  first component (mixture of two compounds)

27 - 41  barbaloin.

Aqueous fractions

1 - 4  barbaloin

28 - 40  mainly third component with traces of
slow-running impurity.

The aqueous phases which contained third component were combined and evaporated to 50 ml. This fraction was run in a counter-current of twenty transfers with butanol. The butanol fractions were examined and it was found that the third component appeared alone in fractions 5 - 13. These fractions were combined with the corresponding aqueous fractions and evaporated to 20 ml. This solution was freeze dried giving a brown solid. This was taken up in water, charcoaled and again freeze dried. A cream coloured solid was obtained which could not be crystallised.

The solid ran as a single spot on a chromatogram run with butanol saturated with water.

An ultra-violet spectrum of the solid was run in ethanol and showed the following peaks (See appendix Fig.3).

$$\lambda_{\text{max}} (m\mu)$$

245

253

296
Periodate Uptake of Third Component obtained by counter-current.

The third component obtained above showed the following periodate uptake determined by the micro method described page

Uptake Results molecular weight = \(3.2 \times 10^{-3}\)

Therefore for an uptake of 1 a molecular weight of 311 is required.

and for an uptake of 2 a molecular weight of 622 is required.

Hydrolysis of Third Component

Hydrolysis with 2N sulphuric acid at 100° for 2 hours showed no splitting of the molecule (Cf. hydrolysis of third component from column page 117).
FIG. 3

3rd COMPONENT
(From Counter-Current)
12.08 mg/lt.

\[ \lambda_{\text{max}} 245 \]
\[ \lambda_{\text{max}} 253 \]
\[ \lambda_{\text{max}} 296 \]
FIG. 4

p-COUMARIC ACID

log E 4.24
max 304

log E 4.03
max 224

λ (mm)

200 250 300 350 400

3.0 3.5 4.0 4.5