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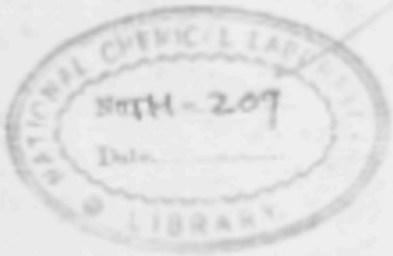


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# CHEMICAL INVESTIGATION OF SOME INDIAN PLANTS

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A
THESIS
SUBMITTED TO
THE UNIVERSITY OF POONA
for
THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY

by
Anil Chandra Ghosh, M.Sc.

National Chemical Laboratory
POONA 8
1963

# A C K N O W L E D G M E N T

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November 4, 1963
POONA-8

( Anil Chandra Ghosh )

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C O N T E N T S



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

# S U M M A R Y

A review of the chemistry of bianthraquinones and related compounds has been presented in Part A of this chapter.

Four compounds have been isolated from the root bark of Cassia siamea Lam., which is described in Part B. From the n-hexane extract, two compounds identified as lupeol and chrysophanol have been obtained. The benzene extract afforded two new hydroxyanthraquinone derivatives. The structure of one of them provisionally named as cassiamin, has been studied and it has been shown to be related to bianthraquinones.

In Part C, the nuclear magnetic resonance spectroscopic studies of twenty seven hydroxyanthraquinones and their derivatives have been described and the results discussed.

The syntheses of three new hydroxyanthraquinone derivatives have been presented in Part D.

# C H A P T E R  I  (PART  A)

## CHEMISTRY OF THE ANTHRAQUINONES AND RELATED COMPOUNDS

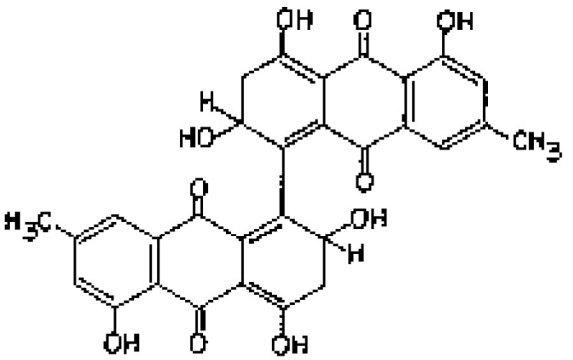
Among the naturally occurring quinone derivatives, anthraquinones are by far the largest in number and are also widely distributed - in fungi, lichens, higher plants, insects, and even in minerals. While a considerable number of potential uses of anthraquinone derivatives have been suggested, the main chemical interest appears to have been confined to the elucidation of their structures.[1-3]

During the last decade, however, a new group of fungal pigments with high molecular weight has come to light. They have been designated bianthraquinones, indicating that their structures contain two anthraquinone units.[1,4]

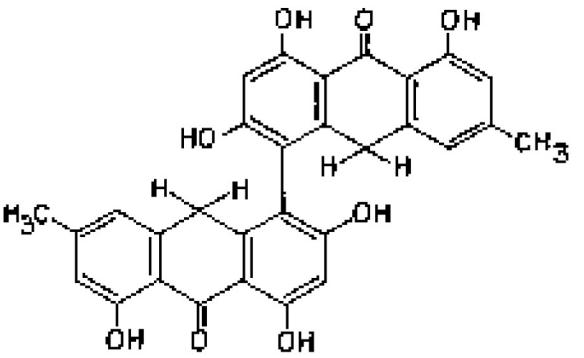
It is interesting to note that most of these mould products are associated particularly with Divaricatum-asymmetricum section of the Penicillia, and more especially with the species Penicillium islandicum Sopp which produce a most remarkable group of colouring matters.

The story of the chemistry of bianthraquinones probably starts with the isolation of aurofusarin in 1937 from Fusarium culmorum by Raistrick and co-workers.[5] But very little was known about the constitution of this compound except that it had an empirical formula, $C_{30}H_{20}O_{12}$, contained two methoxyl groups and did not melt below 360°. From the general properties, it was suspected to be a polyhydroxy bianthraquinone.[6]

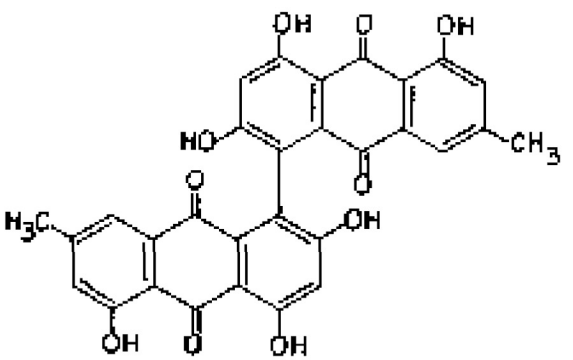
In 1949, Prof. Raistrick[6] in his Bakerian lecture made a remarkable statement; "A number of mould colouring matters, steadily increasing in number and having high melting points, are almost certainly polyhydroxy dianthraquinones, and some of them are clearly derivatives of emodin". Two compounds, rugulosin (I)[6] and penicilliopsin (II)[7] which Raistrick mentioned in his lecture, was of fundamental importance from the structural point of view. In retrospect, this early work can be considered to possess little more than historical importance, but it served well to establish the fact that rugulosin and penicilliopsin, although behaving as closely related to anthraquinones, were more complex than the simpler members of the series. The chemistry of penicilliopsin is of particular interest as here lies the missing link of two complex quinone systems, mainly skyrin (XII) a mould product[4] and hypericin[8-10] (IV) the interesting photodynamic constituent of the plant Hypericum perforatum Linn and other Hypericaceae.



I



II



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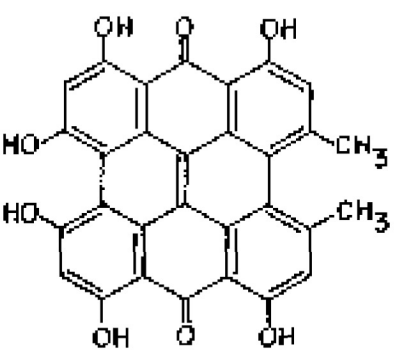
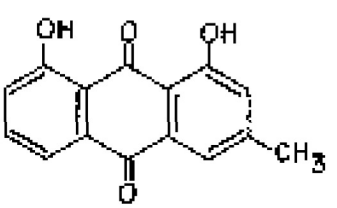
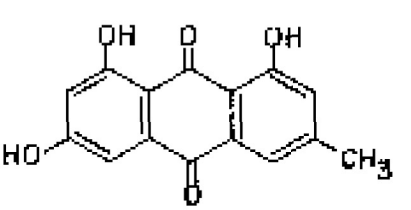


IV



V



VI

The first systematic and serious investigation on the bianthraquinones started almost simultaneously and independently by Raistrick[11-13] and his group in London and Shibata and his school in Tokyo.[14-18] In 1953, from Endothia parasitica[14] (Murr.) Anderson and Anderson, a plant pathogenic fungus which causes an epidemic ddisease chestnut-blight, Shibata and co-workers isolated two colouring matters and designated them endothianin and radicalisin. Later on it was noticed that all the properties of endothianin and radicalisin resemble those of skyrin of Penicillium islandicum Sopp and rugulosin of P.rugulosum Thom respectively, which had been studied earlier by Raistrick and co-workers, but was not published at that time. Since then, a number of bianthraquinones and closely related compounds have been isolated, all from the moulds and their structures have been elucidated. They have been listed in Table I.

# TABLE I

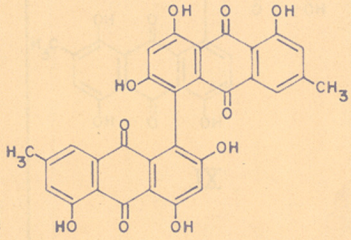
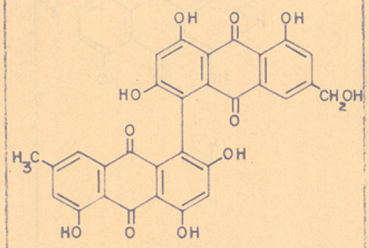
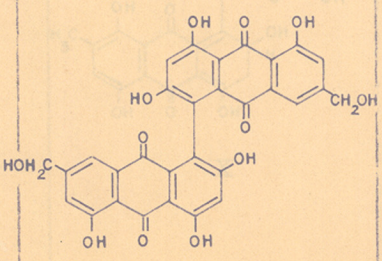
| Name | Structure | Occurrence | Ref.No. |
|---|---|---|---|
| Skyrin |  III | Penicillium islandicum Sopp | 11,15,17,18 |
| | | P.rugulosum Thom | 13 |
| | | P.brunneum Udagawa | 20 |
| | | P.wortmanni Klöcker | 13 |
| | | P.tardum Thom | 19 |
| | | Penicilliopsis clavariaeformis Solms-Laubach | |
| | | Endothia parasitica (Murr.) Anderson and Anderson | 14,15,21 |
| | | E.fluens Shear and Stevens | 15 |
| Oxyskyrin |  VII | P.islandicum Sopp | 18,22 |
| | | Endothia parasitica (Murr.) Anderson and Anderson | |
| Skyrinol |  VIII | P islandicum Sopp | 18 |

Table I Contd.

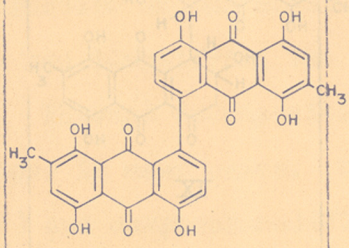
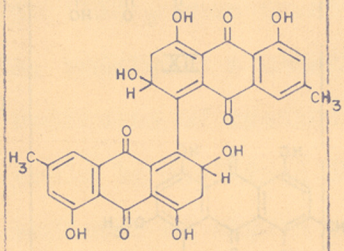
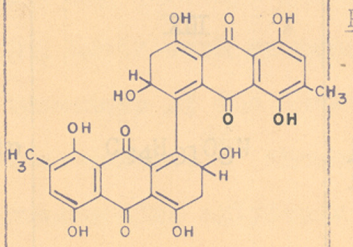
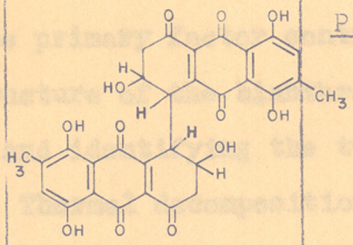
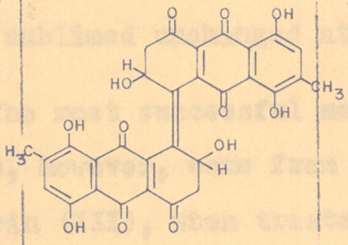
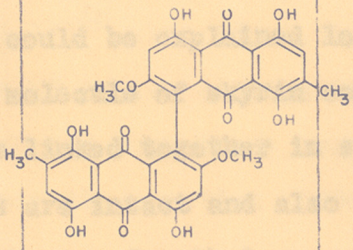
| Name | Structure | Occurrence | Ref. No. |
|---|---|---|---|
| Iridoskyrin |  IX | P islandicum Sopp | ,23,24 |
| Rugulosin |  I | P.rugulosum Thom | 13,14,23, 25,26 |
| | | P.wortmanni Klöcker | |
| | | P.tardum Thom | 19 |
| | | P.brunneum Udagawa | 20 |
| | | Endothia parasitica (Murr.) Anderson and Anderson | 14 |
| | | Endothia fluens Shear and Stevens | 14 |
| Luteoskyrin |  X | P.islandicum Sopp | 18,26-31 |

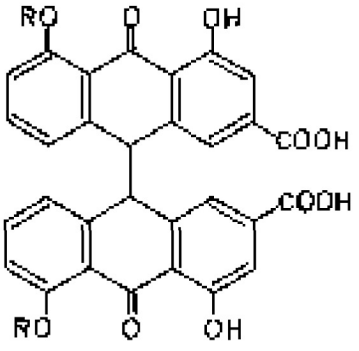
Table I Contd.

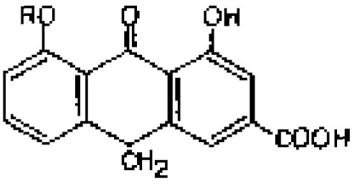
| Name | Structure | Occurrence | Ref. No. |
|---|---|---|---|
| Rubroskyrin |  XI | P.islandicum Sopp | 12,13,18, 26,29,31 |
| Lumiluteo skyrin |  XII | Photo-oxidation product of luteoskyrin | 28, 32 |
| Fusaroskyrin |  XIII | Fusarium Sp. | 33 |
| Erythroskyrin | $C_{24}H_{31}O_6N$ | Penicillium islandicum Sopp | 12 |
| Aurofusarin | $C_{30}H_{20}O_{12}$ | Fusarium culmorum | 5, 6 |

## CONSTITUTION

The primary factor controlling the determination of the structure of the bianthraquinones resides in obtaining and identifying the two simpler anthraquinone moieties. Thermal decomposition of the molecule serves this purpose in many cases. Thus rugulosin (I) gave emodin (V) and chrysophanol(VI).[13,35] But iridoskyrin could be sublimed unchanged at 260° at high vacuum.[12]

The most successful method of obtaining the two fragments, however, came from the surprising observation that skyrin (III), when treated with alkaline sodium dithionite, undergoes a smooth reductive cleavage to give two molecules of emodin[11] This experimental evidence could be explained logically by postulating that the molecule of skyrin consists of two emodin molecules linked together in such a way that both quinone groupings are intact and also the six hydroxyl groups as well as the two C-methyl groups. It may also be mentioned that during their work on sennosides A & B (XIVa) the active glycosides of the laxative senna drugs of commerce, Stoll and co-workers[34] showed that these substances and their corresponding aglycones sennidines A & B (XIVb) undergo reductive cleavage on treatment with alkaline sodium dithionite to give (XVa and XVb).




XV a $R = C_6H_{11}O_5$

XV b $R = H$

XIV a $R = C_6H_{11}O_5$ SENNOSIDES A and B

XIV b $R = H$ SENNIDINES A and B

While most of these bianthraquinones readily undergo reductive cleavage by the action of sodium dithionite, drastic conditions are sometimes necessary in certain cases.[23] Thus iridoskyrin[12] and 4,4'-dihydroxy-bianthraquinone (1,1')[16] were first reported to be stable against the action of sodium dithionite. Later studies by Shibata et al.[23] showed that these compounds also undergo reductive fission when the reaction mixtures were heated.

A few other methods have also been used for the structural elucidation of these compounds. Identification of the pigments in microchemical scale by paper chromatography has been widely employed by Shibata and his school.[18,28] Studies of the infrared and ultraviolet spectra have been invariably valuable, but the final structures of the compounds were confirmed mainly from the exhaustive chemical and degradative experiments. Thus rugulosin, on heating with

copper chromite and quinoline[13] afforded emodin and chrysophanol. Treatment of rugulosin with zinc and hydrochloric acid gave chrysophanol.[25] Lumiluteoskyrin, which was resistant to reductive cleavage by alkaline sodium dithionite, on heating with zinc and hydrochloric acid yielded islandicin.[32] Rubroskyrin on treatment with zinc and glacial acetic acid[12] gave a reduced product which on thermal decomposition or on treatment with conc. $H_2SO_4$ gave irideskyrin.[13,26] Rugulosin on catalytic hydrogenation in presence of palladium black gave tetrahydrorugulosin, which did not give back rugulosin on aerial oxidation, but could be thermally decomposed in vacuo to give chrysophanol.[13,25] The pyridine addition product of skyrin also undergoes a cleavage by hydrogenation in presence of palladium black giving rise to emodin.[12] Zinc dust distillation of both rugulosin[13] and penicillicopsin[7] yielded 2-methylanthracene.

Compounds having enolic hydroxyls are mostly soluble in sodium carbonate[12] and sometimes even in sodium bicarbonate.[24,25,28] Rugulosin, lumiluteoskyrin etc. easily form diacetates by the action of acetyl chloride or acetic acid.[25,28] On prolonged heating with sodium hydroxide solution, rugulosin afforded chrysophanol.[13]

It may be expected that certain properties of the simpler anthraquinones will undergo modifications on condensation to the bianthraquinones, presumably because

of the interaction of the two units in the system. Thus iridoskyrin, though arising from two islandicin moiety does not exhibit any fluorescence[12] in glacial acetic acid. Magnesium acetate in alcohol, which normally gives characteristic colour reaction with hydroxy-anthraquinones having at least one α-hydroxyl group[35] produces an orange colour with skyrin and oxyskyrin, violet red with iridoskyrin and green colour with rubroskyrin, a positive indication of their quinonoid structure. But rugulosin, luteoskyrin, erythroskyrin, and penicilliopsin do not respond to the magnesium acetate test showing their non-quinonoid structures.[4] But isoluteoskyrin is reported to give a sky-blue colour with magnesium acetate.[32]

The action of concentrated sulphuric acid on the bianthraquinones and the related compounds has been proved to be very interesting. All the compounds having non-quinonoid structures are readily dehydrated to give compounds having true quinonoid properties. Thus rugulosin gave dianhydrorugulosin.[13,23] Rubroskyrin was converted to iridoskyrin[12] while luteoskyrin produced islandicin and iridoskyrin.[24] Reduced rubroskyrin, however, produced iridoskyrin and islandicin[12] on treatment with conc. $H_2SO_4$. This dehydration reaction could also be effected by formic acid.[24]

A peculiar colour reaction was observed on treating skyrin with conc. $H_2SO_4$. Skyrin described by Raistrick[11] was dimorphic,- crystallising either as orange reds or hexagonal yellow plates. The former gave a deep cherry-red colour with conc. $H_2SO_4$, which within few seconds changed to stable emerald-green. The latter, however, gave immediately an emerald-green colour with conc. $H_2SO_4$. Raistrick considered that the green colouration of skyrin solution resulted from the interaction between β-hydroxyls of the two emodin moieties.

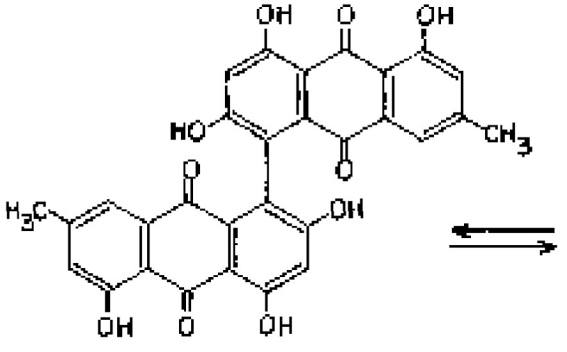
He further observed that skyrin formed a methylated or ethylated compound with methanol or ethanol either on long standing in the alcoholic solution, or by boiling a solution of hexa-acetyl skyrin with alcohol containing conc. $H_2SO_4$. Dimethyl and diethyl skyrins, having two alkoxyls, were insoluble in aqueous carbonate showing the blocking of the two β-hydroxyls, but they were readily hydrolysed by alkali or by glacial acetic acid to regenerate skyrin.

Shibata, however, prepared genuine β,β'- dimethyl-ether of skyrin by the action of diazomethane on skyrin and showed that this was resistant to alkaline hydrolysis.[18]

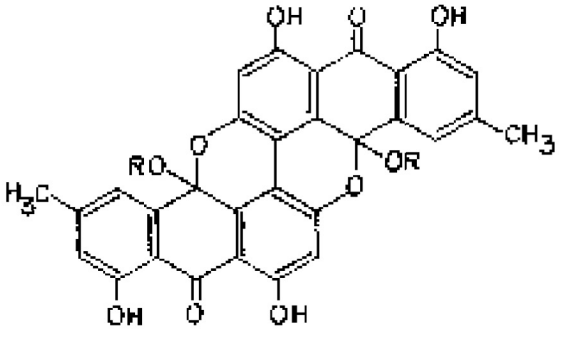
This puzzling behaviour of skyrin (III) with conc. $H_2SO_4$ and alcohol was successfully explained by Shibata and co-workers by postulating a pseudoisomerisation

of the structure. They proposed[16] the structural formulae (XVIa and XVIb) for the isomerised compounds and named them pseudoskyrin (XVIa R=H) and pseudoskyrin dialkyl ether (XVIb R=$CH_3$ or $C_2H_5$). Further study was made by Tanaka[36] who showed that on treatment with cold conc. $H_2SO_4$ for a few minutes, skyrin itself was isomerised to pseudoskyrin (XVIa).

Brockmann[10] postulated that the pyranol (XVIa) and its methyl ether (XVIb) would in conc. $H_2SO_4$ give a doubly positive pyrylium cation (XVII).

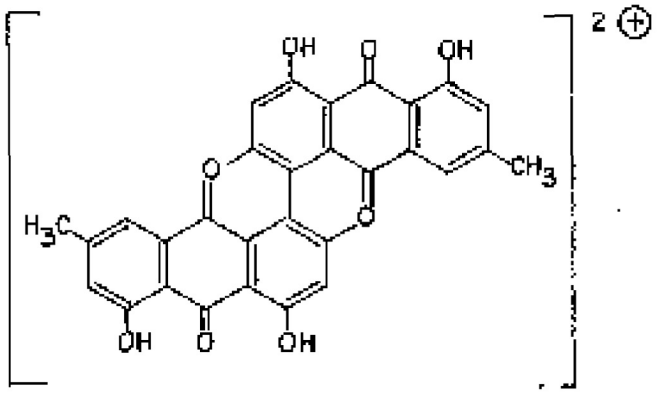


III

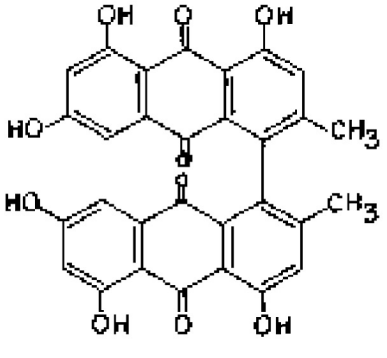


XVI a R = H

XVI b R = $CH_3$ or $C_2H_5$



XVII

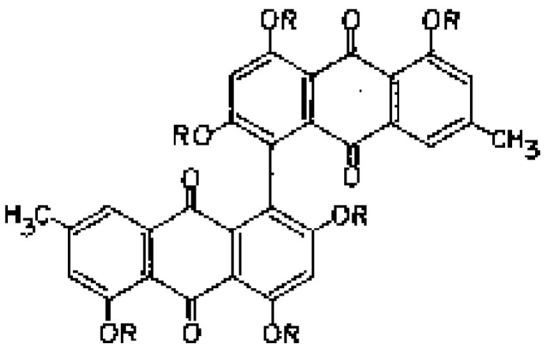


XVIII

He observed that the initial red colour of skyrin with conc. $H_2SO_4$ was similar to that given by the synthetic compound (XVIII) and explained that since no pyrylium cation could be formed in structure (XVIII) on treatment with conc. $H_2SO_4$, the colour remained red only.

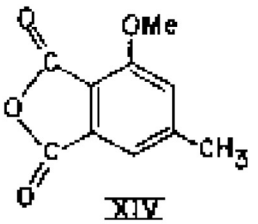
Although the change of colouration from red to green in conc. $H_2SO_4$ is a characteristic reaction indicating pseudoisomerisation of skyrin, it was subsequently found by model experiments that the phenomenon depends on the presence of free hydroxyls in the 'para' position as well as 'ortho' position of the bianthraquinone linkage as in skyrin.[4]

After getting the two fragments, the question remains as to how to locate the carbon-carbon linkage. That the C-C linkage present in substituted dianthrones could be broken by reductive cleavage was shown by Stoll and co-workers.[34] The problem of the constitution of skyrin thus resolved itself into the difficult decision as to which of the free positions[1,3,6,8] in each of the two molecules of emodin (VI) are joined together in the skyrin molecule(III). The only chemical evidence was that γ-coccinic acid methyl ether (XIX) was obtained by the oxidation of the methyl ether of skyrin[14] (IIIa). This excluded the possibility of linkage at 1 or 3 position of the anthraquinone molecule and strongly suggested that the linkage would be present between

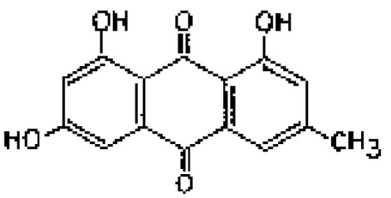


III R = H

III a R = $OCH_3$



XIV



VI

8 and 8' position (methyl in 2) of the emodin moieties of skyrin.[16] The C-C linkage in skyrin was finally proved by the synthesis of β,β'-dimethyl ether of skyrin by the Ullmann condensation of 8-bromo-emodin trimethyl ether followed by partial demethylation.[37]

After the structure of skyrin was established, the elucidation of structures of other compounds in this series was achieved with greater ease, mainly through their inter-relationship, which have been schematically shown in Fig.1.

It can be expected that skyrin and its derivatives should show optical activity due to restricted rotation of molecule in analogy with that observed in some bianthraquinones and bianthracene derivatives by Bell et al.[38,39] However, no optical rotation has been observed in skyrin and its derivatives so far.[16] But rugulosin and luteoskyrin showed optical rotation.

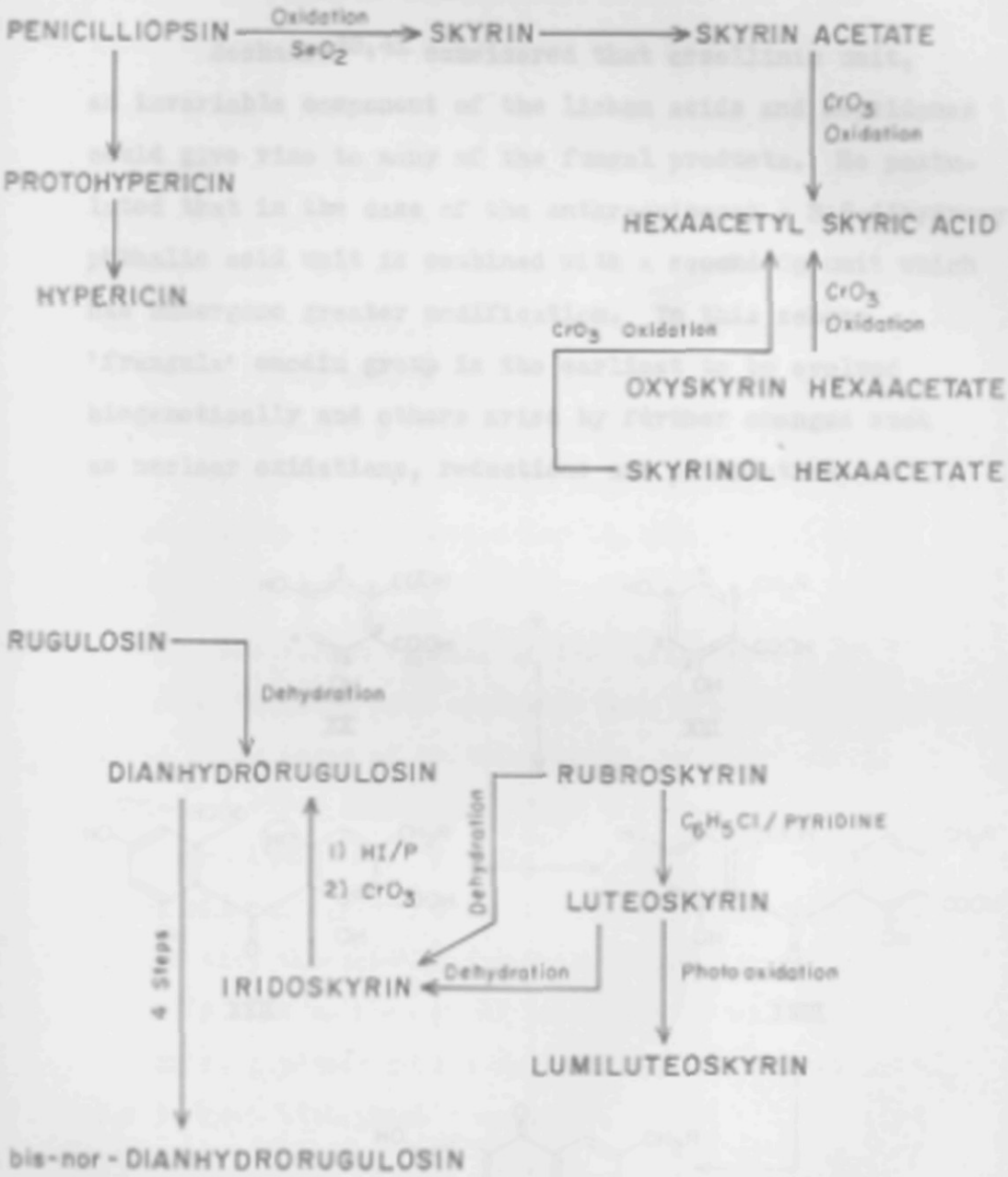
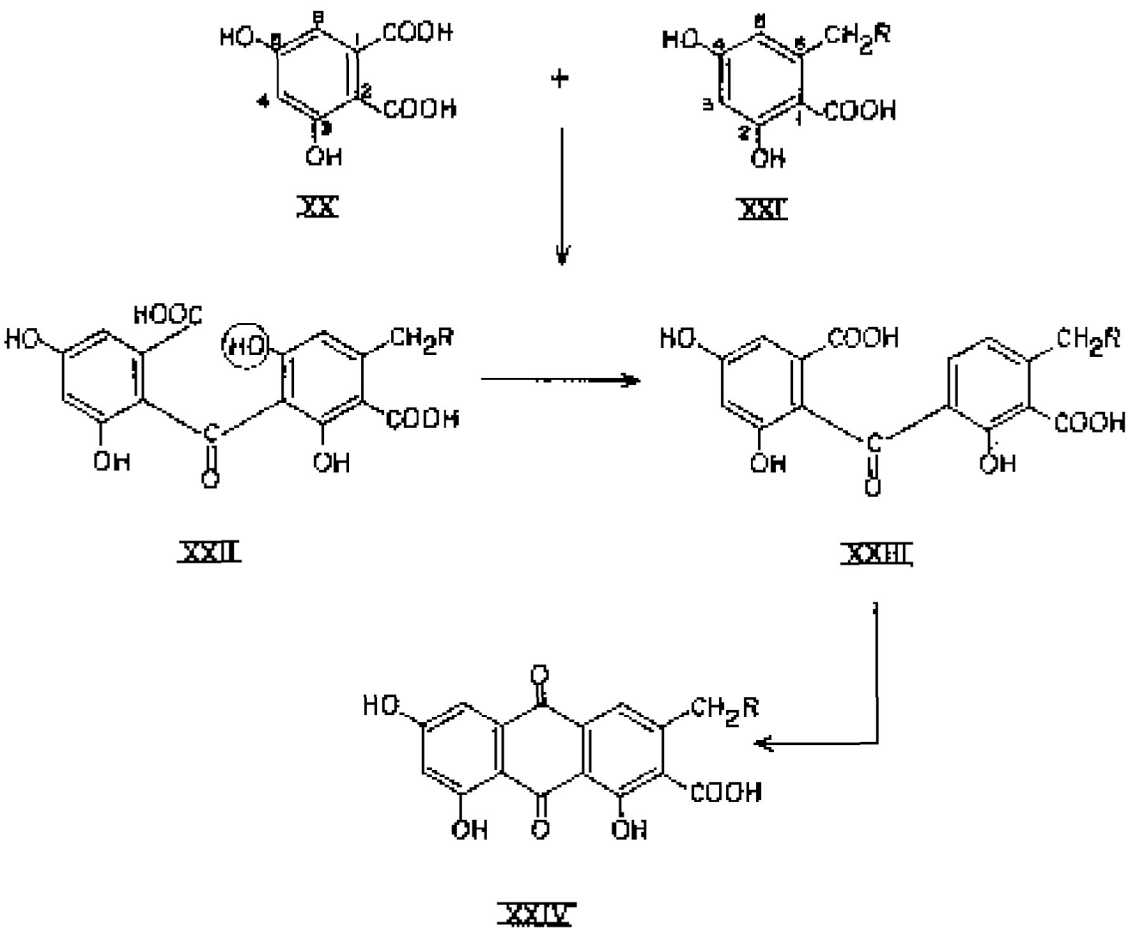

PENICILLIOPSIN
Oxidation
SeO2
SKYRIN
SKYRIN ACETATE
CrO3
Oxidation
PROTOHYPERICIN
HEXAACETYL SKYRIC ACID
HYPERICIN
CrO3 Oxidation
CrO3
Oxidation
OXYSKYRIN HEXAACETATE
SKYRINOL HEXAACETATE
RUGULOSIN
Dehydration
DIANHYDRORUGULOSIN
RUBROSKYRIN
C6H5Cl / PYRIDINE
1) HI/P
2) CrO3
Dehydration
LUTEOSKYRIN
4 Steps
IRIDOSKYRIN
Dehydration
Photo oxidation
LUMILUTEOSKYRIN
bis-nor - DIANHYDRORUGULOSIN

FIG-I-I

## BIOGENESIS

Seshadri[40,41] considered that orsellinic unit, an invariable component of the lichen acids and depsidones could give rise to many of the fungal products. He postulated that in the case of the anthraquinones a 3:5-dihydroxyphthalic acid unit is combined with a second C8 unit which has undergone greater modification. In this scheme 'frangula' emodin group is the earliest to be evolved biogenetically and others arise by further changes such as nuclear oxidations, reductions and methylation.




XX XXI

XXII XXIII

XXIV

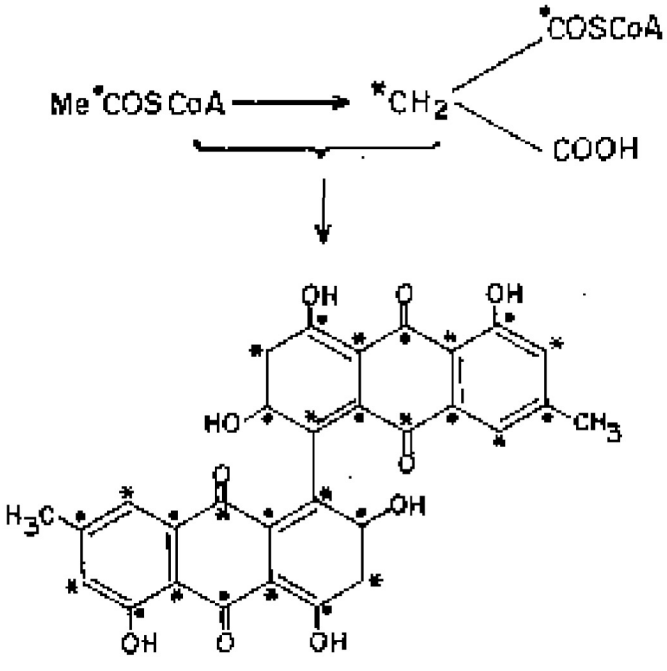
However, no experimental evidence has been obtained so far to substantiate this hypothesis.

The acetate hypothesis of biosynthesis with which the names of Collie,[42] Robinson,[43] and Birch[44] are chiefly associated, claim that many of the fungal metabolites and in particular the quinones, are built up from chains of acetate units combined in some cases with methylation by $C_1$ units. This theory, which originated as a result of inductive reasoning from structural comparison of a large number of natural products has been extensively supported by experimental evidences using $^{14}C$ and $^{18}O$ labelled acetate as the precursor.[45-48]

The recent finding of the participation of malonate in fatty acid synthesis has, however, brought about a new trend of thinking about the biosynthesis of acetate-derived compounds. From the experimentally confirmed examples of 6-methyl salicylic acid,[49,50] orsellenic acid,[51,52] oxytetracycline,[51] the generalization that the so-called acetate derived compounds are actually made up from an acyl-coenzyme A derivative acting as a primer on the condensation of malonyl units, can be made with great confidence.

Recently, Gatenbeck,[53] from the results of experiments performed with a culture of Penicillium islandicum Sopp has shown that in the biosynthesis of

islandicin, one activated acetate unit initiates the condensation of seven malonate units to form the anthraquinone in an overall reaction. The biosynthesis of rugulosin in Penicillium cyclopium was studied by Shibata and Ikekawa.[54] Their studies showed that rugulosin is biosynthesised by the head-to-tail condensation of fourteen malonate units with release of carbon-dioxide and two units of acetate which form preferentially the terminal C2-units.




RUGULOSIN

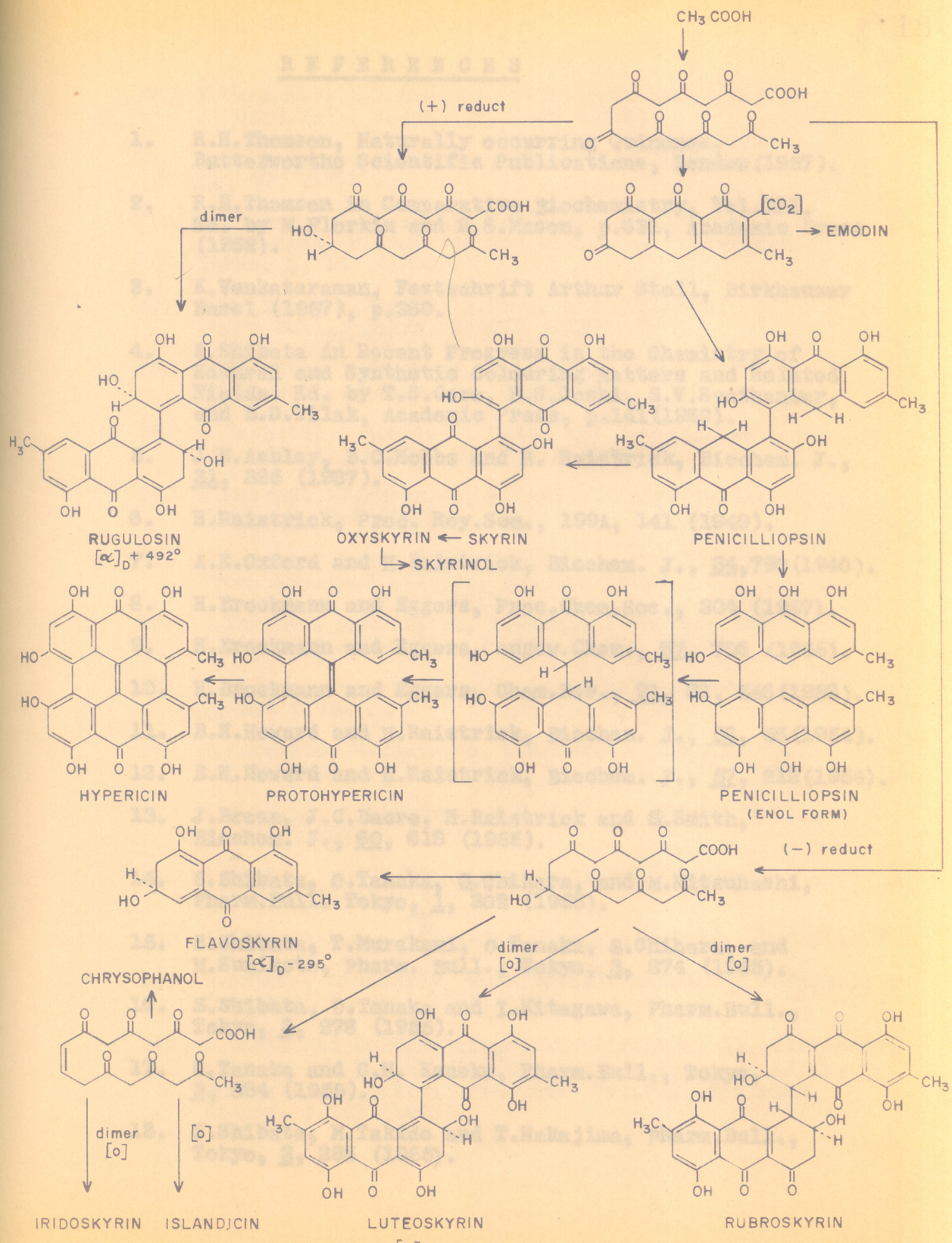
TH-209



It can be easily seen that the chemical structure of the pigments discussed in this chapter suggests a close biosynthetical inter-relation between them. An interesting pattern of the biosynthetical stages was put forward by Shibata[4] to elucidate the formation of anthraquinones, bianthraquinones and the related pigments of Penicillium islandicum, P.rugulosum and some other related fungi. This has been summarised and represented in Fig.2.

It may be noted that hypericin has strikingly close relationship with these fungal products. From the present state of knowledge, however, it is not possible to say if in nature the bianthraquinones and pigments of the hypericin family arise by similar route, though the similarity between their structures can hardly be fortuitous. It is quite possible that future will bring to light more compounds, which may serve as missing links of the biosynthetic schemes for these two types of complex quinones.

KV
547.673(043)
GHO


CH3 COOH
(+) reduct
dimer
[CO2]
EMODIN
RUGULOSIN
[α]D + 492°
OXYSKYRIN
SKYRIN
SKYRINOL
PENICILLIOPSIN
HYPERICIN
PROTOHYPERICIN
PENICILLIOPSIN
(ENOL FORM)
(−) reduct
FLAVOSKYRIN
[α]D - 295°
CHRYSOPHANOL
dimer
[o]
dimer
[o]
dimer
[o]
[o]
IRIDOSKYRIN
ISLANDICIN
LUTEOSKYRIN
[α]D -880°
RUBROSKYRIN

FIG·1·2

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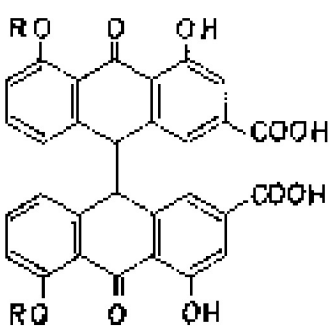
# C H A P T E R I (PART B)

## CHEMICAL INVESTIGATION OF CASSIA SIAMEA LAM.

## CONSTITUTION OF CASSIAMIN

The senna drugs consisting of the plants of Cassia species have been well-known for centuries as one of the most reliable laxatives.[1-2] Besides this, numerous uses e.g. cooling agent in eye affection, remedy against leprosy, tumour damaging capacity etc. are attributed to Cassia plants and biological preparation of the pericarp named as 'Senokot' is also reported.[2-5]

Notwithstanding the reputations which the Senna drugs have enjoyed for several centuries, very little information on the chemical investigation of the active constituents are available. Dragendorff[6] in 1566 attributed the activity of senna to a substance called Cathartic acid, which was subsequently found[4,7,8] to be a mixture of anthraquinones and their glycosides. Stoll and co-workers[3,9] have made outstanding contribution in the chemistry of senna drugs by isolating two main active principles in pure crystalline forms, which were designated as sennosides A and B (Ia). By a series of careful experiments, they established the structures of both these stereoisomerides and also those of the aglycones sennidines A and B (Ib).

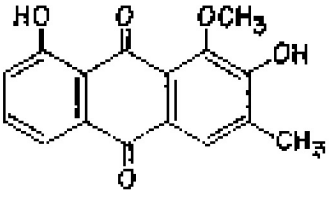



I a $R = C_6H_{11}O_5$
SENNOSIDES A and B

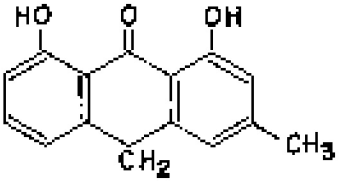
I b $R = H$
SENNIDINES A and B

A third active glycoside in small amount was also isolated[10,11] but its constitution has not been reported.

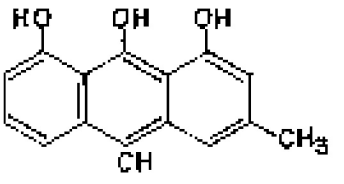
It may be mentioned that chemical examination of senna has been confined to two main varieties - Cassia angustifolia and Cassia acutifolia.[3,4] The isolation of rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid) has been reported from Cassia angustifolia, C. reticulata, C.fistula and C. alata.[1] Recently Takido[12] reported the isolation of chrysophanol, emodin and a new anthraquinone, obtusifolin from Cassia obtusifolia. The latter was shown to have the constitution(II).




II




ANTHRONE FORM




ANTHRANOL FORM

III

Except the isolation of chrysarobin (III) from the wood[13,14] and γ-sitosterol from the seeds[15] of Cassia fistula, no work appears to have been done with this plant.

## PRESENT WORK

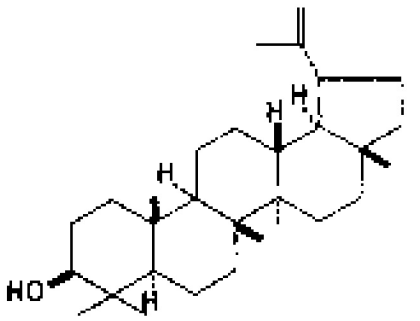
The present work was undertaken with the root bark of *Cassia siamea* Lam. (Leguminosae), a plant which grows in plenty in the State of Maharashtra. As a result of the present investigation, four compounds - one triterpene and three hydroxyanthraquinone derivatives, have so far been isolated.

### Examination of the n-hexane extract

The root bark on extracting with n-hexane produced, a dark brown solid, which was chromatographed over neutral alumina and eluted with different solvents. From the pet. ether (40-60°) eluate, a solid was obtained, which crystallised from ether-methanol as colourless needles, $C_{30}H_{50}O$, m.p. 210-211°; $(\alpha)_D^{20}$ + 26°. It gave a positive Liebermann-Burchard test (violet), a yellow colour with tetranitromethane and a light red colour with trichloroacetic acid. The infrared spectrum showed strong absorption at 3600, 3430, 1025 $cm^{-1}$ (hydroxyl), 1640 and 887 $cm^{-1}$ (characteristic of a vinylidine group $>C=CH_2$).[16] From the monoacetyl derivative, $C_{32}H_{52}O_2$, m.p. 218°, $(\alpha)_D^{20}$ + 46° and the benzoate, $C_{37}H_{54}O_2$, m.p. 266°, $(\alpha)_D^{20}$ + 62°, the compound was found to be identical with lupeol (IV), a pentacyclic triterpene. The compound showed no depression in melting point when admixed with an authentic sample

of lupeol and their IR spectra were superimposable.

It may be mentioned that this is the first triterpene isolated so far from the Cassia plants.



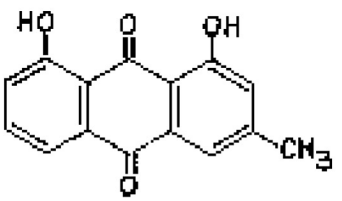

IV

After the separation of lupeol, the column was eluted with benzene, when a pink band gradually separated. From the benzene-ethyl acetate eluate, on removal of the solvent under vacuum, an orange red coloured compound was obtained which crystallised from ethanol as dark yellow leaflets, $C_{15}H_{10}O_4$, m.p. 196°. The homogeneity of the compound was confirmed from thin layer chromatography, using benzene-acetone (80:20) as the solvent system which showed a single spot ($R_f$ 0.92). It gave a positive Börntrager's test for hydroxyanthraquinones,[17] a brown red colour with ferric chloride and an orange colour with methanolic magnesium acetate.[18] It was insoluble in aqueous sodium carbonate, but dissolved readily in sodium hydroxide solution giving a reddish violet colour. It gave reddish violet colour in concentrated sulphuric acid.

The compound easily formed a diacetate, $C_{19}H_{14}O_6$, m.p. 207° and a dimethyl ether, $C_{17}H_{14}O_4$, m.p. 195-196°, thus showing the presence of two hydroxyl groups in the molecule. Kuhn-Roth estimation showed the presence of one C-CH$_3$ group.

The infrared spectrum of the compound showed strong absorptions at 1672 cm$^{-1}$ (unchelated carbonyl), 1635 cm$^{-1}$ (chelated carbonyl) and 1598 cm$^{-1}$ (phenyl band).[19] The ultraviolet spectrum showed λmax. at 227, 255, 278, 287, and 427 mμ (log ε 4.62, 4.36, 4.02, 4.04, 4.06), indicating the compound to be a 1,8-dihydroxy-anthraquinone derivative,[30] (Fig. 1.6). The dimethyl ether showed λmax. at 232, 267, 275 and 388 mμ (log ε 4.64, 4.5, 4.2, 3.97), (Fig. 1.7).

All the above properties of the compound indicated similarity with those of chrysophanol (V).




V

The nuclear magnetic resonance spectra of the diacetate and the dimethyl ether, determined in deutero-chloroform using tetramethylsilane as internal reference, was in full agreement with the above conclusion.

Electronic integration confirmed the presence of a total of fourteen protons in both the derivatives. The methyl ether spectrum showed a single signal at 6.1 $\tau$ (6) indicating two methoxyl groups and a signal at 7.63 $\tau$ (3) indicating an aromatic methyl group. Correspondingly, two signals at 7.6 $\tau$ (6) (two acetoxy groups) and at 7.66 $\tau$(3) (one aromatic methyl group) was discernible from the spectrum of the acetate. The spectra of the parent hydroxyanthraquinone, determined in dioxane, showed two signals at - 1.31 and - 1.14 $\tau$ representing two chelated hydroxyl groups.

An authentic sample of chrysophanol was obtained through the courtesy of Prof. K. Venkataraman, which showed no depression in mixed melting point with the compound. The infrared and UV spectra were superimposable.

Examination of the benzene extract

The benzene extract of the root bark after removal of the solvent under vacuum afforded an orange-red solid in about 1% yield. Preliminary colour reactions showed the presence of hydroxy-anthraquinone derivatives in the pigment. By thin-layer chromatography, three distinct spots corresponding to Rf: 0.98, 0.73 and 0.53 were obtained. All of them showed orange to pink coloured spots when sprayed with alcoholic magnesium acetate indicating the presence of at least three compounds in the

crude benzene extract. The compounds having Rf values 0.82, 0.73 and 0.63 were designated as Compounds A, B, and C respectively. But the separation and isolation of these compounds in pure form on a preparative scale presented considerable difficulties. It was, however, observed that from the crude extract, the Compound A could be separated by repeated extraction with n-hexane, but the solvent extraction method was not successful in the separation of the other two compounds. The use of dilute alkali in the separation of these compounds was of little effect. Both the compounds were insoluble in sodium bicarbonate. They slowly went into solution in hot sodium carbonate and were highly soluble in dilute sodium hydroxide solution giving intense violet colour.

Different chromatographic techniques reported for the separation of anthraquinones were attempted. The chromatography over ignited magnesia in acetone solution was used by Briggs[21] for the isolation of a number of anthraquinone pigments from the plants of Conrema genus. Magnesium carbonate has been used to separate the pigments from Cenatula pentinata.[22] Calcium hydrogen phosphate was used mainly by the Japanese group of workers for the isolation and purification of hydroxyanthraquinone derivatives.[12,37] But in the present case, none of the above methods even with the use of different solvent systems was satisfactory. Florex column also did not give any good separation.

Comparatively better results were achieved by chromatography over silica gel. By elution with benzene followed by benzene-ethyl acetate and finally ethyl acetate, pure samples of Compounds A and B could be obtained, though the last fractions consisted of the mixture of Compounds B and C. With neutral alumina using the same solvent system, the separation of Compounds A and B was also satisfactory. But the isolation of Compound C could be achieved in a very small amount only. The yields of the hydroxyanthraquinones were low; considerable amount of the compounds being firmly held by the column.

Compounds A and B obtained by the above methods were purified by rechromatography.

From the colour reactions and other physical and chemical properties, Compound A was readily identified as chrysophanol.

Compound B was, however, found to be a new anthraquinone derivative and the name 'Cassiamin' is proposed for it.

## CONSTITUTION OF CASSIAMIN

Cassiamin was obtained from the ethyl acetate fraction of the silica gel chromatography as well as from the bright reddish orange band of the alumina chromatography by elution with the same solvent. It could be crystallised from tetrahydrofuran-methanol-ether as bright orange yellow microcrystals or from acetone-benzene as red microcrystals. The two types of crystals, both melting with decomposition at 356-57° (darkening from 340°) gave identical Rf values (0.73) in thin-layer chromatography. Their infrared and ultraviolet spectra were superimposable and the mixed melting point was undepressed.

Cassiamin could be sublimed at high vacuum ($10^{-3}$ mm at 260-270°) when orange red micro-crystals were obtained. It could also be purified by acetylation and subsequent alkaline hydrolysis of its acetate.

### Properties and colour reactions of Cassiamin

Cassiamin is insoluble in pet.ether, n-hexane, cyclohexane and carbontetrachloride, sparingly soluble in ether, methanol, ethanol, benzene, and toluene; moderately soluble in acetone, chloroform, ethyl acetate and glacial acetic acid. It is easily soluble in tetrahydrofuran, dioxane, cyclohexanone and pyridine.

Cassiamin gives characteristic colour reactions of hydroxyanthraquinones. It gives a positive Borntrager test (pink). It is insoluble in aqueous sodium bicarbonate (5%) and cold sodium carbonate (5%). In sodium carbonate it slowly goes into solution on heating, giving a violet coloured solution. In aqueous ammonia also it dissolves slowly giving a violet colour. However, in dilute sodium hydroxide (5%) or potassium hydroxide (5%) it readily dissolves giving an intense violet colour. With concentrated sulphuric acid, cassiamin produces an intense reddish violet colour, which remains unchanged by the addition of boric acid. Magnesium acetate in alcohol produces a pink colour with cassiamin.[18]

Indication of a true quinonoid structure was also obtained by the observation of the deep violet colour of cassiamin in sodium hydroxide changing to orange red on treatment with sodium dithionite. On passing air through the alkaline solution, the original violet colour is readily restored. The violet colour in ammonia is similarly changed to orange red by the addition of zinc dust.

Cassiamin gives no characteristic colour with zirconium nitrate in dilute hydrochloric acid solution. This observation rules out the possibility of cassiamin having two hydroxyls in ortho positions.[23] In glacial acetic acid solution of cassiamin, no fluorescence has

been observed in bread day light indicating that the α-hydroxyl groups in cassiamin do not have a 1,4-relationship.[18,24] However, a light greenish yellow fluorescence could be observed under ultraviolet light. Cassiamin in ethanolic solution gives only a brown-red colour with ferric chloride. It does not respond to Bargellini test, which is characteristic of vicinal trihydroxy groups.[26]

Cassiamin does not show any characteristic bluish green colour with furfural and concentrated sulphuric acid, thus ruling out the possibility of its being an anthrone derivative.[26] The hydroxyanthrones as well as hydroxydianthrones normally give a yellow colour with sodium hydroxide,[3,27] while cassiamin immediately forms an intense violet solution in sodium hydroxide.

Molecular formula of Cassiamin

Elementary analysis of cassiamin and its derivatives agreed more or less with the formula, $C_{17}H_{12-14}O_5$ or $C_{30}H_{18}O_9$ for cassiamin. It contained no nitrogen. Kuhn-Roth oxidation showed cassiamin to contain 6.0% C-methyl group which may indicate the presence of at least one C-methyl group with a $C_{17}$ formula and at least two with the $C_{30}$ formula. Cassiamin contained no

methoxyl group. Active hydrogen determination was not possible due to its insolubility in diglyme.

Molecular weight determination by Rast method was not successful due to the insolubility of cassiamin in camphor or exaltone.

On methylation with dimethyl sulphate-potassium carbonate-acetone, cassiamin afforded an orange yellow methyl ether, m.p. 295-98°. Acetylation with acetic anhydride and a few drops of perchloric acid or concentrated sulphuric acid yielded a yellow acetate, m.p. 180-83° (softening from 170°).

The molecular formula of cassiamin could be effectively determined from the NMR spectra of cassiamin and its derivatives.

The NMR spectrum of cassiamin in dioxane solution showed four signals at -2.29, -2.14, -1.97 and -1.83,* all exchangeable with $D_2O$, which could be attributed to four chelated hydroxyl groups.

The NMR spectrum of the methyl ether in deutero-chloroform (Fig. 1.3) showed three signals in the methoxyl region at 6.01, 6.05 and 6.3 and one signal at 7.85 in the aromatic methyl region. The intensities of these four signals were in the ratio 1:2:2:2 respectively.

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* Chemical shifts indicated are all in $\tau$ scale. Tetramethylsilane was used as the internal reference.

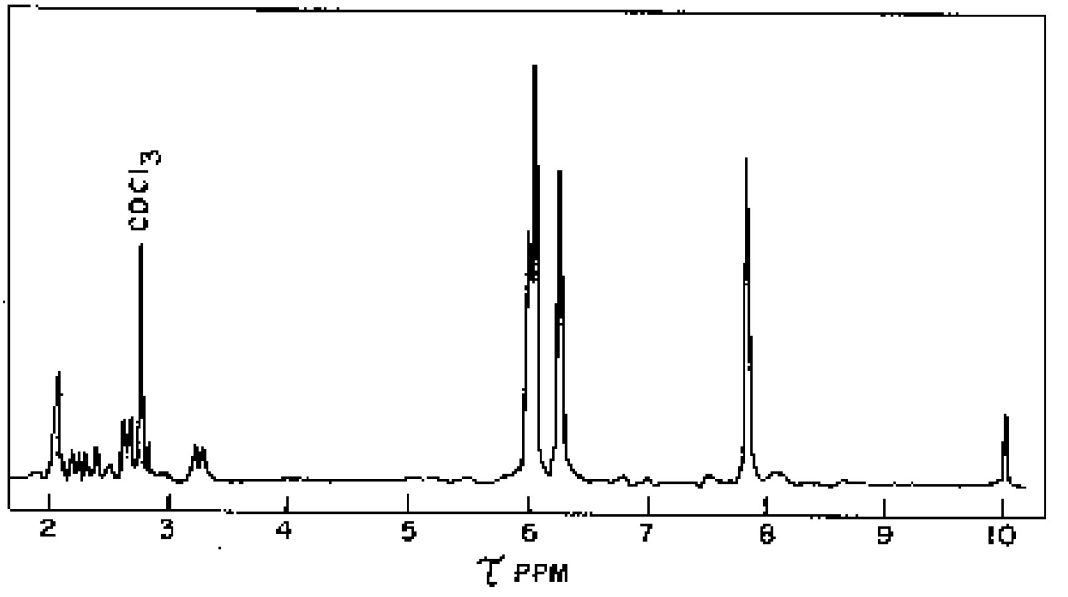



FIG-1-3

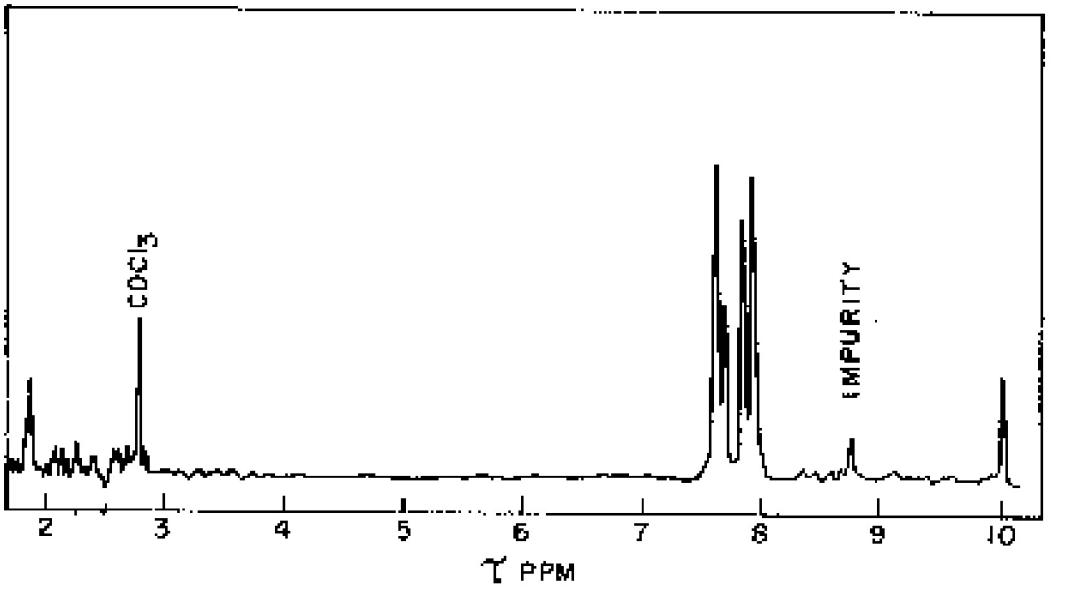



FIG-1-4

In the spectrum of the acetate (Fig. 1.4) there were no signals in the region 6 to 7 $\tau$ showing the absence of any -CH$_2$O- grouping in the molecule. There were signals at 7.6, 7.66, 7.84 and 7.93 with the intensity ratio of 2:1:2:2.

From the comparative study of the spectra of the methyl ether and the acetate, it was evident that the group of signals at 6.01, 6.05 and 6.3 in the methyl ether spectrum were shifted in the acetate spectrum to positions 7.6, 7.66 and 7.84 respectively. The hydroxyl groups in cassiamin which were methylated in the former were all acetylated in the latter case. Therefore, if the weaker signal at 6.01 in the methyl ether spectrum and that at 7.66 in the acetate spectrum represent single methyl groups, it can be assumed that cassiamin has five hydroxyl groups and two methyl groups in the aromatic rings. The fact that four signals were observed in the region - 1.83 to - 2.29 would make it likely that four out of the five hydroxyls are chelated to carbonyl groups.

Proton count by electronic integration further showed that there are seven aromatic protons in both the methyl ether and the acetate of cassiamin.

This conclusion automatically rules out the $C_{17}$ formula for cassiamin as this cannot accommodate eighteen protons (seven aromatic protons, five hydroxyls

and two aromatic methyls) shown by the NMR spectra. Therefore, only the other formula, $C_{30}H_{18}O_9$ can be assigned to cassiamin. These findings were substantially supported later by other methods. The thermister micromethod, which depends upon the application of electro-osmotic technique,[28] showed cassiamin to have a high molecular weight, though the results were rather anomalous.

The correctness of the molecular formula for cassiamin was finally and decisively proved by mass spectroscopic method (kindly determined by Dr. J. S. Shannon, Division of Coal Research, C.S.I.R.O., Australia), which showed the molecular weight of cassiamin to be 522, exactly as required by the formula, $C_{30}H_{18}O_9$.

The molecular formula, $C_{30}H_{18}O_9$ places cassiamin as an unusually complex hydroxyanthraquinone derivative. Further data, as will be seen later, showed cassiamin to be closely related to bianthraquinones. It may be noted that all the bianthraquinones so far isolated are essentially mould products. Cassiamin, should, therefore, be the first example of a hydroxy anthraquinone derivative having such a high molecular weight so far isolated from the plant world.

## Ultraviolet and Infrared spectra of Cassiamin and its derivatives

The ultraviolet and visible spectra of cassiamin and its derivatives also indicate that it is a hydroxy-anthraquinone. The light absorption spectra of cassiamin in different solvents show the following maxima(Table I) (Fig. 1.5 & 1.6).

### T A B L E I

| Solvent | $\lambda_{max}$, mμ | log ε |
|---|---|---|
| Ethanol | 226,259,288, 445 | 4.92,4.80,4.69, 4.61 |
| Concentrated sulphuric acid | 226,270,280, 310,330,505, 535,600 | 4.26,4.55,4.51, 4.3,3.97,4.36, 4.46,3.79 |
| 5% Sodium hydroxide | 236,260,290, 320,540 | 4.9,4.7,4.64, 4.59,4.52. |

Absence of absorption near 320 mμ indicates that cassiamin is not a 1,4-naphthoquinone derivative, since all the known 1,4-naphthoquinones show characteristic absorptions in this region.[29-31] The naphthoquinone structure for cassiamin is further ruled out by the

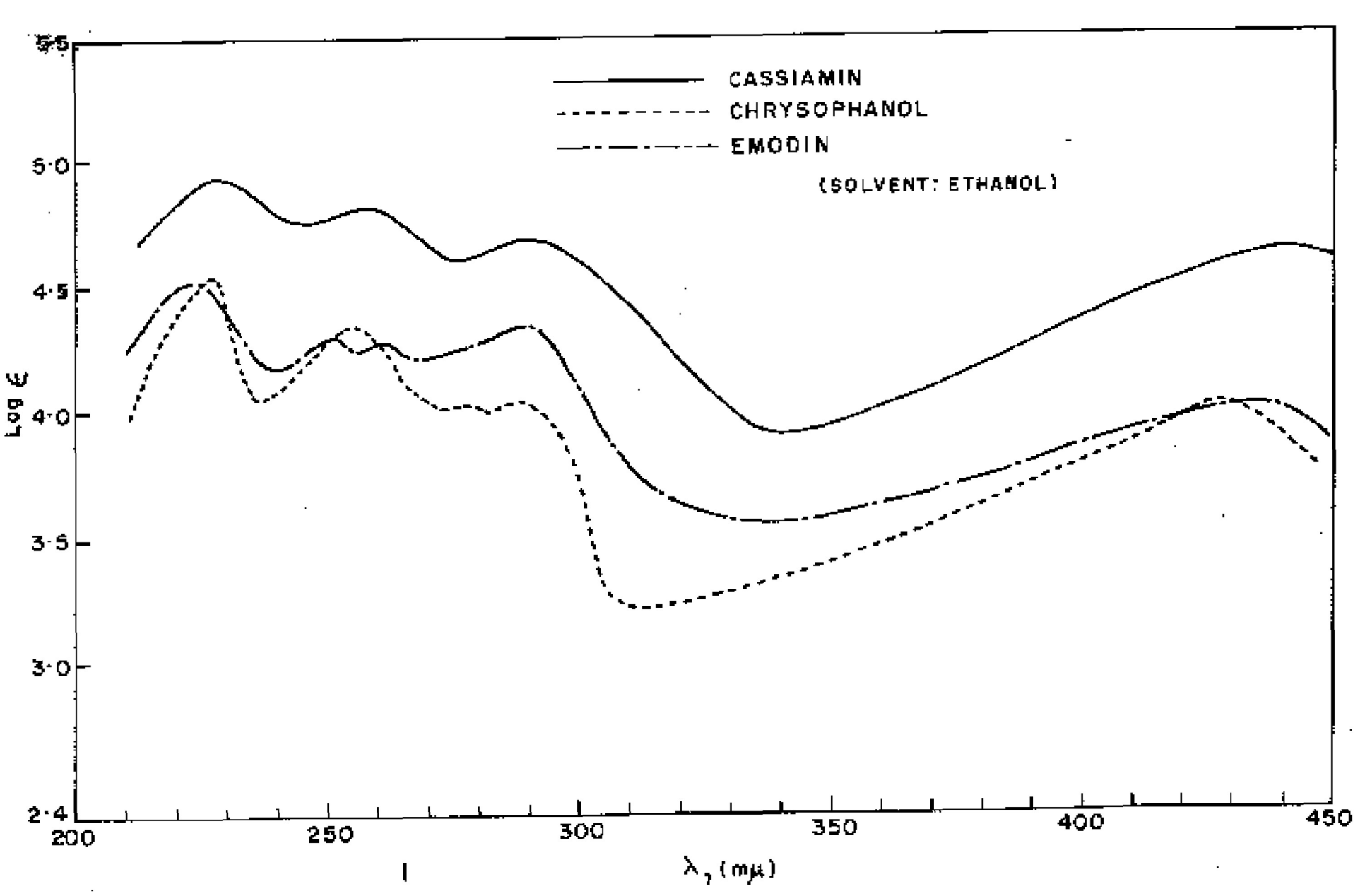

CASSIAMIN
CHRYSOPHANOL
EMODIN
(SOLVENT: ETHANOL)
5·5
5·0
4·5
4·0
3·5
3·0
2·4
Log ε
200
250
300
350
400
450
λ, (mμ)

FIG. I-5

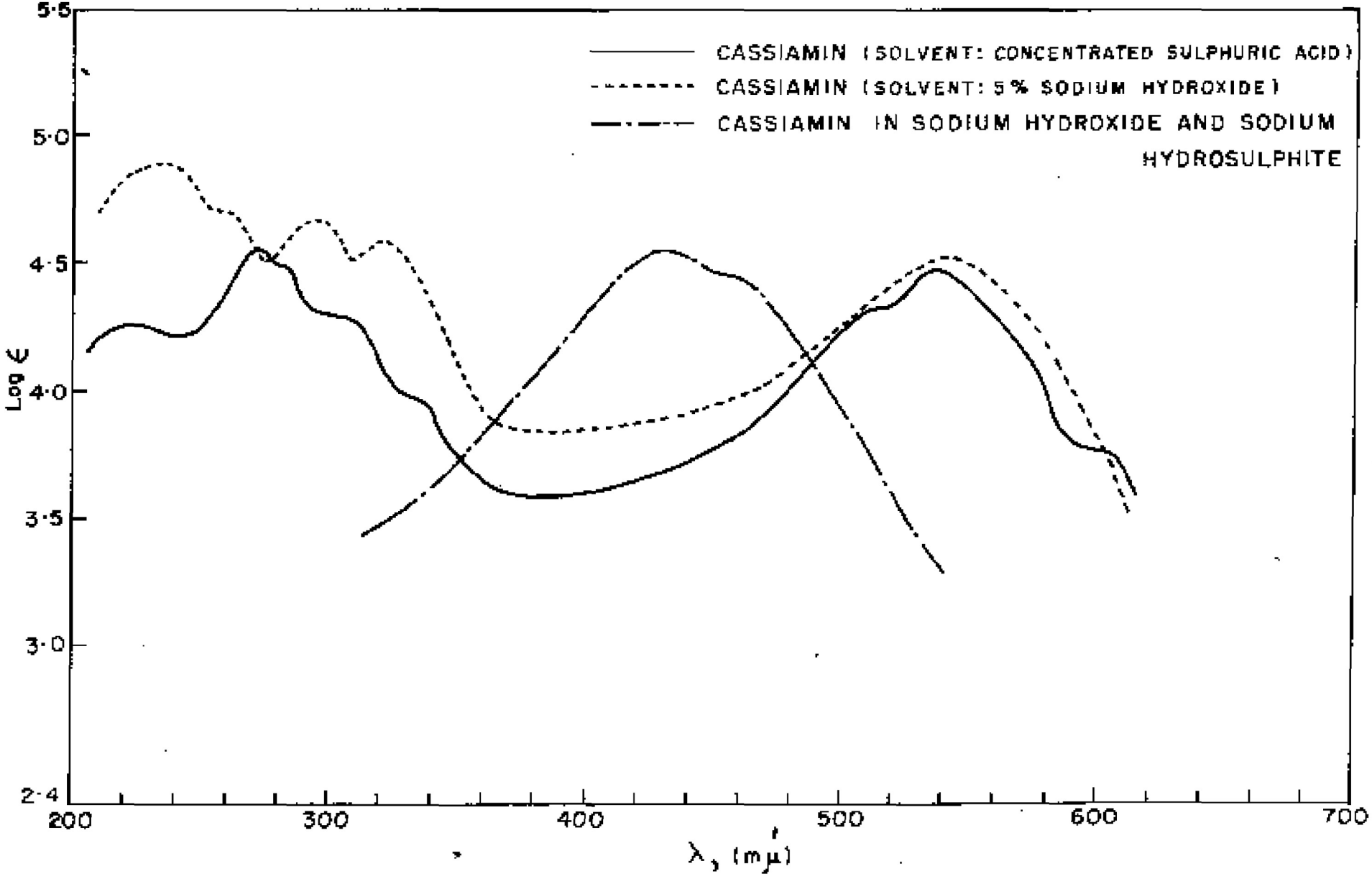

CASSIAMIN (SOLVENT: CONCENTRATED SULPHURIC ACID)
CASSIAMIN (SOLVENT: 5% SODIUM HYDROXIDE)
CASSIAMIN IN SODIUM HYDROXIDE AND SODIUM HYDROSULPHITE
Log ε
5·5
5·0
4·5
4·0
3·5
3·0
2·4
200
300
400
500
600
700
λ, (mμ)

FIG. 1·6

spectrum of its reductively acetylated product which shows λmax at 250, 264, 272, 355, 375 and 390 mμ. (log ε 4.66, 4.84, 5.03, 3.88, 3.92, 3.83) (Fig. 1.9).

Brockmann and Budde[32] observed that the absorption spectra of the reductively acetylated quinones and hydroxyquinones resemble the spectra of the parent hydrocarbons. It was further shown by Patwardhan[33] that reductively acetylated hydroxy-naphthoquinones do not absorb beyond 350 mμ. The present results from the UV spectra showed that cassiamin is more likely to be an anthraquinone derivative.

It was shown by Venkataraman and co-workers[33,34] that quinones in their leuco form show a characteristic absorption in the visible region. This observation can be utilised to determine the basic ring skeleton of quinones, as the leuco naphthoquinones and anthraquinones absorb at different wave lengths. The leuco vat spectra of cassiamin also indicate it to be an anthraquinone derivative. It has λmax at 432, 460 mμ (log ε 4.53, 4.46) (Fig. 1.6).

As will be seen from Table I , cassiamin itself shows maxima at 228, 259, 288 and 445 mμ, which is in agreement with a 1,8-dihydroxyanthraquinone chromophore.[30] Briggs and collaborators[35] found that in the ultraviolet spectra of hydroxyanthraquinones, the major absorption band due to the carbonyl group of the quinonoid nucleus

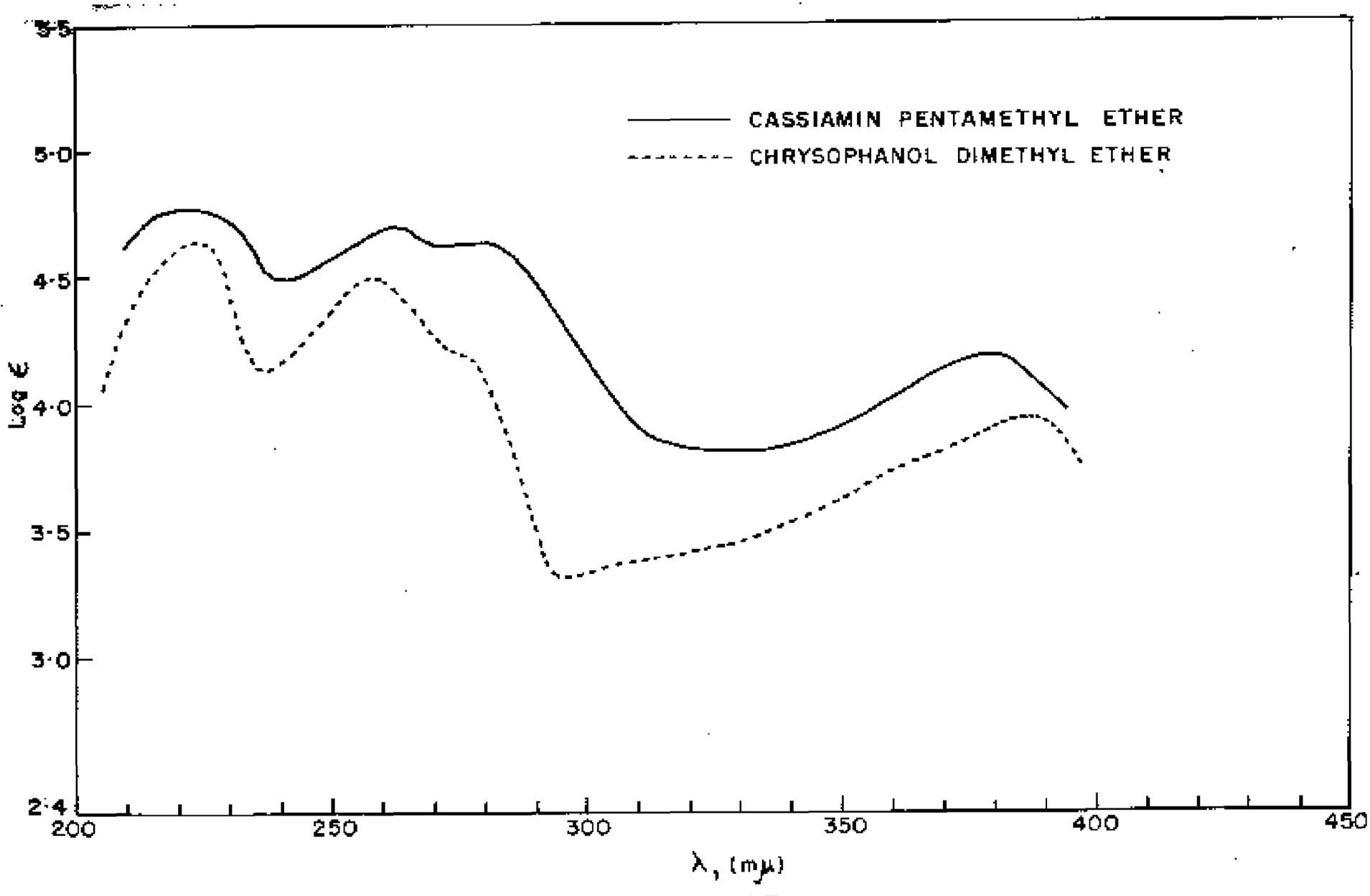
is greatly affected by the number of free α-hydroxyl groups, the presence of β-hydroxyl groups having little effect. Thus in hydroxyanthraquinones containing no free α-hydroxyl , this band occursnear 360 mμ, with one free α-hydroxyl near 410 mμ and with two such groups near 430 mμ. This observation was fully supported by Birkinshaw.[30] He also showed that in the anthraquinones having three or four free α-hydroxyl groups, the above mentioned bands give place to new bands in the 490, 510 mμ region. Ikido[28] showed further that hydroxyanthraquinones with 1,4-dihydroxy groups in the molecule exhibit a shift in the visible region, the absorption maxima appearing above 480 mμ.

From the above discussion, it would be logical to assume that cassiamin does not contain a system having three free α-hydroxyl groups in the same anthraquinone unit. A 1,4-dihydroxyanthraquinone system is also likely to be absent in cassiamin.

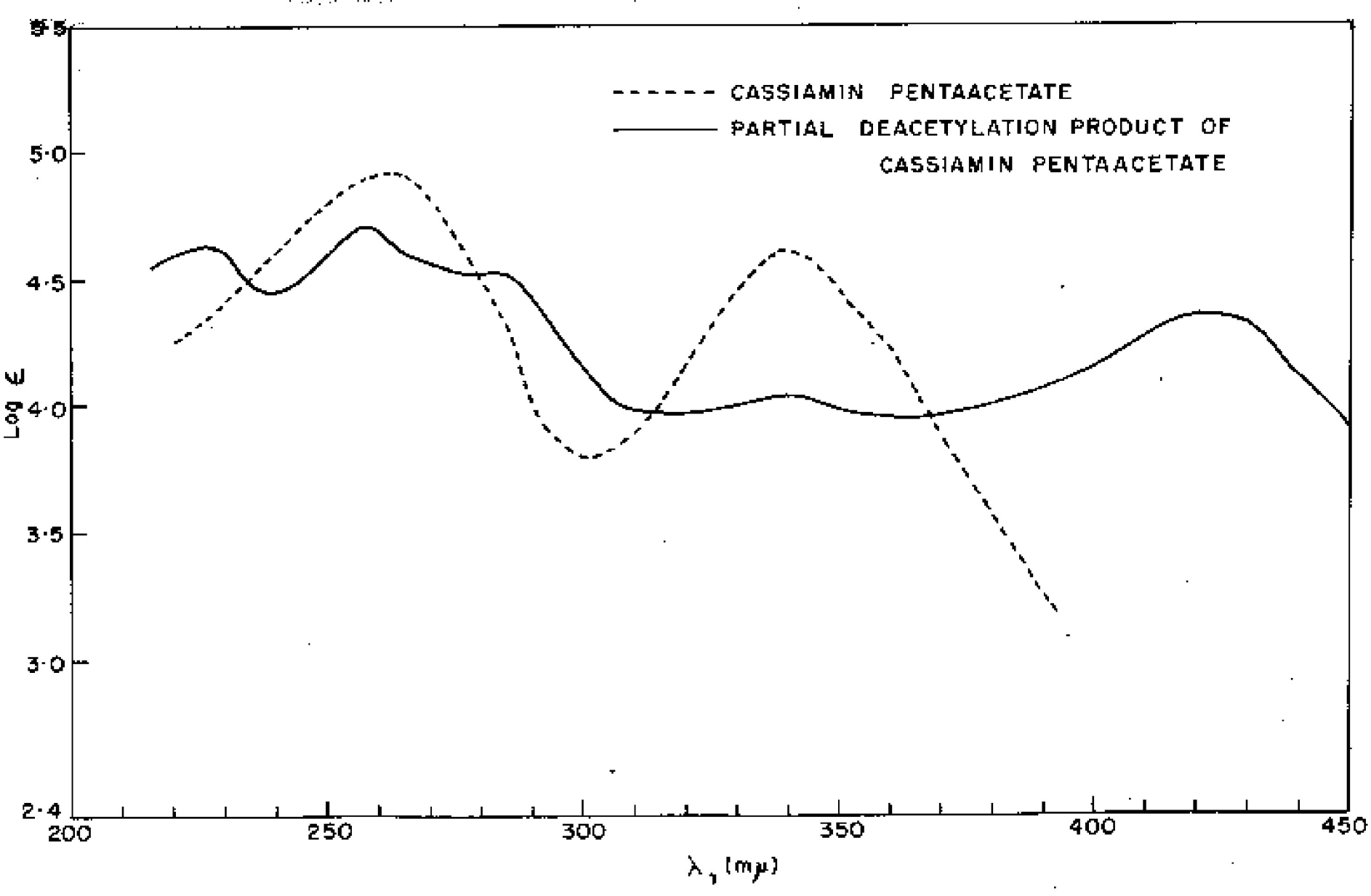
The absorption spectra of the methyl ether and acetate of cassiamin has been shown in Table II (Fig. 1.7 and 1.8).

TABLE II

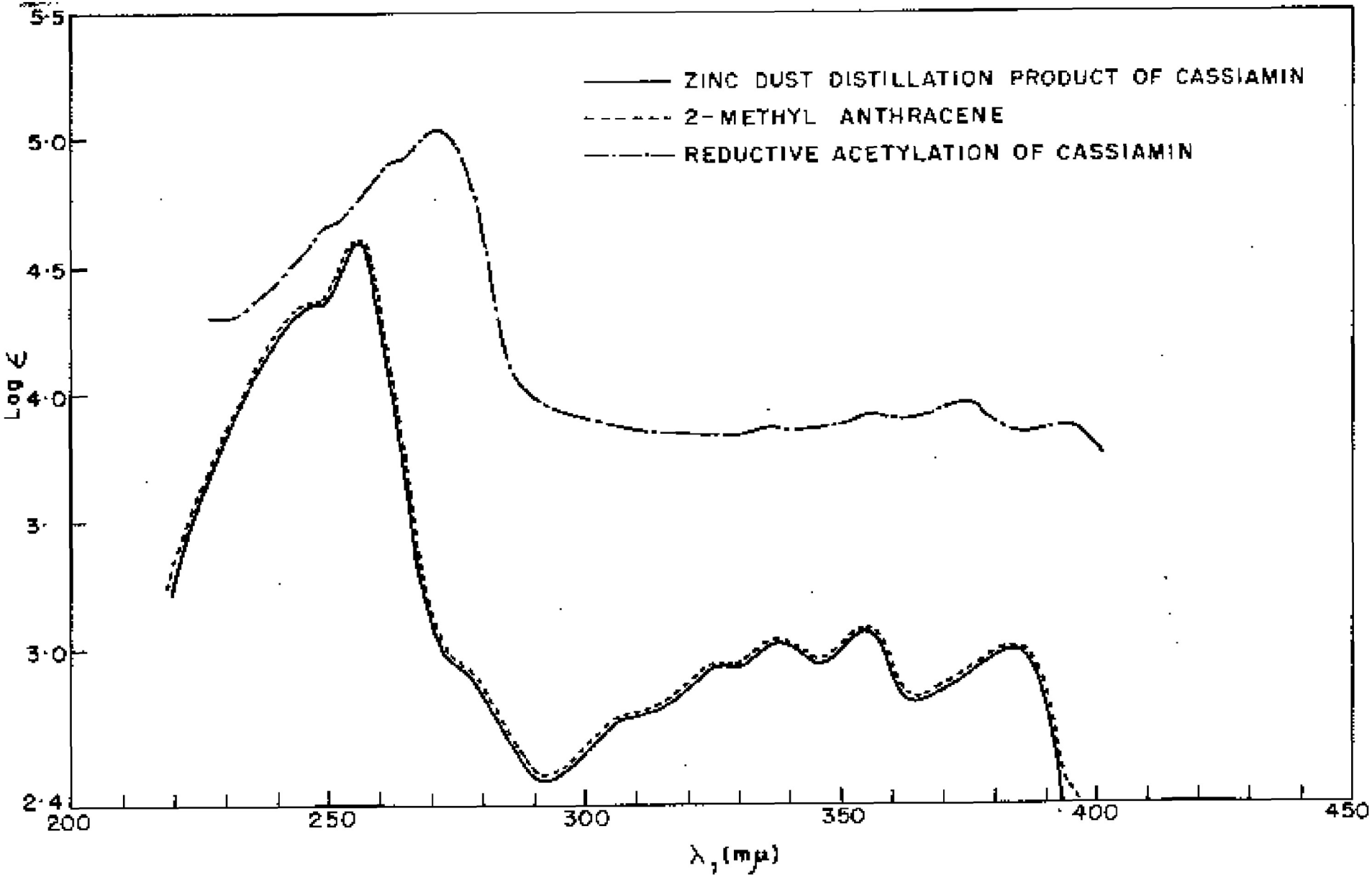
| Compound | $\lambda_{max}$, mμ | log ε |
|---|---|---|
| Cassiamin pentamethyl ether | 323,262, 280,230 | 4.75, 4.69 4.61, 4.13 |
| Cassiamin pentaacetate | 262,240 | 4.9, 4.6 |


CASSIAMIN PENTAMETHYL ETHER
CHRYSOPHANOL DIMETHYL ETHER
Log ε
5·5
5·0
4·5
4·0
3·5
3·0
2·4
200
250
300
350
400
450
λ, (mμ)

FIG·1·7


CASSIAMIN PENTAACETATE
PARTIAL DEACETYLATION PRODUCT OF CASSIAMIN PENTAACETATE
Log ε
5·5
5·0
4·5
4·0
3·5
3·0
2·4
200
250
300
350
400
450
λ, (mμ)

FIG·1·8


ZINC DUST DISTILLATION PRODUCT OF CASSIAMIN
2-METHYL ANTHRACENE
REDUCTIVE ACETYLATION OF CASSIAMIN
Log ε
5·5
5·0
4·5
4·0
3·
3·0
2·4
200
250
300
350
400
450
λ, (mμ)

FIG·1·9

The methyl ether of cassiamin shows no absorption maxima above 380 mμ indicating the absence of any free α-hydroxyl group. The spectrum of the acetate of cassiamin also leads to similar conclusion that cassiamin is a hydroxyanthraquinone derivative.

The light absorption spectra of the bianthraquinone derivatives have not been extensively studied.[37,38] It is interesting to note that dianhydro-rugulosin which is built up from two chrysophanol units, has a very similar spectrum to that of the latter. But the absorption in dianhydrorugulosin is far more intense than that of chrysophanol. Similar relationship exists in the case of bis-nor-dianhydrorugulosin and chrysazin and also in iridoskyrin and islandicin.

The infrared spectra of cassiamin and its derivatives are also in good agreement with the assumption that cassiamin contains a 1,8-dihydroxy-anthraquinone system.[19,39-41] The infrared spectrum of cassiamin in nujol mull (Fig. 1.10) shows peak at 3380 $cm^{-1}$ (hydroxyl), 1672 $cm^{-1}$ (unchelated carbonyl), 1623 $cm^{-1}$ (chelated carbonyl) and 1600 $cm^{-1}$ (phenyl). The pentamethyl ether in nujol mull (Fig. 1.11) and chloroform solution shows absorption bands at 1672 $cm^{-1}$ (unchelated carbonyl) and 1600 $cm^{-1}$ (phenyl band), whereas the acetate of cassiamin in nujol mull (Fig. 1.12) shows peaks at 1769 $cm^{-1}$ (acetate), 1670 $cm^{-1}$ (unchelated carbonyl) and 1598 $cm^{-1}$ (phenyl).

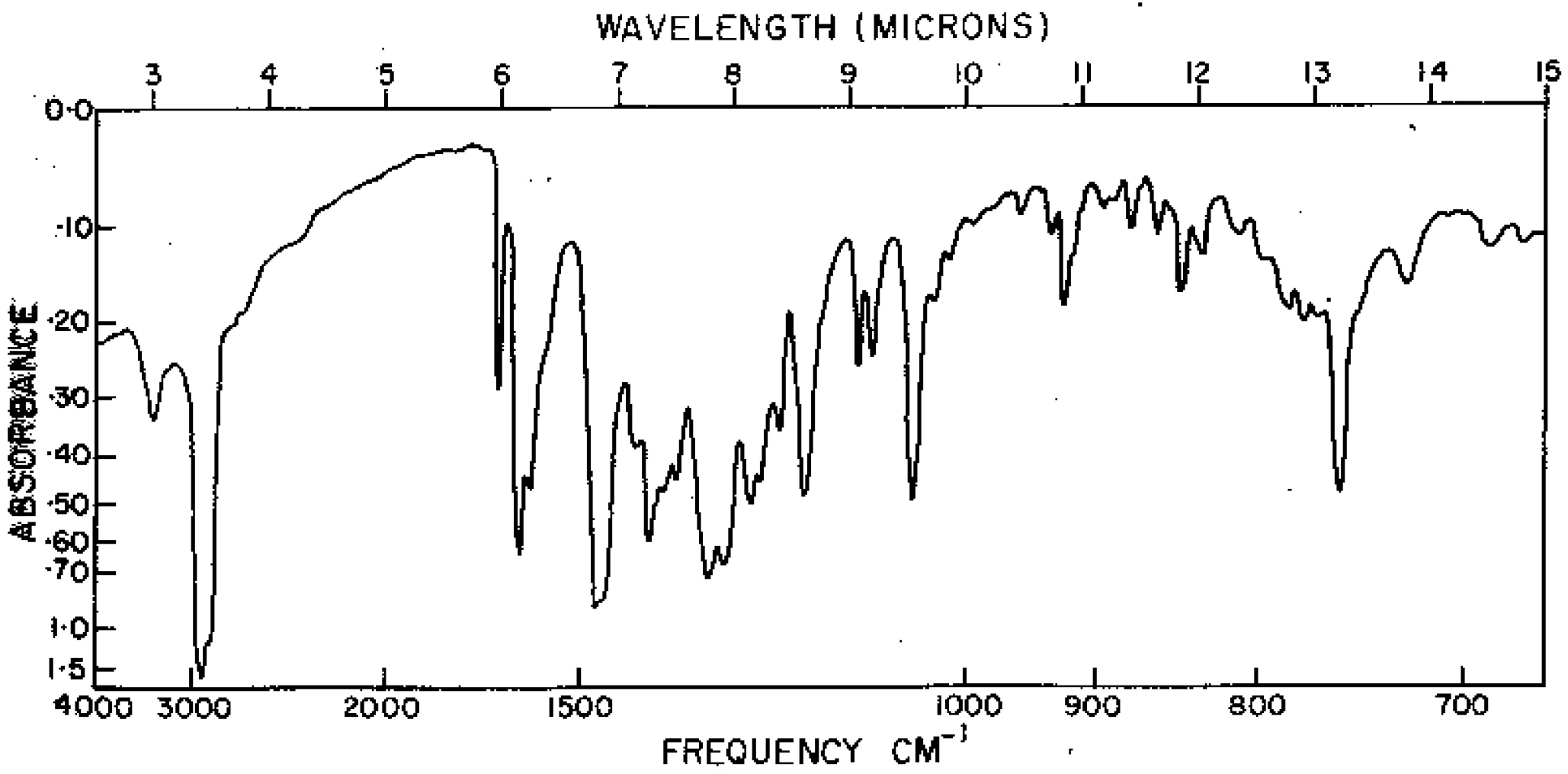

WAVELENGTH (MICRONS)
3
4
5
6
7
8
9
10
11
12
13
14
15
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
ABSORBANCE
4000
3000
2000
1500
1000
900
800
700
FREQUENCY CM⁻¹

FIG. 1.10.

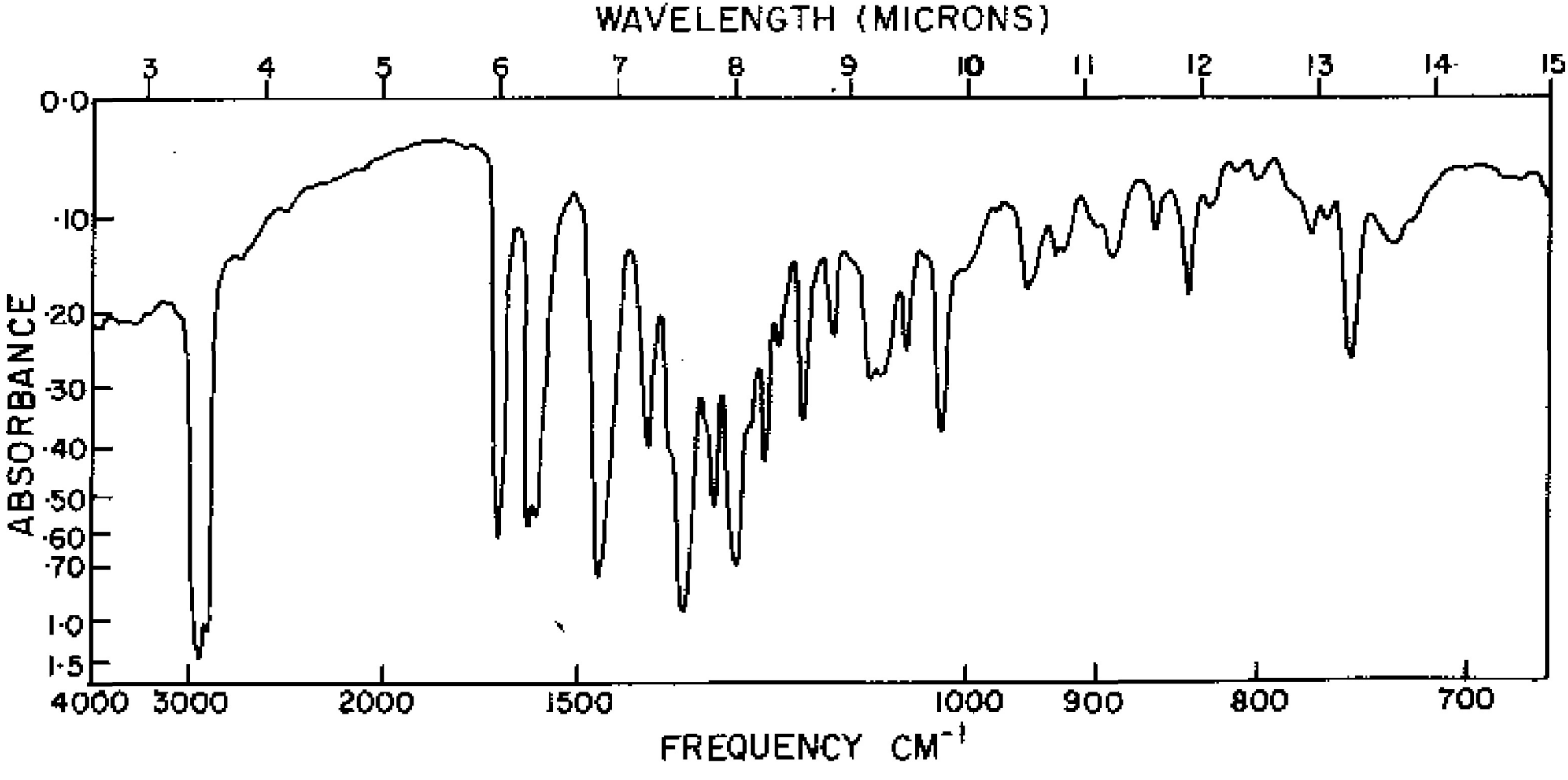

WAVELENGTH (MICRONS)
3
4
5
6
7
8
9
10
11
12
13
14
15
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
ABSORBANCE
4000
3000
2000
1500
1000
900
800
700
FREQUENCY CM⁻¹

FIG. I.II.

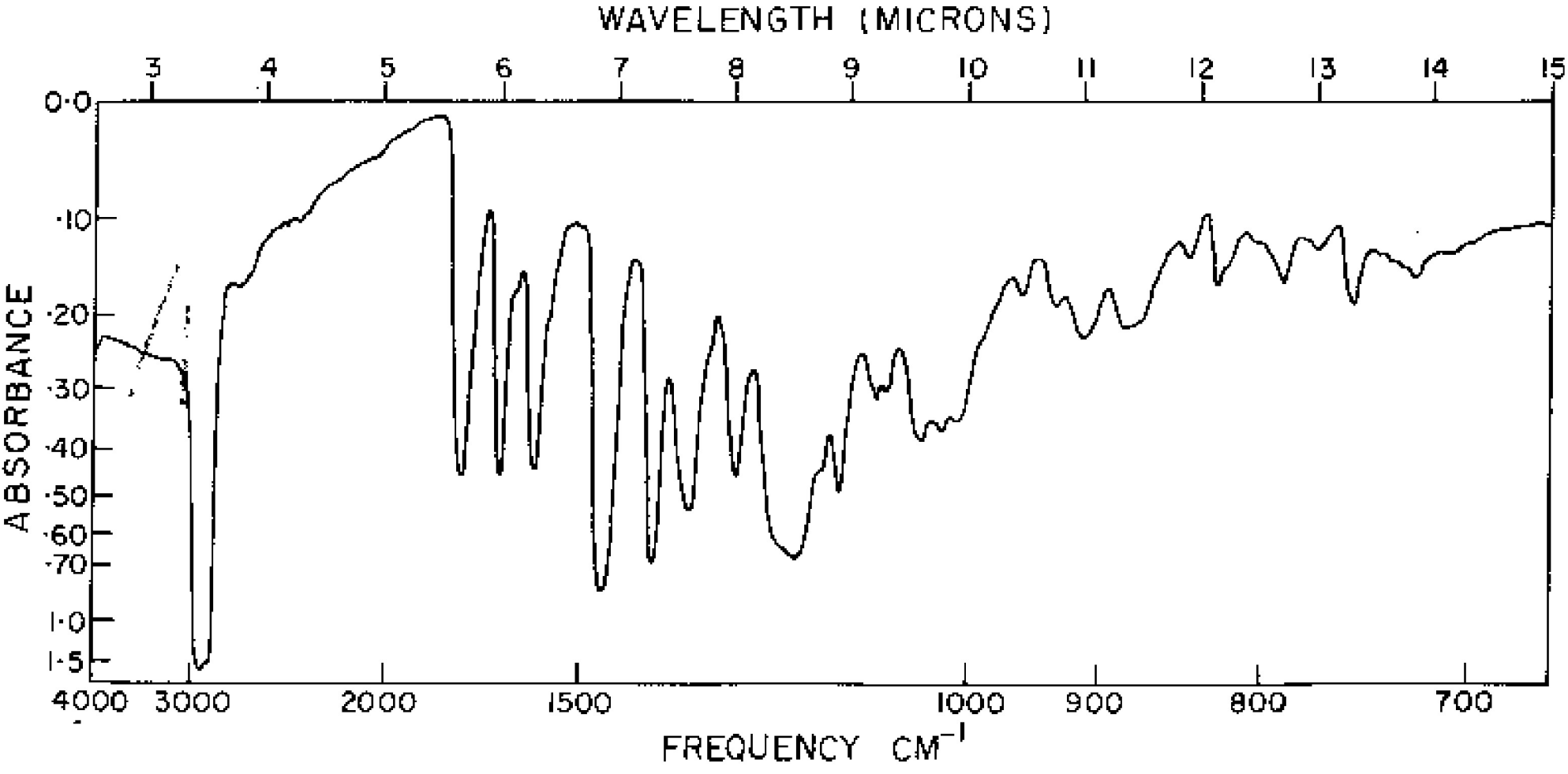

WAVELENGTH (MICRONS)
3
4
5
6
7
8
9
10
11
12
13
14
15
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
ABSORBANCE
4000
3000
2000
1500
1000
900
800
700
FREQUENCY CM⁻¹

FIG. 1.12.

The absence of absorption near 1623 $cm^{-1}$ in the last two compounds clearly indicates that the chelating hydroxyl groups have all been substituted. Cassiamin in dioxane shows peaks at 3600, 1672, 1625 and 1600 $cm^{-1}$.

Further, a 1,8-dihydroxy structure is also to be expected on phytochemical grounds since all hydroxy-anthraquinones so far obtained from the cassia plants have at least two hydroxyls in 1,8 positions.[1]

A remarkable property of cassiamin is its optical activity. The pentaacetate of cassiamin showed optical rotation $(\alpha)^{30}_{589}$ - 169.6° (measured on a Perkin-Elmer 141 Polarimeter).

Cassiamin is stable against the action of strong alkali and concentrated sulphuric acid; it could be recovered unchanged even after heating it with concentrated sulphuric acid for 2 hours and 10% sodium hydroxide for 6 hours on a water bath (after acidification).

Zinc dust distillation of cassiamin yielded 2-methyl anthracene, m.p.206° identified from its infrared (Fig.1.13) and ultraviolet spectra (Fig.1.9) in comparison with an authentic sample. The mixed melting point of both the samples was undepressed. It may be mentioned that Rugulosin[41] and Penicilliopsin,[42] which are closely related to bianthraquinones also give 2-methyl-anthracene on zinc dust distillation.

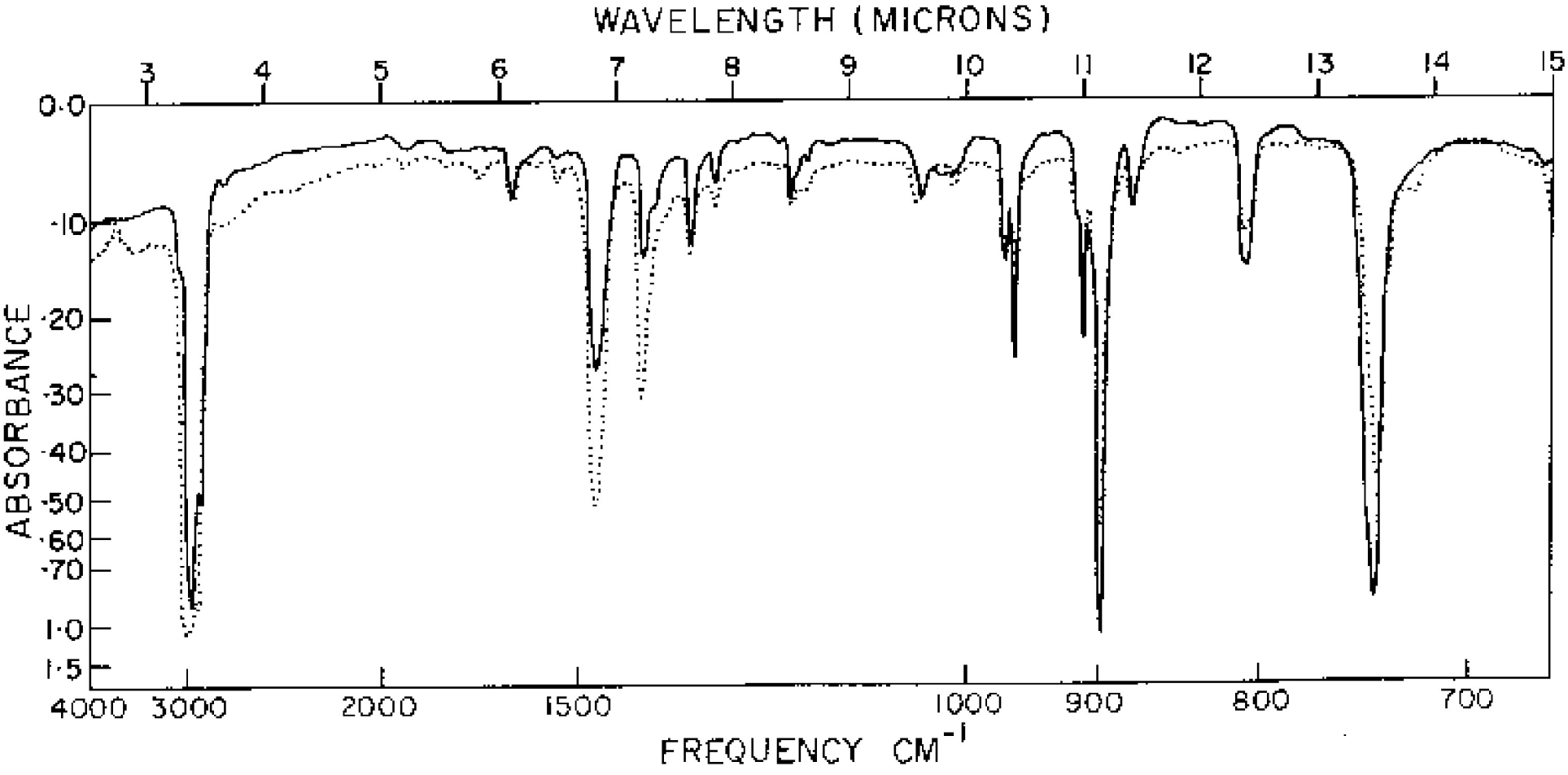



FIG. I. 13.

IR SPECTRA OF: ——— 2-METHYLANTHRACENE. ····· ZINC DUST DISTILLATION PRODUCT OF CASSIAMIN.

As described earlier, cassiamin on methylation with dimethylsulphate-potassium carbonate-acetone, produced an orange yellow pentamethyl ether, $C_{35}H_{28}O_9$, m.p. 296-98°. It was insoluble in sodium carbonate and sodium hydroxide solutions and gave no specific colour with alcoholic magnesium acetate.

Methylation of cassiamin with diazomethane in ethereal solution produced a compound which analysed for a monomethyl ether, $C_{31}H_{20}O_9$, m.p. 188-90°. It was insoluble even in hot sodium carbonate solution (unlike cassiamin). This indicated that a β-hydroxyl group may be present in cassiamin which accounts for its solubility in warm aqueous sodium carbonate solution. The monomethyl ether was easily soluble in dilute sodium hydroxide solution giving a violet colour. With alcoholic magnesium acetate, it produced a pink colour. The ultra-violet spectrum of the compound showed λmax. at 227,264, 276, 285, 410-420 mμ (log ε 4.42, 4.66, 4.53, 4.54, 4.18). The infrared spectrum showed peaks at 1672 $cm^{-1}$ (unchelated carbonyl), 1623 $cm^{-1}$ (chelated carbonyl), and 1600 $cm^{-1}$ (phenyl) (Fig. 1.14).

With acetic anhydride in presence of concentrated sulphuric acid or perchloric acid, cassiamin easily formed a penta-acetate, $C_{40}H_{28}O_{14}$, m.p. 182-83° (softening from 170°).

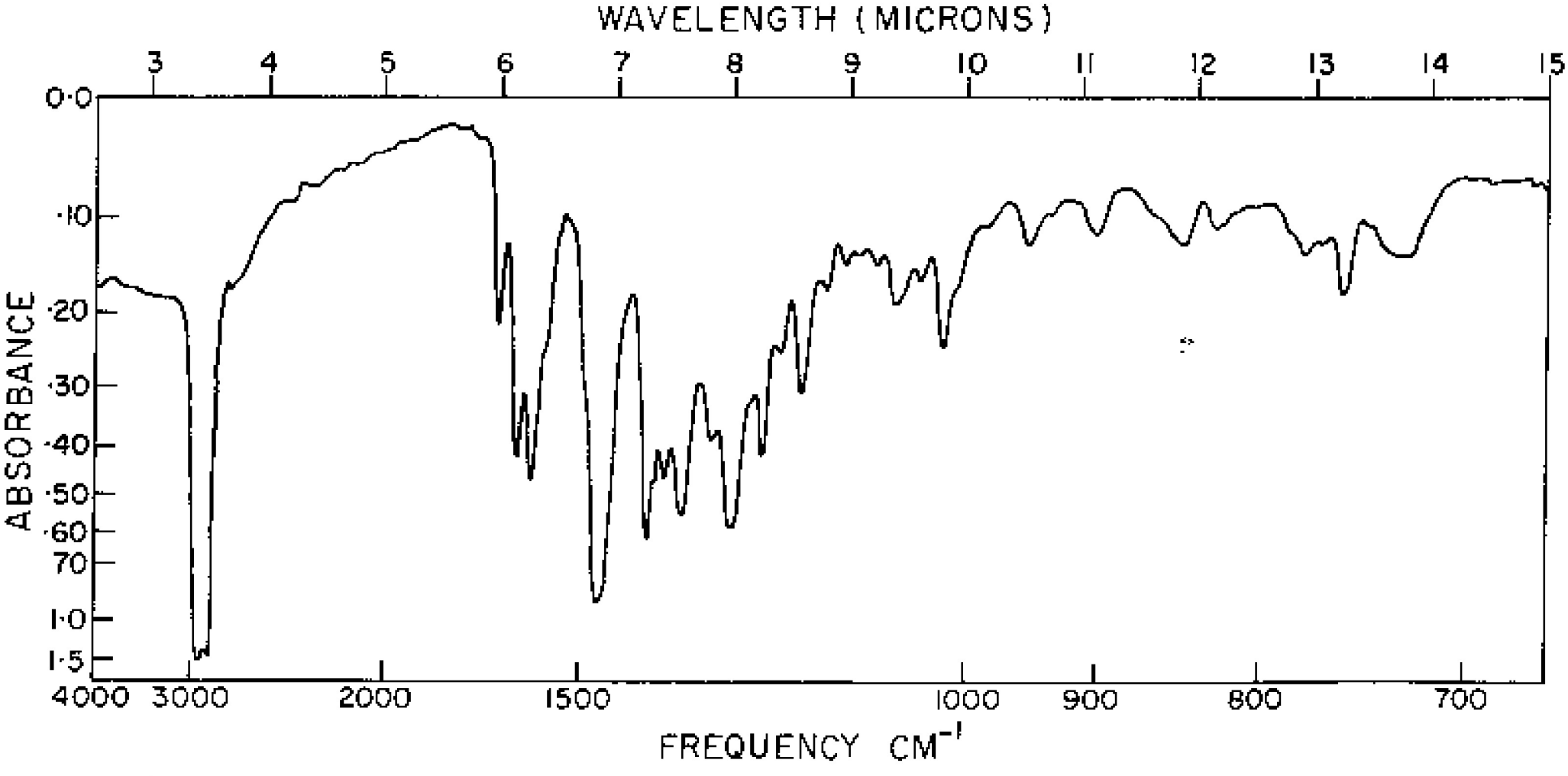
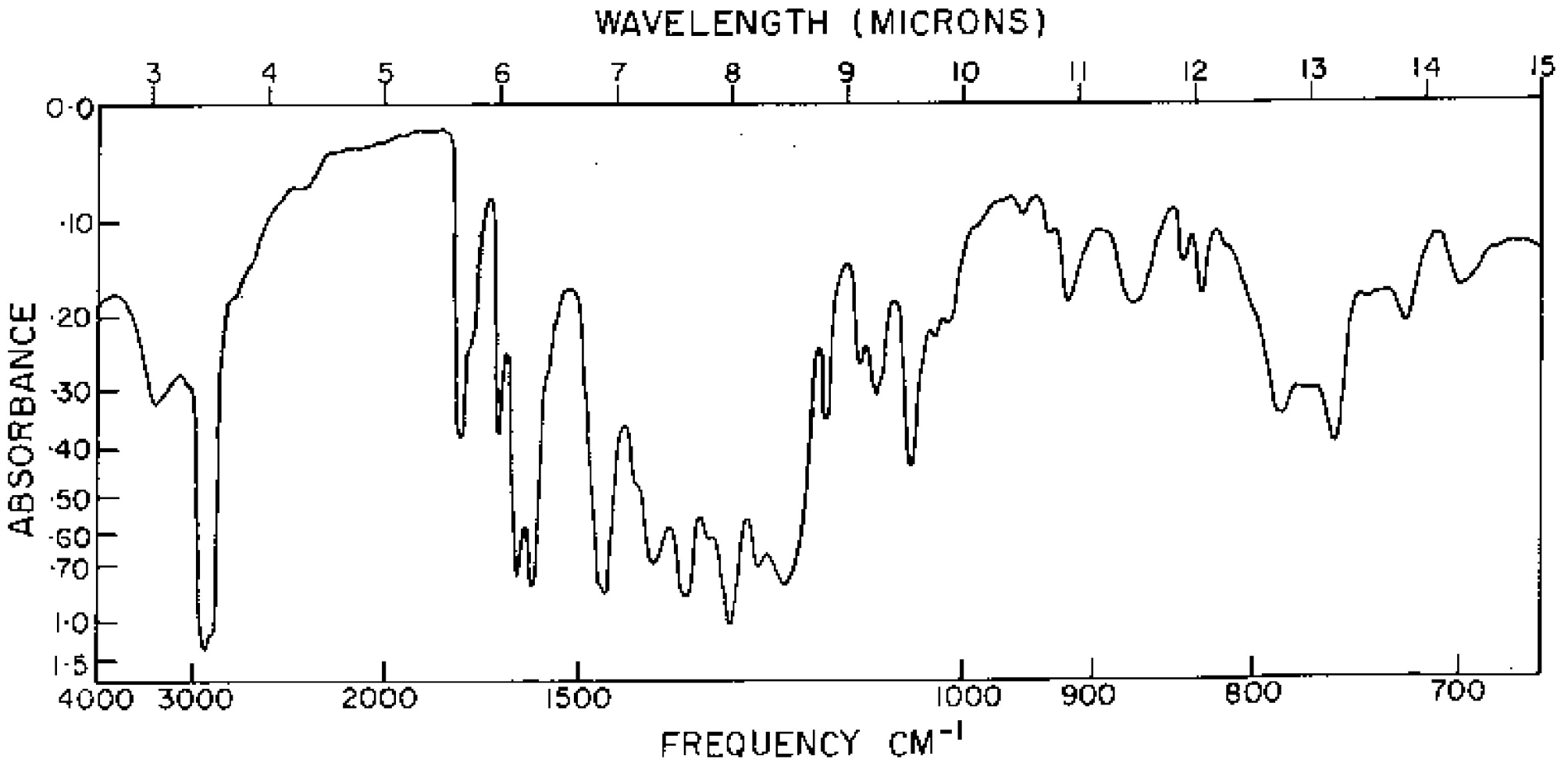

WAVELENGTH (MICRONS)
3
4
5
6
7
8
9
10
11
12
13
14
15
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
ABSORBANCE
4000
3000
2000
1500
1000
900
800
700
FREQUENCY CM⁻¹

FIG. 1.14.

The penta-acetate could be deacetylated by heating with aqueous sodium hydroxide (8%) to produce cassiamin. But attempt to deacetylate the penta-acetate by refluxing with methanol containing a few drops of concentrated sulphuric acid afforded a partially deacetylated product. The yellow compound melting at 212-14° (softening at 206-207°), analysed for cassiamin diacetate, $C_{34}H_{32}O_{11}$. The light absorption spectrum of the compound showed λmax. at 226, 288, 284, 340, and 415-425 mμ (log ε 4.6, 4.7, 4.01, 4.34) (Fig. 1.8). The infrared spectrum in nujol mull showed peaks at 1768 $cm^{-1}$ (phenolic acetate), 1670 $cm^{-1}$ (unchanged carbonyl), 1624 $cm^{-1}$ (chelated carbonyl), and 1598 $cm^{-1}$ (phenyl) (Fig. 1.15). The NMR spectrum of the compound taken in deuterochloroform showed two signals at - 2.58 τ (1) and - 2.5 τ (1), indicating the presence of two chelated hydroxyls in the compound.

Cassiamin is reasonably stable against the action of oxidising agents like chromic acid or alkaline silver oxide. After shaking a dimethyl formamide solution of cassiamin with chromic acid and a few drops of concentrated sulphuric acid for 24 hours, cassiamin could be recovered unchanged. Oxidation of cassiamin with alkaline silver oxide afforded a dark coloured compound. The crude compound gave a positive test with potassium iodide - potassium iodate reagent (violet), which indicated the


WAVELENGTH (MICRONS)
3
4
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9
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11
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14
15
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
ABSORBANCE
4000
3000
2000
1500
1000
900
800
700
FREQUENCY CM⁻¹

FIG. 1.15.

presence of a carboxylic group. But the acid could not be isolated in pure state. Most of the original cassiamin was recovered unchanged.

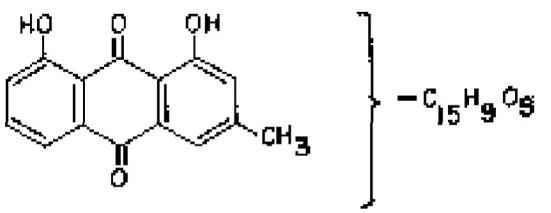
Chromic acid oxidation of the acetate of cassiamin in glacial acetic acid and acetic anhydride also failed to give any conclusive result. The presence of carboxylic group in the product was detected by potassium iodide-potassium iodate reagent. On deacetylation of this compound with aqueous alkali, a small amount of the product melting above 360° was obtained which gave similar colour reactions as cassiamin. It was soluble in sodium bicarbonate (6%) and gave a positive test for carboxylic group. But it could not be isolated in sufficient amount for characterisation. In most of the experiments, cassiamin could be recovered unchanged after deacetylation of the chromic acid oxidation product of cassiamin penta-acetate.

As indicated before, cassiamin gives in cold a vat with sodium dithionite in sodium hydroxide, from which the original compound could be recovered by re-oxidising with air. In the preliminary experiments, which were carried out at room temperature, no appreciable changes in the product could be detected. It may be mentioned that both iridoskyrin and 4,4'-dihydroxy-bianthraquinone-(1,1') were first believed to be stable

against the action of alkaline sodium dithionite.[37,43,44] More drastic conditions were necessary in those cases.

A solution of cassiamin in pyridine with aqueous sodium dithionite and sodium hydroxide was heated on a water bath for 6 hours. Examination of the product on thin layer chromatography indicated the presence of two spots (Rf 0.92 and 0.73). The compound having the Rf: 0.92 was collected and purified by column chromatography over alumina. The orange red compound, m.p. 194-95° was identified as chrysophanol. The infrared (Fig.1.16) and ultraviolet spectra were superimposable with an authentic sample of chrysophanol and there was no depression in mixed melting point.

The isolation of chrysophanol from the sodium dithionite reaction product of cassiamin gave a positive indication that this unit is present in the parent compound. It may be mentioned that the ultraviolet spectra of cassiamin and chrysophanol are very similar, though the absorption in the former are much more intense than the latter. The partial structure of cassiamin could now be conveniently expressed as -




VI

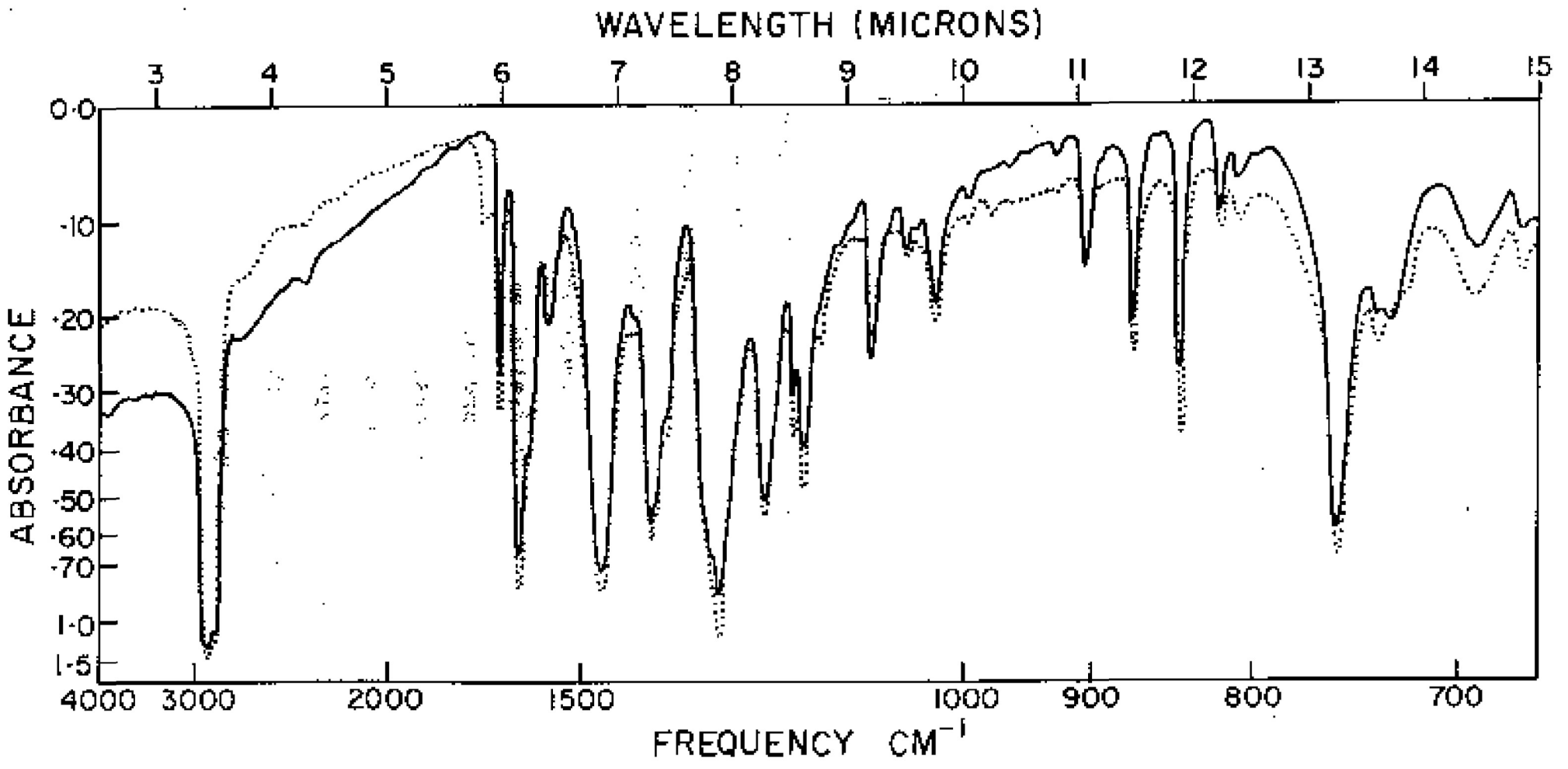



FIG. I.16. IR SPECTRA OF: —— CHRYSOPHANOL, ····· CHRYSOPHANOL FROM THE REDUCTIVE CLEAVAGE PRODUCT OF CASSIAMIN.

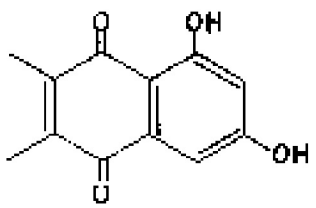
Since cassiamin contains five hydroxyl groups and two aromatic methyl groups, it may be expected that the other part of the molecule would account for remaining three hydroxyls and one aromatic methyl group. Attempt to isolate the second fragment gave an impure product; it was obtained from the sodium carbonate soluble fraction of the reductive cleavage product. It gave a positive Bomtrager's reaction. On thin layer chromatography, the compound moved along with cassiamin and pure emodin ($R_f$ =0.73). Since the compound could not be further purified due to its poor yield, a direct comparison with emodin was not possible. But all its properties strongly suggested its similarity with emodin. It gave a reddish pink colour with alcoholic magnesium-acetate and reddish violet colour with sodium hydroxide and concentrated sulphuric acid. All these colour reactions were given by a pure sample of emodin.

Unlike parent cassiamin, it was easily soluble in cold sodium carbonate indicating the presence of a β-hydroxyl group in the compound. It did not show any characteristic colour with zirconium nitrate thus ruling out the possibility of its having two hydroxyls in ortho positions. A 1,4-dihydroxy system in the compound was also less probable since it did not show any fluorescence in glacial acetic acid in broad day light.

The ultraviolet spectra of the compound was very similar to emodin and cassiamin. The infrared spectra in nujol mull showed peaks at 1670 and 1620 $cm^{-1}$ indicating that it was a 1,8-dihydroxyanthraquinone derivative, but the resolution in the finger print region was poor.

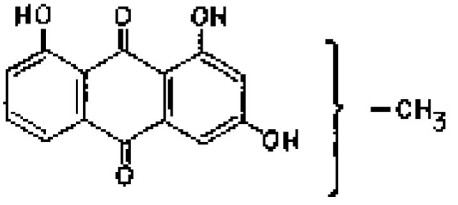
It may be recalled that the presence of four chelated hydroxyls in cassiamin are discernible from the NMR spectrum. Since two of these hydroxyls are present in chrysophanol unit (in 1,8 positions), it would be logical to suppose from the foregoing discussion that the other two chelated hydroxyls, which are present in the second fragment has also a 1,8-dihydroxy relationship. This would place the fifth hydroxyl in cassiamin as a β-hydroxyl in the second unit which also accounts for the formation of monomethyl ether of cassiamin by the action of diazomethane.

Further, the presence of a doublet at 3.36 τ (1) with a coupling constant of 2.8 cps in the pentamethyl ether of cassiamin would strongly suggest the presence of a system (VII) in cassiamin (vide Chapter I-C).




VII

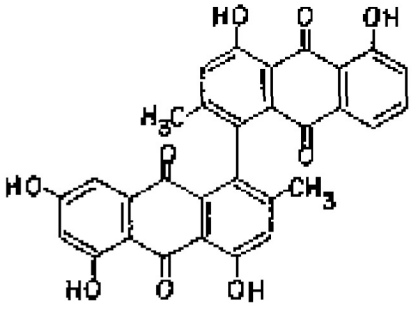
This would suggest the partial structure of the second fragment in cassiamin to be (VIII). Since none of



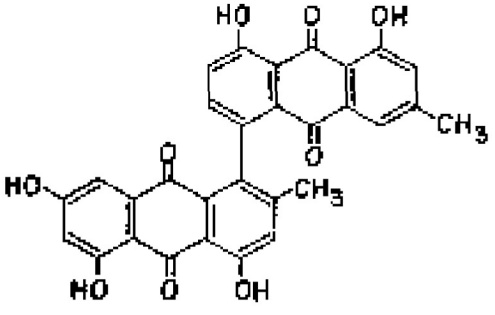
VIII

the hydroxyanthraquinones so far isolated from plants have a $\alpha$-methyl substitution, the methyl group in the second fragment should be in 6 or 7 positions and considering all the facts the most probable formula of this unit would be 1,6,8-trihydroxy-3-methylanthraquinone (emodin).

Provisionally accepting that the second fragment is emodin, the problem of the constitution of cassiamin now resolves itself into the difficult decision as to how the two units are joined together in the cassiamin molecule. At present, no experimental evidence is available to decide this point, so that, of the possible structures of cassiamin, (IX) and (X) are advanced merely to indicate the type of structure which cassiamin may possibly possess.



IX



X

Either of these structures offers a satisfactory explanation of many of the properties of cassiamin that have been recorded. Although from the present state of our knowledge, such a conclusion must be accepted with reservation, there seems to be little doubt that the complete elucidation of the structure of this complex quinone will open a new field of interest in the chemistry of naturally occurring quinones.

Compound C

Very little work could be carried out on the constitution of the compound C having $R_f$ value 0.69. All attempts to obtain a pure crystalline compound in sufficient amount has failed so far. The purest sample was an orange red solid which remained unchanged upto 280°. At higher temperature, it darkened without showing any definite melting point. The colour reactions were very similar to cassiamin, but the ultraviolet spectra of the compound in various solvents were substantially different from cassiamin (Table III).

TABLE III

| Solvent | $\lambda_{max}$ , mμ |
|---|---|
| Ethanol | 226, 268, 305, 396-405 |
| Concentrated sulphuric acid | 328, 262-272, 286, 330, 400-415, 500, 530, 600 |
| 5% sodium hydroxide | 225-235, 266, 320~, 410, 525 |

~ Inflexion

The infrared spectrum of Compound C in nujol mull showed peaks at 3380 $cm^{-1}$ (hydroxyl), 1626 $cm^{-1}$ (chelated carbonyl), and 1602 $cm^{-1}$ (phenyl).

From all the properties so far studied, it seems probable that this is also a new hydroxyanthraquinone derivative having a high molecular weight.

## E X P E R I M E N T A L

For analysis, the compounds were dried over phosphorous pentoxide under vacuum at 100° for 5-6 hours. All melting points are uncorrected. Specific rotations were taken in chloroform solution. The infrared spectra were recorded on a Perkin-Elmer Infracord Spectrophotometer, Model 137E; the compounds were examined as paraffin mulls unless otherwise stated. The ultraviolet spectra were determined on a Perkin-Elmer 350 Spectrophotometer in 95% ethanol solution. The NMR spectra were determined in deuterochloroform solution on a Varian A-60 Spectrophotometer. The NMR spectrum of cassiamin was taken in dioxane solution. In all cases, tetramethylsilane was used as the internal reference.

Neutral alumina of different grades standardised according to the method of Brockmann[48] was used in column chromatography.

A mixture of silica gel (prepared and standardised in this laboratory by Dr. D.N. Sen and co-workers) and calcium sulphate (B.D.H.) in the ratio 85:15 was used for preparing plates for thin layer chromatography. Benzene-acetone (80:20) was used as the solvent system.

The powdered dry root bark (2 kg) of Cassia-siamea Lam was exhaustively extracted (Soxhlet) with n-hexane, followed by benzene. The different extracts were collected and examined separately.

n-Hexane extract:
Isolation of lupenol and chrysophanol

From the n-hexane extract after removal of the solvent, a dark red solid was obtained (15 g., 0.75%). A portion (8 g) of this was taken in pet.ether (40-60°) and passed through a column of alumina (Grade III, 100 g). Elution with pet.ether (40-60°) afforded an almost colourless solid. It was followed by benzene (40 x 10 ml), benzene-ethyl acetate (50:50) (40 x 10 ml) and ethyl acetate respectively, when a pink band gradually moved. From the benzene-ethyl acetate fraction, a second compound (orange red) was obtained.

The yellowish solid obtained by elution with pet.ether was purified by rechromatography over a second column of alumina. It was finally crystallised from a mixture of ether-methanol as colourless needles, m.p. 210-212°, $(\alpha)_D^{30}$ + 36° (c, 2.5). IR spectrum: 3500, 3420, 1640, 1196, 1145, 1110, 1047, 1025, 987, 977, and 887 $cm^{-1}$.

Analysis: Found: C, 83.8; H, 11.5.

$C_{30}H_{50}O$ requires: C, 84.4; H, 11.8%.

The acetate (m.p. 218°; $(\alpha)_D^{20}$ + 46°, and the benzoate m.p. 265°, $(\alpha)_D^{20}$ + 62°, of lupeol were prepared according to the methods described in Chapter III.

Isolation of chrysophanol

The second compound obtained from the benzene-ethyl acetate was purified by chromatography over a column of calcium hydrogen phosphate in benzene. On crystallisation from ethanol, the compound was obtained as dark yellow leaflets, m.p. 196° (TLC* Rf: 0.92) (lit.[45] m.p. 196°). It showed all the colour reactions of hydroxyanthraquinones. IR spectrum: 1672, 1625, 1598, 1567, 1270, 1206, 1163, 1085, 1023, 903, 870, 840, 820, and 755 $cm^{-1}$.

Analysis: Found: C, 70.6; H, 4.1

$C_{15}H_{10}O_4$ requires: C, 70.9; H, 4.0%.

Methylation of chrysophanol

Chrysophanol (500 mg) in dry acetone (100 ml) was refluxed with anhydrous potassium carbonate (3 g) and dimethyl sulphate (1.5 ml) for 20 hr. The mixture was filtered and washed with hot acetone (50 ml). The filtrate and the washings were distilled off to dryness. The residue on crystallisation from ethanol gave orange needles (500 mg), m.p. 195-96°.

* TLC = thin layer chromatography.

Analysis: Found: C, 73.4; H, 5.3. $-OCH_3$ 20.8.

$C_{17}H_{14}O_4$ requires: C, 72.3; H, 5.0. $-OCH_3$ 10.9%.

Acetylation of chrysophanol

Chrysophanol (200 mg) was dissolved in acetic anhydride (10 ml) and a few drops of boron-trifluoride etherate were added at room temperature. After 24 hr., the solution was poured into crushed ice (50 g). The yellow solid was filtered, washed and dried (230 mg). Crystallisation from ethanol gave yellow plates, m.p. 207°. IR spectrum: 1767, 1670, 1595, 1360, 1200, 1025 and 892 $cm^{-1}$.

Analysis: Found: C, 67.7; H, 4.0; $-COCH_3$ 34.1.

$C_{19}H_{14}O_6$ requires: C, 67.4; H, 4.2%. $-COCH_3$ 25.4%.

Examination of the benzene extract:
Isolation of chrysophanol, Cassiamin and Compound C

After exhaustive extraction with n-hexane, the root bark of Cassia siamea was extracted with benzene (soxhlet) for several days. The orange red solution, after removal of the solvent, afforded an orange red solid (20 g). Thin layer chromatography showed three spots corresponding to $R_f$: 0.92, 0.73 and 0.53 (designated as Compounds A, B and C respectively). An aliquot portion of the solid (8 g) was extracted with

500 ml. of n-hexane for 48 hr (soxhlet). The n-hexane solution (brown) on removal of the solvent yielded an orange-yellow solid (0.3 g) which was readily identified as chrysophanol.

The insoluble orange red solid, containing Compound B (cassiamin) and Compound C, however, could not be separated by solvent extraction procedure. Different columns using ignited magnesia (in acetone solution) calcium hydrogen phosphate (benzene-chloroform) were not satisfactory for the separation of the two compounds. Chromatography over silica gel and neutral alumina column (Grade IV) gave better results.

The orange red solid (5 g) was passed through a column of silica gel (100 g) in benzene solution. Elution was continued successively with benzene, benzene-ethylacetate and ethyl acetate; 10 X 50 ml fractions of each were collected. From the benzene-fraction, an orange yellow solid was obtained which after recrystallisation from ethanol melted at 193-94°. It was subsequently identified as chrysophanol.

The benzene-ethyl acetate fraction afforded an orange yellow solid (1 g) ($R_f$: 0.73) which was further purified by rechromatography over a second column. The

latter fractions obtained by elution with benzene-ethyl acetate and ethyl acetate were evidently mixtures, since they gave two spots on thin layer chromatography (Rf : 0.72 and 0.52).

Cassiamin could also be obtained by chromatography of the crude benzene extract (5 g) over neutral alumina (grade IV, 100 g) using successively the solvent systems of benzene, benzene-ethyl acetate, ethyl acetate, and ethyl acetate-acetic acid. The pink band gave chrysophanol on elution with benzene-ethyl acetate. Elution of the orange red band with ethyl acetate and subsequent removal of the solvent yielded cassiamin (0.8 g). On continuing the elution with ethyl acetate, a mixture of cassiamin and Compound C was obtained. Finally the column was washed with ethyl acetate containing a few drops of acetic acid, when mainly the mixture of the two compounds was obtained (2.5 g).

Cassiamin (Rf: 0.72) obtained by either of these methods was crystallised from tetrahydrofuran-methanol mixture, as orange yellow micro plates, m.p. 366-67° (darkening from 340°). It gave a deep violet solution in sodium hydroxide and a reddish violet solution in concentrated sulphuric acid. IR spectrum: 3320, 1672, 1623, 1600, 1285, 1262, 1227, 1192, 1162, 1103, 1087, 1049, 956, 922, 893, 875, 859, 842, 830, 782 and 755 cm-1, (in nujol mull).

Analysis: Found: C, 68.8; H, 3.6. C-$CH_3$ 5.0, 5.2
-$OCH_3$ absent, Mol.wt. 622 (by mass spectroscopic method).

$C_{30}H_{18}O_8$ requires: C, 68.9; H, 3.5. C-$CH_3$ 5.7%, Mol. wt. 522.

Compound C.

Attempts to isolate the Compound C ($R_f$: 0.53) in pure form by further chromatography gave an orange brown solid, which was crystallised from tetrahydrofuran-methanol as orange red micro plates, m.p.>300° (darkening from 280°). It was insoluble in sodium bicarbonate, but dissolved slowly in 5% sodium carbonate on heating. It was readily soluble in sodium hydroxide solution giving an intense violet colour. In concentrated sulphuric acid, it formed a reddish violet solution. IR spectrum: 3280, 1625, 1602, 1335, 1270, 1155, 1152, 1104, 1058, 982, 926, 860, 843 and 767 $cm^{-1}$.

Action of concentrated sulphuric acid on cassiamin

A solution of cassiamin (0.05 g) in concentrated sulphuric acid (2 ml) was heated on a water bath for 3 hr. It was cooled and poured over ice (10 g). The separated solid was filtered, washed with water and dried (0.04 g). Crystallisation from tetrahydrofuran gave orange yellow micro plates, m.p. 363-64° (TLC, $R_f$: 0.73). It was identified as cassiamin.

Action of 10% sodium hydroxide on cassiamin

A solution of cassiamin (50 mg) in 10% sodium hydroxide (5 ml) was heated on a water bath for 6 hr. The violet solution was cooled, acidified and the precipitated solid was collected. Crystallisation from tetrahydrofuran-methanol gave orange yellow micro-plates, m.p. 352-54°. It was identified as cassiamin.

Zinc dust distillation of cassiamin

Cassiamin (0.3 g) was intimately mixed with zinc dust (10 g) and the mixture was packed in a silica tube together with pumice stones to impart porosity to the packing. The tube was uniformly heated at 400-420° and a steady stream of hydrogen was passed through the tube. After 4 hr. the straw-coloured sublimate (0.03 g) from the cooler part of the tube was collected and crystallised from alcohol in almost colourless leaflets, m.p. 205°. The mixed melting point with an authentic sample of 2-methylanthracene was undepressed. IR spectrum: 1636, 1303, 1271, 1178, 1040, 987, 960, 909, 898, 875, 808 and 742 $cm^{-1}$.

Analysis: Found: C, 93.5; H, 6.4.

$C_{15}H_{12}$ requires: C, 93.7; H, 6.3 %.

Methylation of cassiamin

Cassiamin (1 g) in anhydrous acetone (300 ml) was refluxed with freshly distilled dimethyl sulphate (10 ml) and freshly ignited potassium carbonate (20 g) for 20 hr. The mixture was filtered hot, the residue washed well with hot acetone and the acetone distilled off from the filtrate. The orange product was collected, dried and taken in benzene. The benzene solution was chromatographed over a column of alumina. The orange yellow solid obtained after removal of benzene eluate was recrystallised several times from tetrahydrofuran-methanol mixture to give bright orange yellow micro-plates, m.p. 295-98°. IR spectrum: 1672, 1600, 1592, 1320, 1279, 1247, 1211, 1163, 1089, 1056, 890, 841 and 782 $cm^{-1}$.

Analysis: Found: C, 70.8; H, 5.2. $-OCH_3$ 24.8.
$C_{35}H_{28}O_9$ requires: C, 70.9; H, 4.8 % $-OCH_3$ 26.1.

Methylation of cassiamin with ethereal diazomethane

Nitrosomethyl urea (2.5 g) was added to a mixture of aqueous (50% KOH) (8 ml) and ether (100 ml) at 8°. The yellow ethanol diazomethane solution was dried over KOH pellets and decanted to an ethereal solution(500 ml) containing cassiamin (0.5 g). The mixture was kept at 0° for 48 hr. The excess of diazomethane was destroyed by a few drops of glacial acetic acid and the separated

orange red solid was filtered off. The ether solution was concentrated and the products obtained were mixed with the orange red solid. After several recrystallisations from ethanol, orange red microscopic crystals were obtained, m.p. 188-90°. It formed a reddish violet solution with concentrated sulphuric acid. IR spectrum: 1672, 1623, 1598, 1321, 1260, 1212, 1162, 1063, 1020, 950, 900, and 842 cm$^{-1}$.

Analysis: Found: C, 69.7; H, 4.2.

$C_{31}H_{20}O_9$ requires: C, 69.4; H, 3.8 %.

Acetylation of cassiamin

Method A. Cassiamin (0.5 g) in acetic anhydride (20 ml) was treated with a few drops of perchloric acid at room temperature. This was allowed to stand overnight and then poured over crushed ice. The yellow precipitate was collected, washed and dried (0.52 g). It was crystallised from ethanol in pale yellow microscopic plates, m.p. 182-83° (softening from 170°), $(\alpha)^{30}_{589}$ = - 169.6° (c, 1.0) (measured on a Perkin-Elmer Polarimeter in chloroform solution). IR spectrum: 1769, 1670, 1598, 1318, 1248, 1178, 1133, 1088, 1042, 910 and 825 cm$^{-1}$.

Analysis: Found: C, 65.8; H, 4.0, -COCH$_3$ 30.4; Mol.wt.687,738(thermister micro-method).

$C_{40}H_{28}O_{14}$ requires: C, 65.6; H, 3.9 -COCH$_3$ 29.3%. Mol.wt. 732.

Method B. A mixture of cassiamin (0.5 g) in acetic anhydride (20 ml) and a few drops of concentrated sulphuric acid was refluxed for 2 hr. The solution was cooled, and poured over crushed ice. The precipitate was collected, washed and dried (0.53 g). It crystallised from ethanol in pale yellow microscopic plates, m.p. 182-83° (softening from 170°).

The infrared and ultraviolet spectra were identical with the acetate obtained by the method A and there was no depression in mixed melting point.

Deacetylation of cassiamin

Cassiamin acetate (0.4 g) was heated for 5 hr. with aqueous 5% sodium hydroxide (40 ml) on a boiling water bath in an atmosphere of nitrogen. The reaction mixture was cooled and acidified with dilute sulphuric acid and the resulting precipitate was coagulated by heating on a water bath for 30 minutes. The resulting solid was filtered, washed and dried. Crystallisation from tetrahydrofuran methanol gave orange yellow microplates, m.p. 355-56° (darkening from 340°). It was identified as cassiamin.

Analysis: Found; C, 68.8; H, 3.6.

$C_{30}H_{18}O_9$ requires: C, 68.9; H, 3.5%.

Partial deacetylation of cassiamin acetate

A solution of cassiamin acetate (0.2 g) in methanol (100 ml) and a few drops of concentrated sulphuric acid was

refluxed for 3 hr. The solution was concentrated to a little volume and then poured into water. The yellow solid was collected, washed and dried. It crystallised from ethanol as yellow micro-plates, m.p. 312-14° (softening at 306-7°). IR spectrum: 3320, 1768, 1670, 1624, 1586, 1318, 1382, 1180, 1136, 1080, 1046, 918, 870, 830, 785 and 756 $cm^{-1}$.

Analysis: Found: C, 67.17; H, 3.94, $-COCH_3$ 12.
Mol.wt. 649 (thermister micro-method).

$C_{34}H_{22}O_{11}$ requires: C, 67.4; H, 3.6, $-COCH_3$ 14%.
Mol. wt. 602.

Mild chromic acid oxidation of cassiamin

A mixture of cassiamin (0.1 g), dimethyl formamide (4 ml), $CrO_3$ (0.1 g) and concentrated sulphuric acid (2-3 drops) was shaken at room temperature for 24 hr. The mixture was diluted with water, the product was collected, washed and dried (0.08 g). It was methylated by dimethyl sulphate (1 ml), anhydrous potassium carbonate (1 g) and acetone (20 ml). The methylated product was chromatographed over a short column of alumina. The benzene eluate was evaporated in vacuo and the resulting solid (0.06 g) crystallised from chloroform-methanol as orange yellow micro plates, m.p. 293-94°. It was identified as cassiamin pentamethyl ether.

Chromic acid oxidation of cassiamin penta-acetate

Cassiamin penta-acetate (0.5 g) was dissolved at 55-60° in a mixture of glacial acetic acid (25 ml) and acetic anhydride (25 ml). The temperature was maintained at 55-60°, while a solution of $CrO_3$ (1 g) in water (1 ml) and glacial acetic acid (25 ml) was added with constant stirring over a period of 0.5 hr. The bath temperature was then raised to 65-70° and maintained at this temperature for 3 hr. The deep green solution was poured into water (300 ml) and left in a refrigerator overnight, after which this was extracted with chloroform, (3 x 200 ml). The reddish brown chloroform solution was washed with water and then extracted with 5% sodium bicarbonate solution (5 x 50 ml).

Sodium bicarbonate soluble fraction

The sodium bicarbonate soluble fraction was acidified with dilute hydrochloric acid and re-extracted with chloroform. The chloroform solution was washed with water and dried over anhydrous sodium sulphate. The dark yellow coloured compound, obtained after the removal of chloroform (0.04 g) could not be obtained in pure state by crystallisation. It was therefore deacetylated by heating with 5% sodium hydroxide solution for 3 hr and the deacetylated product (0.02 g) was recrystallised from

tetrahydrofuran-methanol as dark yellow microscopic plates, m.p. >350°. It showed a positive potassium iodide-potassium iodate test (violet) for carboxylic acid. But the sample was not sufficient for analysis. IR spectrum: 3330, 1688, 1660, 1608, 1260, 1200, 1175, 1114, 1082, 910, and 848 cm-1.

Sodium bicarbonate insoluble fraction

After extraction with sodium bicarbonate, the chloroform layer was washed well with water, and dried over sodium sulphate. On removal of the solvent, a dark yellow solid (0.3 g) was obtained. This was deacetylated by heating with 5% sodium hydroxide solution for 4 hr. and the orange red solid obtained on acidification was crystallised from tetrahydrofuran-methanol; orange micro-plates, m.p. 383-86° (darkening from 340°). This was identified as sassiamin.

Analysis: Found: C, 68.6; H, 3.8.

$C_{30}H_{18}O_9$ requires : C, 68.9; H, 3.5%.

Silver oxide oxidation of sassiamin

Silver nitrate (1.36 g) in water (10 ml) was treated with caustic soda (0.34 g) in water (10 ml). The separated silver oxide was filtered, washed free from nitrate and transferred to a beaker containing water (30 ml). Sodium hydroxide (1.36 g) was added

with vigorous stirring and the temperature of the mixture was maintained at 75°. Cassiamin (0.4 g) was introduced and the stirring was continued for 1 hr. The mixture was filtered hot. The residue of silver was washed well with hot water. On acidification of the filtrate and washings, a brown precipitate was obtained. This was treated with 5% sodium bicarbonate solution. Acidification of the filtered solution yielded a gummy brown solid (0.03 g) which could not be purified further by crystallisation. The crude compound gave a positive test for carboxyl group with potassium iodide-potassium iodate reagent (violet).

The sodium bicarbonate insoluble fraction (0.03 g) was crystallised from tetrahydrofuran-methanol as orange yellow microplates, m.p. 353-53°. It was readily identified as cassiamin.

Air oxidation of the alkaline vat of cassiamin

Method A. Cassiamin (150 mg) was dissolved in 2% sodium hydroxide solution (20 ml) and the excess of sodium hydrosulphite (1 g) was added in small portions at room temperature with occasional stirring. After 0.5 hr. the solution was filtered and oxidised by passing air. The solution was acidified and the yellowish precipitate extracted with chloroform. The chloroform

solution dried, and evaporated in vacuo. The product crystallised from tetrahydrofuran-methanol mixture as orange red microcrystals, m.p. 353-56° (TLC $R_f$: 0.73). It was identified as cassiamin.

Method B. Cassiamin (1 g) was taken in pyridine (5 ml) and 8% sodium hydroxide (3 ml) and sodium dithionite (10 g) was added to it. The mixture was heated with occasional stirring on a water bath for 6 hr. It was cooled, acidified and extracted with chloroform. The chloroform solution was washed with water and then extracted with 5% sodium carbonate (5 x 20 ml).

Chloroform extract

From the chloroform solution, on removal of the solvent, an orange red solid was obtained which gave two distinct spots ($R_f$ 0.92 and 0.73) on thin layer chromatography. This was extracted with n-hexane 100 ml(reflux) and the n-hexane extract, after concentration to a little volume was passed through a column of alumina (neutral, grade IV; 10 g). Elution was continued with benzene and benzene-ethylacetate. From the benzene-ethyl acetate fraction, an orange red solid (0.03 g) was obtained. This on crystallisation from ethanol gave dark yellow leaflets, m.p. 194-96° (TLC $R_f$: 0.92).

It was readily identified as chrysophanol.

Analysis : Found: C, 70.4; H, 4.2.

$C_{15}H_{10}O_4$ requires: C, 70.8; H, 3.9%.

The n-hexane insoluble fraction (0.6 g) was recrystallised from tetrahydrofuran-methanol as orange yellow micro plates, m.p. 354-55° (darkening from 340°). This was identified as cassiamin.

Cold sodium carbonate soluble fraction

The aqueous sodium carbonate solution was acidified and extracted with chloroform. The organic layer was separated, washed with water and dried over sodium sulphate. On removal of the solvent, an orange red compound (0.015 g) was obtained. It melted at 235-40° (darkening at 222°). Further attempt to purify this material by recrystallisation did not afford any crystalline compound. On thin layer chromatography, it gave a single spot (Rf: 0.73). Pure emodin also showed the same Rf value when the same solvent system (benzene-acetone 80:20) was used.

Reductive acetylation of cassiamin

Reductively acetylated cassiamin was prepared by refluxing a mixture of cassiamin penta-acetate (0.05 g), acetic anhydride (10 ml), freshly fused sodium acetate (0.3 g) and zinc dust (0.6 g) for 2 hr. The product was recrystallised from ethanol, m.p. 228-230°.

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# C H A P T E R I (PART C)

## STUDIES ON NUCLEAR MAGNETIC RESONANCE SPECTRA OF HYDROXYANTHRAQUINONE AND THEIR DERIVATIVES

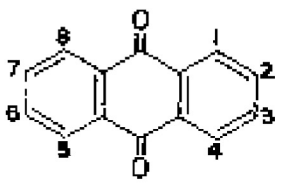
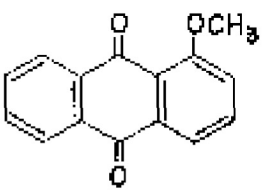
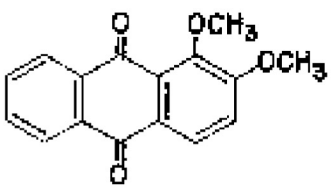
In view of the ever increasing application of nuclear magnetic resonance spectroscopy in the structural elucidation of organic compounds, their day-to-day use cannot be over emphasised. However, the use of this important tool in the field of hydroxyanthraquinone derivatives seems to be yet unexplored. The work described in this chapter embodies a study of the spectra of twenty-seven hydroxyanthraquinones and their derivatives.

Most of these compounds were synthesised in the laboratory of Prof. K. Venkataraman. The purity of the compounds was ascertained from the melting points. The spectra were recorded on a Varian A-60 spectrophotometer. In most cases, deuterochloroform, prepared by the action of sodium deuteroxide on trichloroacetophenone according to the method of P.M.Muir and G.Gopakumar[1] was used as solvent. Tetramethylsilane was used as internal reference in all cases. In some cases of hydroxyanthraquinones, dioxane was used as the solvent.

## Results and Discussion

The spectra of anthraquinones are described in Table I. The chemical shifts indicated are all on the $\tau$ scale.

# TABLE I

| Compound | Chemical shifts (τ) | Peak multiplicity* | No. of hydrogen | Possible assignments |
|---|---|---|---|---|
|  I | 1.73 | m | 4 | 1,4,5,8 H's |
| | 2.22 | m | 4 | 2,3,6,7 H's |
| (in dioxane soln.) | | | | |
|  II | 1.70 to 2.60 | m | 6 | 3,4,5,6,7,8 H's |
| | 2.67 | q | 1 | 2 H |
| | 5.96 | s | 3 | $OCH_3$ |
|  III | 1.93 | m | 3 | 4,5,8 H's |
| | 2.40 | m | 2 | 6,7 H's |
| | 2.65 | d | 1 | 3 H |
| | 6.08 | s | 6 | $OCH_3$ (two) |

* m = multiplet  s = singlet

q = quadruplet  d = doublet

Table I Contd.

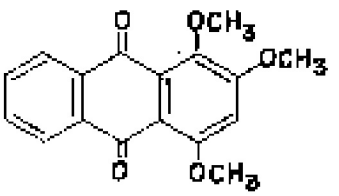
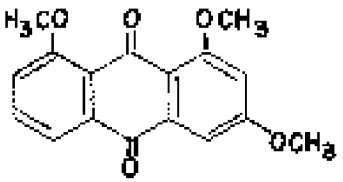
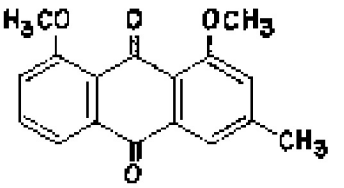
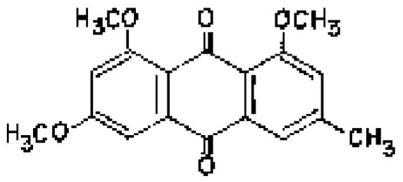
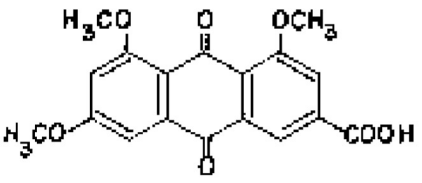
| | | | | |
|---|---|---|---|---|
|  IV | 2.00 | m | 2 | 5,8 H's |
| | 2.42 | m | 2 | 6,7 H's |
| | 3.28 | s | 1 | 3 H |
| | 6.04 | s | 3 | $OCH_3$ at 4 |
| | 6.06 | s | 3 | $OCH_3$ at 1 |
| | 6.12 | s | 3 | $OCH_3$ at 2 |
|  V | 2.20 to 2.80 | m | 4 | 4,5,6,7 H's |
| | 3.30 | d | 1 | 2 H |
| | 6.04 | s | 3 | $OCH_3$ at 8 |
| | 6.07 | s | 3 | $OCH_3$ at 1 |
| | 6.09 | s | 3 | $OCH_3$ at 3 |
|  VI | 2.10 to 2.90 | m | 5 | Ar H's |
| | 6.10 | s | 6 | $OCH_3$ (two) |
| | 7.56 | s | 3 | $CH_3$ |

Table I Contd.



VII

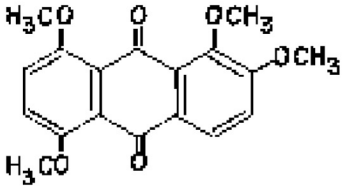
| | | | |
|---|---|---|---|
| 3.42 | ~~s~~ d | 1 | 4 H |
| 3.75 | d | 1 | 5 H |
| 3.95 | d | 1 | 2 H |
| 3.29 | d | 1 | 7 H |
| 6.07 | m | 9 | $OCH_3$ (three) |
| 7.62 | s | 3 | $CH_3$ |



VIII

| | | | |
|---|---|---|---|
| 1.87 | d | 1 | 4 H |
| 2.47 | d | 1 | 6 H |
| 2.55 | d | 1 | 2 H |
| 3.05 | d | 1 | 7 H |
| 5.83 | s | 3 | $OCH_3$ at 1 |
| 5.98 | s | 3 | $OCH_3$ at 8 |
| 5.91 | s | 3 | $OCH_3$ at 6 |

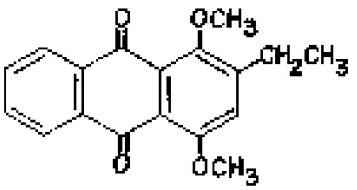
Signal for COOH was too broad to be located



IX

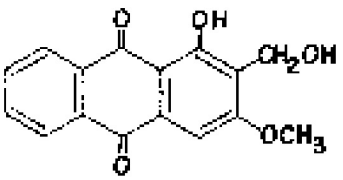
| | | | |
|---|---|---|---|
| 2.00to 3.10 | m | 4 | Ar H's |
| 6.06 to 6.10 | m | 12 | $OCH_3$ (four) |

**Table I Contd.**



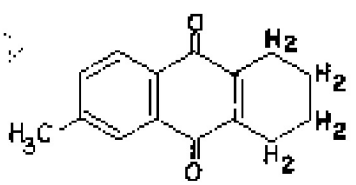
X

| | | | |
|---|---|---|---|
| 1.85 | m | 2 | 5,8 H's |
| 2.20 | m | 2 | 6,7 H's |
| 2.80 | s | 1 | 3 H |
| 6.00 | s | 3 | $OCH_3$ |
| 6.11 | s | 3 | $OCH_3$ |
| 7.22 | q | 2 | $-CH_2-$ |
| 8.72 | t* | 3 | $CH_3$ |



XI

| | | | |
|---|---|---|---|
| 2.70 | s | 1 | chelated OH |
| 1.88 | m | 2 | 5,8 H's |
| 2.33 | m | 2 | 6,7 H's |
| 2.72 | s | 1 | 4 H |
| 5.24 | s | 2 | $-CH_2-O$ |
| 6.04 | s | 3 | $OCH_3$ |
| 7.80 | s | 1 | OH (attached to $-CH_2$) |



XII

| | | | |
|---|---|---|---|
| 1.90to 2.70 | m | 3 | Ar H's |
| 7.50 | m | 4 | 1,4 $CH_2$'s |
| 7.58 | s | 3 | $CH_3$ |
| 8.33 | m | 4 | 2,3 $CH_2$'s |

* t = triplet

Table I Cont'd.

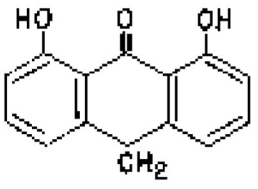
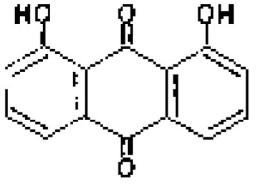
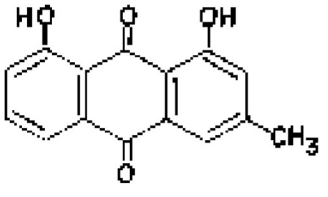
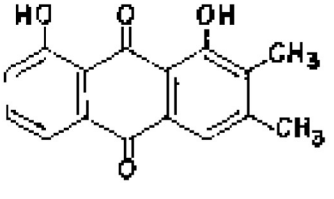
| | | | | |
|---|---|---|---|---|
|  XIII | - 1.18 | s | 2 | OH's (chelated) |
| | 2.60to 3.40 | m | 6 | Ar H's |
| | 6.88 | s | 2 | $CH_2$ |
|  XIV | - 1.84 | s | 2 | OH's(chelated) |
| | 2.40to 2.80 | m | 6 | Ar H's |
|  XV | - 1.90 | s | 1 | OH (chelated) |
| | - 1.80 | s | 1 | OH (chelated) |
| | 2.25 to 2.90 | m | 5 | Ar H's |
| | 7.60 | s | 3 | $CH_3$ |
|  XVI | - 2.35 | s | 1 | OH (chelated) |
| | - 2.07 | s | 1 | OH (chelated) |
| | 2.30to 2.90 | m | 4 | Ar H's |
| | 7.64 | s | 3 | $CH_3$ |
| | 7.77 | s | 3 | $CH_3$ |

Table I Contd.

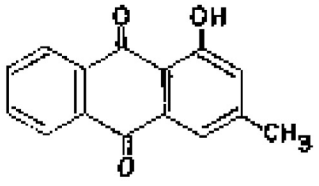
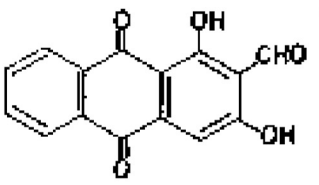
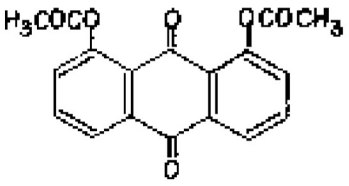
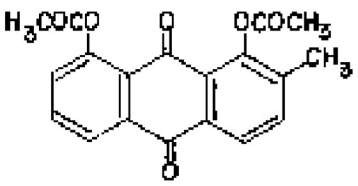
| | | | | |
|---|---|---|---|---|
|  XVII | - 2.60 | s | 1 | OH (chelated) |
| | 1.70 | q | 2 | 5,8 H's |
| | 2.20 | q | 2 | 6,7 H's |
| | 2.33 | s | 1 | 4 H |
| | 2.66 | s | 1 | 2 H |
|  XVIII | - 3.67 | s | 1 | OH (chelated) |
| | - 2.60 | s | 1 | OH at 3 |
| | - 0.17 | s | 1 | CHO |
| | 1.70 to 2.90 | m | 5 | Ar H's |
|  XIX | 1.70 to 2.75 | m | 6 | Ar H's |
| | 7.54 | s | 3 | $OCOCH_3$ |
| | 7.68 | s | 3 | $OCOCH_3$ |
|  XX | 2.00 to 2.80 | m | 5 | Ar H's |
| | 7.62 | s | 3 | $OCOCH_3$ |
| | 7.63 | s | 3 | $OCOCH_3$ |
| | 7.77 | s | 3 | $CH_3$ |

Table 1 Contd.

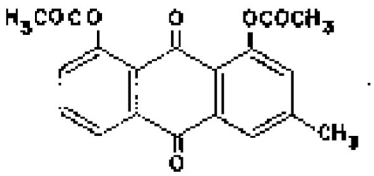
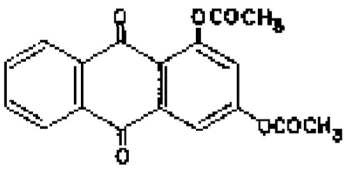
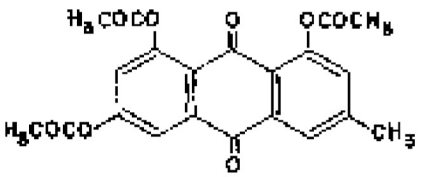
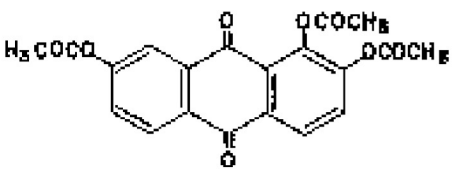
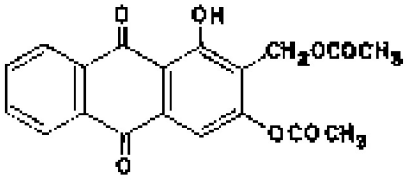
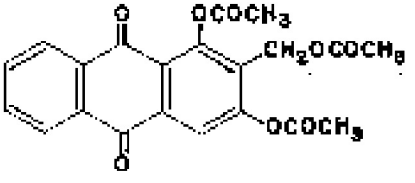
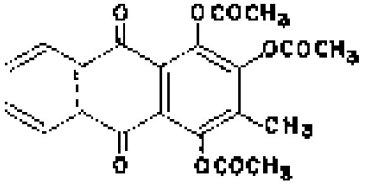
| | | | | |
|---|---|---|---|---|
|  XXI | 1.88 to 2.90 | m | 5 | Ar H's |
| | 7.55 | s | 3 | $CH_3$ |
| | 7.60 | s | 6 | $OCOCH_3$(two) |
|  XXII | 1.90 | m | 2 | 5,8 H's |
| | 2.11 | d | 1 | 4 H |
| | 2.37 | m | 2 | 6,7 H's |
| | 2.85 | d | 1 | 2 H |
| | 7.67 | s | 3 | $OCOCH_3$ at 1 |
| | 7.70 | s | 3 | $OCOCH_3$ at 3 |
|  XXIII | 2.07 | m | 2 | 4,5 H's |
| | 2.81 | m | 2 | 2,7 H's |
| | 7.58 | s | 3 | $CH_3$ |
| | 7.68 | s | 6 | $OCOCH_3$(two)at 1,8 |
| | 7.69 | s | 3 | $OCOCH_3$ at 6 |
|  XXIV | 1.70to 3.70 | m | 5 | Ar H's |
| | 7.66 | s | 3 | $OCOCH_3$ at 1 |
| | 7.69 | s | 6 | $OCOCH_3$ (two) at 2,7 |

Table I Contd.

| | | | | |
|---|---|---|---|---|
|  XXV | - 2.88 | s | 1 | OH (chelated) |
| | 1.84 | m | 2 | 5,8 H's |
| | 2.30 | m | 2 | 6,7 H's |
| | 2.63 | s | 1 | 4 H |
| | 4.80 | s | 2 | $-CH_2-$ |
| | 7.65 | s | 3 | $OCOCH_3$ at 3 |
| | 7.97 | s | 3 | $OCOCH_3$ attached to $-CH_2-$ |
|  XXVI | 1.89 | m | 2 | 5,8 H's |
| | 2.08 | s | 1 | 4 H |
| | 2.35 | m | 2 | 6,7 H's |
| | 4.90 | s | 2 | $-CH_2-$ |
| | 7.55 | s | 3 | $OCOCH_3$ at 1 |
| | 7.66 | s | 3 | $OCOCH_3$ at 3 |
| | 8.04 | s | 3 | $OCOCH_3$ attached to $-CH_2-$ |
|  XXVII | 1.8cto 2.35 | m | 4 | Ar H's |
| | 7.50 | s | 3 | $OCOCH_3$ at 4 |
| | 7.55 | s | 3 | $OCOCH_3$ at 1 |
| | 7.64 | s | 3 | $OCOCH_3$ at 2 |
| | 7.87 | s | 3 | $CH_3$ |

The NMR spectrum of anthraquinone gives two groups of signals centered at 1.73 and 2.32, corresponding to an $A_2B_2$ pattern. In the spectra of its derivatives discussed below only the more apparent changes caused by the substituent groups are considered in addition to the absorptions of the substituents themselves, since these are the most valuable from the structural point of view. When only one ring is substituted, the $A_2B_2$ system due to the other is ignored. A detailed study of the more complex ABC type of pattern has also not been considered. The spectral data of 1,8-dihydroxy-9-anthrone (XIII) and 1,2,3,4-tetrahydro-6-methylanthraquinone (XII) are also presented in the Table and the possible assignments given.

Methoxyanthraquinones

The absorption bands of the methoxyl groups of most of the compounds examined fall in a very narrow region 5.96 to 6.13. Out of these only α-methoxyanthraquinone (II) shows a methoxyl signal below 6 (actually at 5.96). With increasing substitution of methoxyls these signals move slightly upfield. The observed shifts are readily rationalised in terms of the electron donor action of these groups. The methoxyl groups of alizarin dimethyl ether (III) absorb

at 6.08, 0.12 p.p.m., upfield compared to that of α-methoxyanthraquinone, while those of purpurin trimethyl ether (IV) occur at 6.04, 6.06 and 6.12; the latter signals being conveniently assigned to the 4,1 and 2 substituents respectively. Similarly, 1,3,8-trimethoxyanthraquinone (V) shows absorptions at 6.04, 6.07 and 6.09, which are due to the methoxyls at 8,1 and 3 positions. These assignments are made on the consideration that α-methoxyl groups in contrast to those at β-positions, should come under the deshielding effect of the quinone carbonyl functions.[2] However, this seems to be a minor effect since small changes in electron distribution in the aromatic ring seem to compensate for the difference between the two positions arising from the long range effect of the carbonyl groups. Thus, as mentioned earlier, alizarin dimethyl ether (III) shows only one methoxyl signal. In the spectra of chrysophanol dimethyl ether (VI), emodin trimethyl ether (VII) and quinalizarin tetramethyl ether (IX), the methoxyls appear as unresolved bands in the range 6.06 to 6.1.

The aromatic protons of these compounds show characteristic shifts associated with the oxygenation of such molecules.[3] The proton ortho to the methoxyl group in α-methoxyanthraquinone (II)is about 0.45 p.p.m.

upfield from the average position of its other β-protons. The same is true for alizarin dimethyl ether (III) and 1,4-dimethoxy-2-ethylanthraquinone (X). In purpurin trimethyl ether (IV) the proton at position 3 absorb at 3.28,i.e. about 0.86 p.p.m. upfield compared to those at positions 6 and 7, indicating the influence of two methoxyl groups in ortho positions. The similarly situated protons of 1, 3, 8-trimethoxyanthraquinone (V) and emodin trimethyl ether (VII) absorb almost exactly in the same place. Without a finer analysis of the spectra, it is not possible to say if the chemical shifts caused by these groups are additive. However, since they are quite large, they may be of considerable help in structural work for the recognition of oxygenation patterns.

In going from emodin trimethyl ether (VII) to the corresponding acid (VIII) it is observed that all the aromatic signals are shifted downfield and that the difference in chemical shift between the α,- and β-protons in either of the two rings becomes larger. The complete assignments for the aromatic protons of these two compounds are readily given in the light of these considerations.

Acetoxyanthraquinones

The spectra of acetoxyanthraquinones are very similar to those of the methoxy derivatives although the absorption of the aromatic protons are all shifted downfield in comparison with the latter. Thus the proton at position 4 of 1,3-diacetoxyanthraquinone (XXII) absorbs at 2.11, more than 0.2 p.p.m. upfield from the average position of the remaining α-proton signals, and the 2-proton signal appears at 2.88, nearly 0.48 p.p.m. upfield from those due to the rest of the β-proton signals. These diamagnetic shifts of the aromatic proton absorptions are thus weaker than those due to methoxyl groups which are stronger electron donors. Consequently, the differentiation of various types of α- or β- protons becomes more difficult in the spectra of the acetoxy derivatives. Emodin triacetate (XXIII) gives two overlapping bands for the α- and β- pairs of protons. Although the unambiguous assignments for the different signals in these group can be readily given, they clearly suggest the decreased usefulness of acetate derivatives for structural analysis of unknown hydroxy-anthraquinones.

On the other hand, the signals due to α- and β-acetoxy groups themselves are readily differentiated.

The 1 and 3 acetoxy groups of 1,3-diacetoxy anthraquinone (XXII) absorb at 7.57 and 7.7 respectively.

The α-acetoxy groups show up usually at 7.57 ± 0.07 and β-acetoxyls at 7.68 ± 0.02. As in the case of the methoxyanthraquinones, this difference arises from the fact that α-substituents are closer to the carbonyl groups than the β-substituents. The induced magnetic field due to diamagnetic circulations of the carbonyl $\pi$-electrons is parallel to the applied field in and near the plane of the molecule and diminishes rapidly with the increasing distance.

# REFERENCES

1. P.M. Nair and G. Gopakumar - Unpublished work.

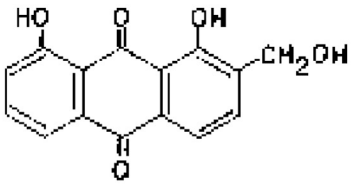
2. L.M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press (1959).

3. J.B. Bredenberg and J.N. Shoolery, Tetrahedron Letters, No. 9, 285 (1961).

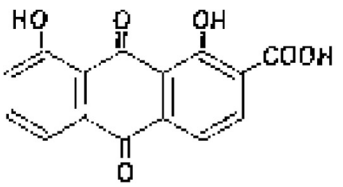
# CHAPTER I (PART D)

## SYNTHESIS OF SOME NEW HYDROXYANTHRAQUINONE DERIVATIVES

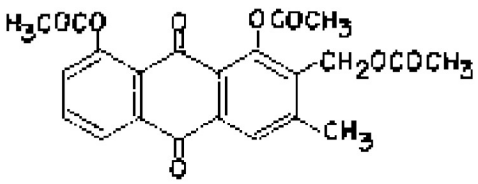
During the progress of work on the structure of cassiamin (vide Part B), it was felt necessary to synthesise a few 1,8-dihydroxyanthraquinone derivatives and study their properties. As a result, the following three new compounds have been prepared: (a) 1,8-dihydroxy-2-hydroxymethylanthraquinone (I); (b) 1,8-dihydroxyanthraquinone-2-carboxylic acid (II); and (c) 1,8-diacetoxy-2-acetoxymethyl-3-methylanthraquinone (III).




I




II




III

The scheme of synthesising these compounds has been represented in Charts 1 and 2. A series of reactions are involved in this scheme, but they normally proceed smoothly giving good yields.

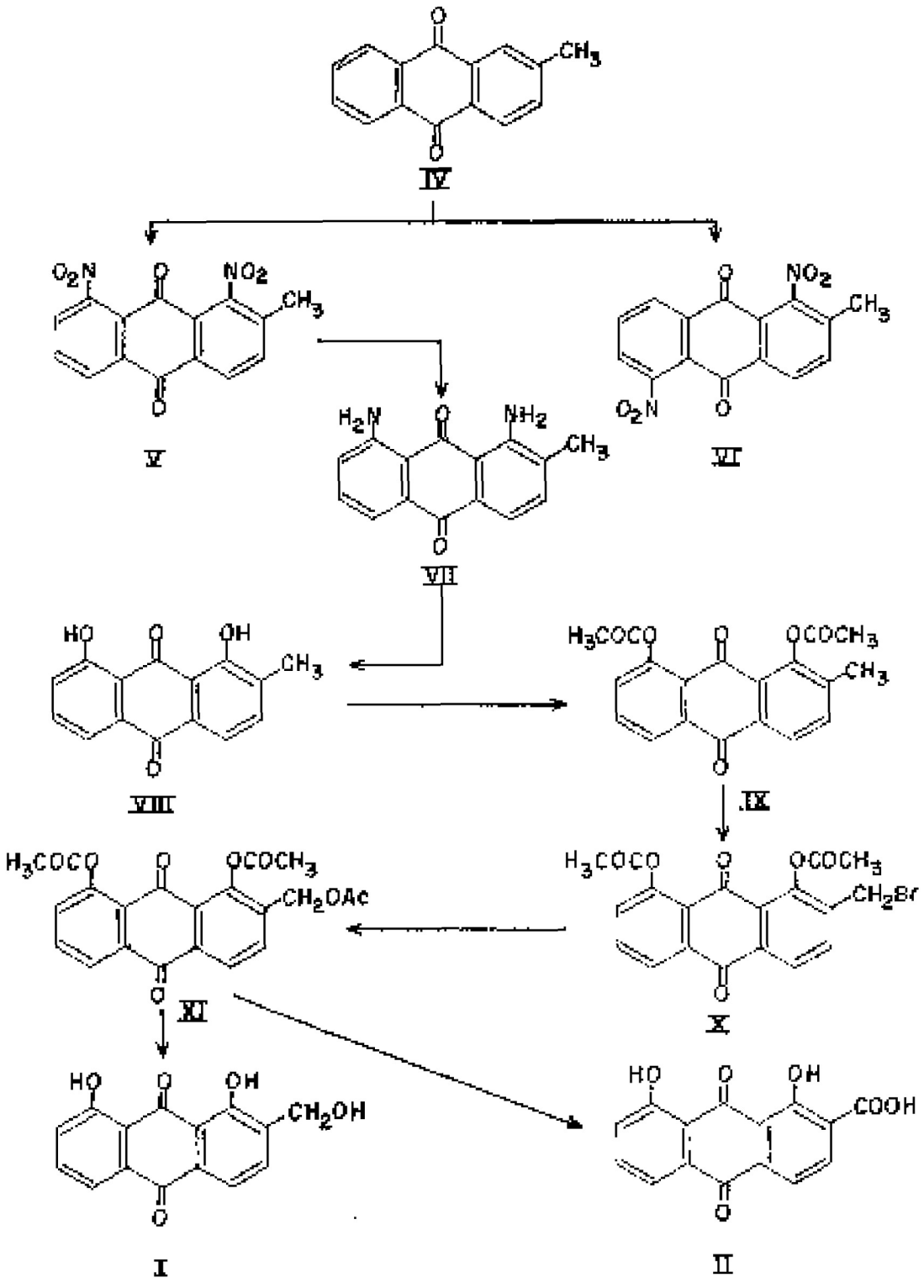



CHART 1

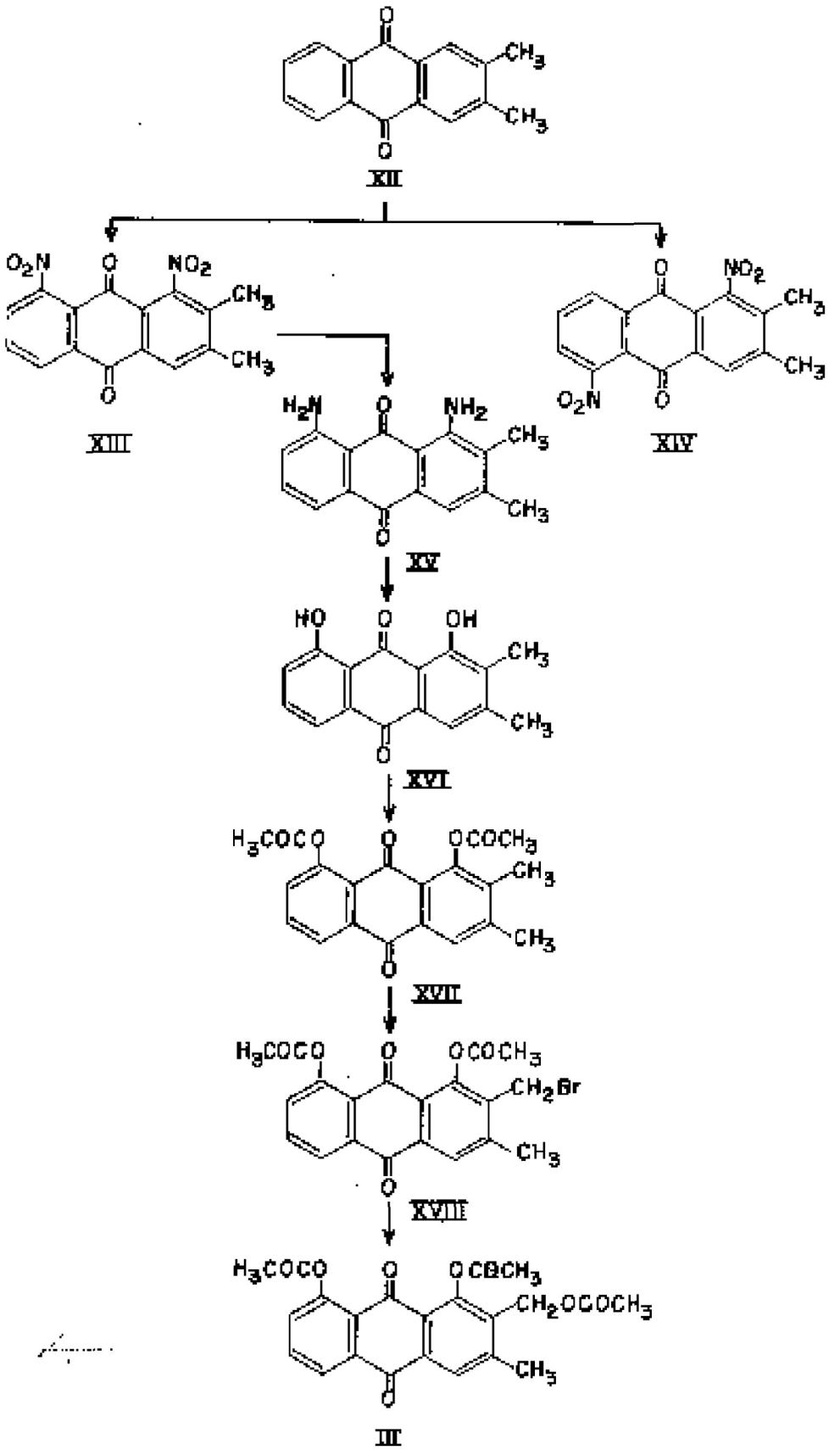



CHART 2

For the syntheses of Compounds (I) and (II), 2-methylanthraquinone (IV), a common dye intermediate was used as the starting material. Nitration of 2-methylanthraquinone to the dinitro stage gave a mixture of 1,8-dinitro- and 1,5-dinitro-2-methylanthraquinone (V & VI), which could be separated easily by taking advantage of their difference in solubility in concentrated sulphuric acid. The 1,8-dinitro compound (V) on reduction with sodium sulphide yielded 1,8-diamino-2-methylanthraquinone (VII) which was purified by chromatography. The latter on diazotisation and subsequent boiling with 40% sulphuric acid afforded 1,8-dihydroxy-2-methylanthraquinone (VIII). This was easily acetylated to the corresponding 1,8-diacetoxy-2-methylanthraquinone (IX).

It may be mentioned here that the replacement of the nitro group by acetoxy group under Friedel-Crafts' conditions in presence of boron-trifluoride etherate, which has been discussed in Chapter II was first attempted on the dinitro compound using different reaction conditions. But both 1,8-dinitro-2-methylanthraquinone as well as its lower homologue, 1,8-dinitroanthraquinone, failed to give any acetate ester under any of these conditions.

However, a general observation was made during this series of studies. The normal method of acetylation of hydroxyanthraquinones consists of the use of sulphuric

acid, perchloric acid, pyridine, and sodium acetate. Boron-trifluoride can also be used conveniently, since all the model experiments showed that the acetylation with acetic anhydride in presence of boron-trifluoride proceeds smoothly. Thus, 1,8-dihydroxyanthraquinone, 1,8-dihydroxy-3-methylanthraquinone (chrysophanic acid), 1,8-dihydroxy-2,3-dimethylanthraquinone gave excellent yields of the corresponding acetates.

In an attempt to obtain 1,8-diacetoxyanthraquinone-2-carboxylic acid (IX) directly from 1,8-diacetoxy-3-methylanthraquinone, the latter was subjected to chromic acid oxidation in acetic acid-acetic anhydride mixture. The methyl group, however, appeared to be surprisingly resistant to oxidation by chromic acid. This behaviour of 1,8-diacetoxy-3-methylanthraquinone is strongly reminiscent of that of the acetates of morindone (1,5,6-trihydroxy-2-methylanthraquinone),[1] cynodontin (1,4,5,6,8-tetrahydroxy-2-methylanthraquinone),[2] and catenarin (1,4,6,8-tetrahydroxy-3-methylanthraquinone).[3] In all these cases, the methyl groups were in ortho positions with respect to one of the acetoxy groups.

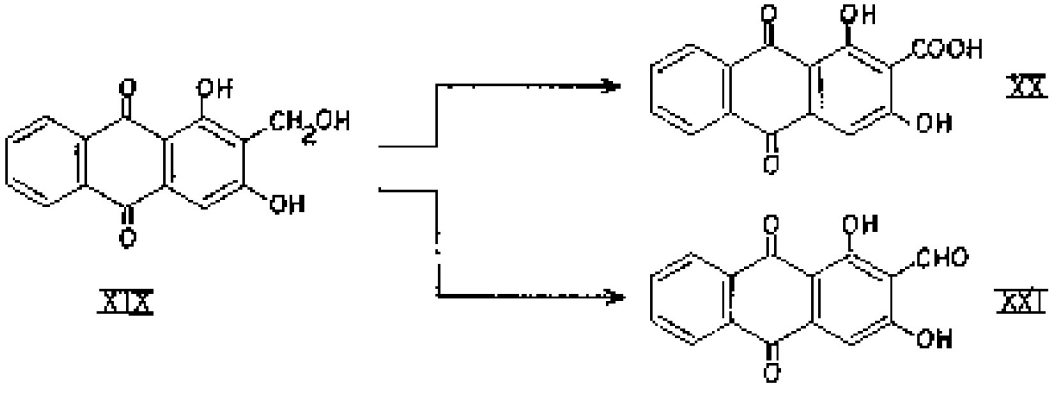
1,8-Diacetoxy-3-methylanthraquinone (IX) was therefore converted by bromination with N-bromosuccinimide and benzoyl peroxide in carbon tetrachloride to the ω-bromo compound. It may be mentioned that N-bromosuccinimide has been used extensively for the bromination

of methylene and methyl groups particularly in the side-chain bromination of aromatic hydrocarbons.[4] However, the first application to an anthraquinone derivative was the bromination of rubiadin diacetate to the corresponding 3-bromo methyl compound by Venkataraman and co-workers.[5]

In the present case, 1,8-diacetoxy-3-methyl-anthraquinone was converted by treatment with N-bromo-succinimide to the ω-bromo compound (X) which on refluxing with sodium acetate-acetic anhydride for two hours yielded 1,8-diacetoxy-3-acetoxymethylanthraquinone (XI). This was hydrolysed by refluxing the methanolic solution with a few drops of concentrated sulphuric acid. The 1,8-dihydroxy-3-hydroxymethylanthraquinone (I) thus obtained was not sufficient for analysis. It was insoluble in cold 5% sodium carbonate, but readily dissolved in 6% sodium hydroxide solution giving a reddish violet colour; with concentrated sulphuric acid it formed a reddish violet solution. Alcoholic magnesium acetate produced a reddish pink colour.

1,8-Diacetoxy-3-acetoxymethylanthraquinone (XI) was next oxidised by silver oxide in presence of sodium hydroxide to 1,8-dihydroxyanthraquinone-2-carboxylic acid (II), m.p. 311-13°.

In a model experiment of silver oxide oxidation in presence of sodium hydroxide, lucidin (XIX) was oxidised as described by Ayyangar.[6] He reported that by oxidation with silver oxide (1 mole) and sodium hydroxide at 75° for 1 hr., lucidin (1 mole) was converted to munjistin (XX) in an yield of 35-40%.




However, the NMR spectrum of the compound obtained by the above procedure revealed that there was no expected carboxyl group in the product. The NMR spectrum in deutero chloroform showed signals at - 3.67 and - 2.6 $\tau$ , both exchangeable with $D_2O$, but a third signal at the aldehyde region at - 0.17 $\tau$ was not exchangeable. This indicated that the oxidation had taken place only upto the aldehyde stage. Finally this compound was identified as nor-damnacanthal (1,3-dihydroxy-2-formylanthraquinone) (XXI).

Recently, similar observation has also been reported by Hirose,[7] who further showed that in the oxidation of lucidin, the use of 1 mole of silver oxide

gave nor-damacanthal in an yield of 37% and with 2 moles of silver oxide, munjistin was formed.

2,3-Dimethylanthraquinone (XII) was used as the starting material for the synthesis of the third compound, 1,8-diacetoxy-2-acetoxymethyl-3-methylanthraquinone (III), (Chart 2). Nitration with 2 moles of nitric acid(d, 1.5) gave a mixture of 1,8-dinitro-2,3-dimethylanthraquinone (XIII) and 1,5-dinitro-2,3-dimethylanthraquinone (XIV) which were separated by their difference in solubility in concentrated sulphuric acid and glacial acetic acid.

Reduction of 1,8-dinitro-2,3-dimethylanthraquinone with sodium sulphide yielded the corresponding 1,8-diamino compound (XV). The latter on diazotization and hydrolysis gave the corresponding dihydroxy compound (XVI), which was acetylated to give 1,8-diacetoxy-2,3-dimethylanthraquinone (XVII). α-Bromination with N-bromosuccinimide and catalytic amount of benzoyl peroxide yielded 1,8-diacetoxy 2-bromomethyl-3-methylanthraquinone (XVIII) which on heating with sodium acetate and acetic anhydride gave 1,8-diacetoxy-2-acetoxymethyl-3-methylanthraquinone(III), m.p. 162-63°.

## EXPERIMENTAL

1,8-Dinitro-2-methylanthraquinone (V), m.p.292-93° and 1,5-dinitro-2-methylanthraquinone, m.p. 345-46°, were prepared by nitrating 2-methylanthraquinone (IV) and separating the two isomers.[8] Reduction of 1,8-dinitro-2-methylanthraquinone with sodium sulphide[9] gave 1,8-diamino-2-methylanthraquinone (VII), m.p.206-07°, in 88% yield.

1,8-Dihydroxy-2-methylanthraquinone (VIII)

1,8-Diamino-2-methylanthraquinone (2.2 g) in concentrated sulphuric acid (35 ml) was cooled to 5° and diazotised with a mixture of sodium nitrite (2.2 g) and concentrated sulphuric acid (40 ml). The diazonium salt solution was poured over crushed ice (200 g) and then added to a boiling solution of 40% sulphuric acid. The mixture was gently boiled for 1 hr., cooled and diluted with water (200 ml). The yellow precipitate was collected, washed and dried (1.8 g). Crystallisation from alcohol gave greyish yellow needles, m.p. 175-76°. IR spectrum: 1665, 1620, 1588, 1270, 1160, 1040 and 848 $cm^{-1}$.

Analysis: Found: C, 70.6; H, 3.9.

$C_{15}H_{10}O_4$ requires: C, 70.9; H, 3.9%.

1,8-Diacetoxy-3-methylanthraquinone (IX)

Method A. A mixture of 1,8-dihydroxy-3-methylanthraquinone (0.6 g) in acetic anhydride (15 ml) and pyridine (0.5 ml) was heated on a water bath for 2 hr. After 24 hr., the reaction mixture was poured into crushed ice (50 g). The yellow solid was filtered, washed with water and dried (0.51 g). Recrystallisation from alcohol gave yellow needles, m.p. 201-202°. IR spectrum: 1754, 1675, 1600, 1325, 1276, 1198, 1042, 1020, 960, 909, 866, and 823 $cm^{-1}$.

Analysis: Found: C, 67.4; H, 4.1.
$C_{19}H_{14}O_6$ requires: C, 67.5; H, 4.2%.

Method B. 1,8-Dihydroxy-?-methylanthraquinone (1 g) was taken in acetic anhydride ( 15 ml) to which a few drops of freshly distilled boron-trifluoride were added. After 24 hr., the reaction mixture was poured over crushed ice (50 g). The yellow solid was filtered, washed and dried. Recrystallisation from alcohol gave yellow needles, m.p. 201-202°.

Infrared spectrum of the compound was superimposable with that of the acetate obtained by the Method A, and there was no depression in mixed melting point.

In model experiments, 1,8-dihydroxyanthraquinone, 1,8-dihydroxy-2,3-dimethylanthraquinone and 1,8-

dihydroxy-3-methylanthraquinone (chrysophanol) were similarly acetylated by acetic anhydride in presence of boron-trifluoride.

Attempted acetylation of 1,8-dinitro-3-methyl anthraquinone with acetic anhydride in presence of boron-trifluoride

To 1,8-dinitro-3-methylanthraquinone (0.2 g) in acetic anhydride, a few drops of boron-trifluoride etherate were added and the reaction mixture kept at room temperature for 48 hr. The solution was poured over crushed ice and the yellow solid formed was filtered, washed and dried. It was crystallised from glacial acetic acid as yellow needles, m.p. 292-93°. Infrared spectra of the recrystallised sample as well as the crude product were superimposable with that of the starting material.

In another experiment, the reaction mixture was heated on a water-bath for 6 hr., but no acetate was formed. Model experiments with 1,8-dinitroanthraquinone also failed to yield the corresponding acetate.

1,8-Diacetoxy-3-bromomethylanthraquinone (X)

1,8-Diacetoxy-3-methylanthraquinone (1.25 g) was refluxed with N-bromosuccinimide (1 g) and benzoyl-peroxide (0.05 g) in carbon tetrachloride (100 ml) for 24 hr. The solvent was distilled off and the residue

washed with hot water and dried (3.4 g). It was chromatographed over a small column of alumina in benzene and the bromine containing fraction was collected. On removal of the solvent, a greyish yellow solid was obtained (0.6 g). Crystallisation from carbon tetrachloride gave pale yellow leaflets, m.p. 185-87°.

1,8-Diacetoxy-2-acetoxymethylanthraquinone (XI)

A mixture of 1,8-diacetoxy-2-bromomethylanthraquinone (0.5 g), freshly fused sodium acetate (0.5 g) and acetic anhydride (5 ml) was refluxed for 2 hr. The reaction mixture was cooled and poured into water. The greyish solid was filtered, washed and dried (0.4 g). Crystallisation from ethanol gave yellow plates, m.p. 192-94°. IR spectrum: 1765, 1678, 1598, 1323, 1228, 1193, 1087, 1040, 1018, 982, 960, 903, 869, 803, and 745 cm-1.

Analysis: Found: C, 63.4; H, 4.3.

$C_{21}H_{16}O_8$ requires: C, 63.6; H, 4.1%.

Attempted chromic acid oxidation of 1,8-diacetoxy-2-methylanthraquinone

To a solution of 1,8-diacetoxy-2-methylanthraquinone (IX) (1 g) in glacial acetic acid (20 ml) and acetic anhydride (20 ml) maintained at 57-60°, a solution of $CrO_3$ (2 g) in water (1.6 ml) and glacial acetic acid

(20 ml) was added in small portions during 0.5 hr. with constant stirring. The reaction mixture was heated for another 3 hr. at 60-65°. The solution was cooled and poured into ice water (200 ml). The yellow precipitate was filtered, washed and dried. Crystallisation from ethanol gave pale yellow needles, m.p. 201-202°. It was identified as the starting material.

Silver oxide oxidation of lucidin (XIX) to nor-damnacanthal (XXI)

Silver nitrate (0.136 g) in water (1 ml) was treated with sodium hydroxide (0.024 g). The precipitated silver oxide was filtered, washed and transferred to a beaker containing 10 ml of water. Sodium hydroxide (0.136 g) was added with vigorous stirring. The temperature of the mixture was maintained at 75° and lucidin (XIX, 0.1 g) was introduced. Stirring was continued for 1 hr. and the mixture filtered hot. The residue of silver was washed well with hot water. The filtrate and washings were acidified and the yellow precipitate was treated with 5% sodium carbonate. Acidification of the filtered solution gave an orange red solid (0.011 g) which showed a positive test for carboxyl group with potassium iodide-potassium iodate reagent. The residue (0.04 g) was recrystallised from ethanol as yellow needles, m.p. 220-221° (lit.[7] m.p. 221-222°) IR spectrum: 1675, 1648, 1634, 1600 and 1582 cm-1.

washed thoroughly with warm water. The filtrate and washings were acidified, and the yellow precipitate was dissolved in 5% aqueous sodium bicarbonate. Acidification of the filtered solution and crystallisation of the precipitate (0.1 g) from glacial acetic acid gave yellow needles, m.p. 211-13°. The compound gave a brown red colour in sodium bicarbonate, a violet colour in sodium carbonate and a reddish violet colour in concentrated sulphuric acid. It gave a reddish purple colour with methanolic magnesium acetate. IR spectrum: 1700, 1665, 1620, 1595, 1280, 1250, 1200, 1160, 1076, 1038, 921, 893, 857, 840 and 750 cm-1.

Analysis: Found: C, 63.1; H, 2.9.
$C_{15}H_8O_6$ requires: C, 63.4; H, 2.8%.

1,8-Dinitro-2,3-dimethylanthraquinone (XIII)

This compound was prepared by nitration of 2,3-dimethylanthraquinone (1 mole, 100%) in sulphuric acid with fuming nitric acid (2.2 moles). The 1,8-dinitro- and 1,5-dinitro-2,3-dimethylanthraquinone (XIII & XIV) were separated by their differential solubilities in concentrated sulphuric acid. 1,8-Dinitro,2,3-dimethylanthraquinone after crystallisation from glacial acetic acid melted at 260-62° (lit.[10] m.p. 262-266°).
Analysis: Found: C, 59.1; H, 2.7; N, 8.4.
$C_{16}H_{10}O_6N_2$ requires: C, 58.9; H, 3.1; N, 8.6%.

1,8-Diamino-2,3-dimethylanthraquinone (XV)

1,8-Dinitro-2,3-dimethylanthraquinone (2.5 g) was made into a paste with sodium sulphide (20 g) and water (1 ml). The paste was diluted with water (100 ml) and the reaction mixture was refluxed for 2 hr. The bright violet red crystalline product was filtered, washed and dried (1.8 g); crystallisation from O-dichlorobenzene gave violet red needles, m.p. 188-190°. IR spectrum: 3400, 3280, 1660, 1588, 1538, 1320, 1260, 1010, and 838 cm-1.

Analysis : Found: C, 72.6; H, 5.2; N, 10.8.

$C_{16}H_{14}N_2O_2$ requires: C, 72.2; H, 5.3; N, 10.5%.

1,8-Dihydroxy-2,3-dimethylanthraquinone (XVI)

A solution of 1,8-diamino-2,3-dimethylanthraquinone (1.6 g) in concentrated sulphuric acid (20 ml) was cooled to 5° and diazotised with sodium nitrite (1.6 g) in concentrated sulphuric acid (15 ml). After 1 hr., the reaction mixture was poured over crushed ice (100 ml). The diazonium solution thus obtained was added in portions to a boiling 40% sulphuric acid solution (75 ml) at 140-45°. The mixture was heated at this temperature for 1 hr. The yellow precipitate was filtered, washed and dried (1.1 g), m.p. 193-94°. IR spectrum: 1665, 1618, 1596, 1262, 1220, 1180, 1158, 1100, 1135, 900, 840, 762.

Analysis: Found: C, 71.1; H, 4.2.

$C_{16}H_{12}O_4$ requires: C, 71.6; H, 4.6%.

1,8-Diacetoxy-2,3-dimethylanthraquinone (XVII)

1,8-Dihydroxy, 2,3-dimethylanthraquinone (0.8 g) was acetylated by acetic anhydride and a few drops of perchloric acid at room temperature for 1 hr. The yellow acetate (0.83 g) was recrystallised from acetic acid as pale yellow needles, m.p. 221-223°. Acetylation of (XVI) with acetic anhydride in presence of boron-trifluoride also produced (XVII) in quantitative yield. IR spectrum: 1760, 1670, 1595, 1276, 1190 and 930 $cm^{-1}$.

Analysis: Found: C, 68.5; H, 4.2.

$C_{20}H_{16}O_6$ requires: C, 68.2; H, 4.6%.

1,8-Diacetoxy-2-bromomethyl-3-methylanthraquinone (XVIII)

A mixture of 1,8-diacetoxy-2,3-dimethylanthraquinone (0.7 g), N-bromosuccinimide (0.41 g), and benzoyl peroxide (0.02 g) in carbon tetrachloride (75 ml) were refluxed for 34 hr. The solvent was removed and the residue washed with water, collected and dried. The yellowish grey compound (0.7 g) was taken in benzene and chromatographed over alumina. The fraction containing the bromo compound was collected. The solvent was removed and the residue (0.4 g) was crystallised from carbon tetrachloride, m.p. 182.5°.

Analysis: Found: Br, 19.5.

$C_{20}H_{15}O_6Br$ requires: Br, 18.6%.

1,8-Diacetoxy-2-acetoxymethyl-3-methylanthraquinone (III)

A mixture of 1,8-diacetoxy-2-bromomethyl-3-methylanthraquinone (0.25 g), freshly fused sodium acetate (0.4 g) and acetic anhydride (10 ml) was refluxed for 2 hr. The reaction mixture was cooled and poured over crushed ice (50 g). The pale yellow product was collected, washed and dried (0.19 g). Crystallisation from ethanol gave yellow needles, m.p. 162-63°. The compound gave a violet solution with 8% sodium hydroxide and a reddish violet solution with concentrated sulphuric acid. With methanolic magnesium acetate it gave a reddish pink colour. IR spectrum: 1760, 1678, 1598, 1330, 1266, 1190, 1083, 1040, 972, 918, 887, 829, 810, and 780 $cm^{-1}$.

Analysis: Found: C, 64.1; H, 4.5. - $COCH_3$ 30.9.

$C_{23}H_{18}O_8$ requires: C, 64.4; H, 4.4%. - $COCH_3$ 31.2%.

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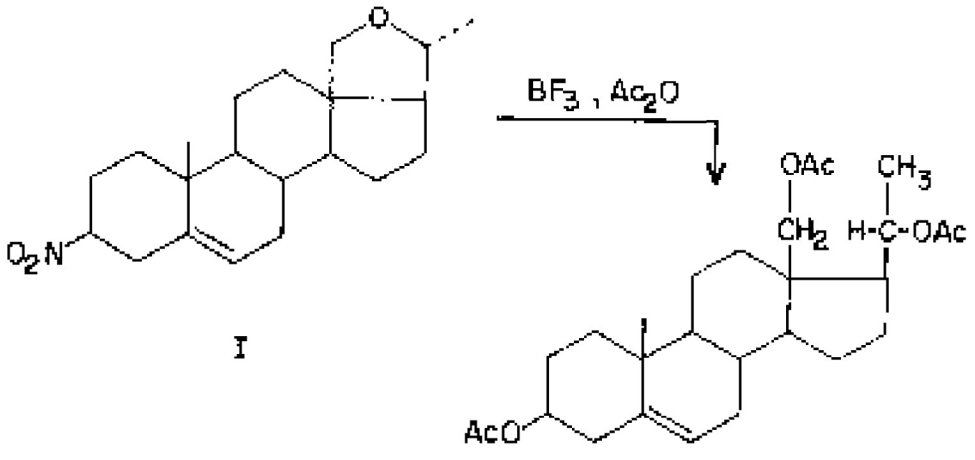
# C H A P T E R II

## A NOVEL BORON TRIFLUORIDE CATALYSED REACTION REPLACEMENT OF A NITRO GROUP BY ACETOXY GROUP UNDER FRIEDEL CRAFTS' CONDITIONS

# S U M M A R Y

The scope and limitations as well as the mechanism of a new reaction of nitro compounds, the replacement of nitro group by acetoxy group under Friedel Crafts' conditions, have been studied. The reaction is found to be a general one for aliphatic nitro compounds but is not applicable in the case of aromatic nitro compounds. The reaction with optically active nitro compounds proceeds mostly with retention of configuration. Nitrite ion could not be detected as a reaction product. The mechanism of the reaction has been studied on the light of the above findings.

Recently, a new reaction for nitro-compounds was discovered in the Division of Biochemistry of this Laboratory. Manas Ram et al.[1] reported that 3β-nitro-18,20β-oxido-5-pregnene (I) when treated with boron-trifluoride and acetic anhydride afforded 3β,18,20*-triacetoxy-5-pregnene (II).




I

II

The boron trifluoride-catalysed replacement of a nitro-group by an acetoxy group at position 3β was rather unexpected and constituted a hitherto unknown reaction in organic chemistry. The reaction appeared to be a general one, since nitromethane (III) was also

reported to yield methyl acetate (IV) under similar treatment.[1]

$$CH_3NO_2 \xrightarrow{BF_3\ ,\ Ac_2O} CH_3OCOCH_3$$

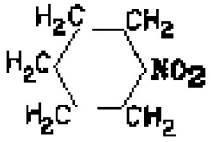
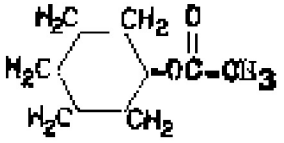
III IV

It was decided to make some further studies on the scope and limitations of the reaction as well as on the mechanism involved. The investigator was particularly interested in using the reaction to synthesise 1,8-dihydroxyanthraquinone derivatives from the easily available 1,8-dinitroanthraquinone - a potential intermediate in the synthesis of several hydroxy-anthraquinone derivatives (vide Chapter I).

Scope of the reaction

Preliminary experiments were carried out with a number of aliphatic and aromatic nitro-compounds using excess of acetic anhydride and equimolar proportions of boron trifluoride and the nitro-compounds. It was found that the reaction proceeded smoothly with aliphatic nitro-compounds at 0-25° and was over in a few minutes giving rise to corresponding acetate esters in 35-55% yields (Table I).

# TABLE I

| Nitro-compounds | Product | Yield* (%) |
|---|---|---|
| $CH_3NO_2$ | $CH_3OCOCH_3$ | 40-50 |
| $CH_3CH_2NO_2$ | $CH_3CH_2OCOCH_3$ | 48 |
| $CH_3-CH(NO_2)-CH_3$ | $(CH_3)_2CHOCOCH_3$ | 50 |
|  |  | 48-55 |
| $CH_3-CH(NO_2)-(CH_2)_5-CH_3$ | $(CH_3)(CH_3(CH_2)_5)CH-OC(=O)-CH_3$ | 36-42 |

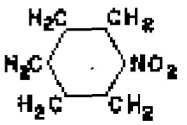
* Yields were estimated from the IR spectra.

Aromatic nitro-compounds were inert to the reaction conditions. Thus nitrobenzene and 4-nitro-biphenyl were recovered unchanged even after refluxing the reaction mixture overnight. Neither was it possible to detect appreciable amounts of 1,8-diacetoxy-anthra-quinone after prolonged treatment of 1,8-dinitro-anthraquinone with boron trifluoride and acetic anhydride. In the case of 1-nitro-naphthalene, however, the formation of a small amount of naphthyl acetate could be detected in IR spectrum.

Stoichiometry

For determining the stoichiometry of the reaction, acetic anhydride was kept in excess but the proportions of the nitro-compound and boron trifluoride were varied. The results are summarised in Table II.

# TABLE II

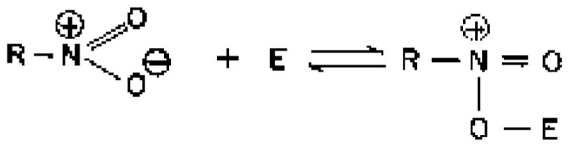
| Nitro-compound | $BF_3$-etherate | Yield of the acetate* (%) |
|---|---|---|
| $CH_3NO_2$ (1 mole) | 1/2 mole | 33 |
| | 1 mole | 49 |
| | 2 moles | 60 |
| | 3 moles | 61 |
|  (1 mole) | 1/2 mole | 37 |
| | 1 mole | 52 |
| | 3 moles | 58 |

* Calculated from the IR spectra.

Higher concentrations of boron trifluoride did not increase the yields significantly.

Mechanism of reaction

Three types of mechanisms could be considered for the replacement reaction. In each of these mechanisms, an electrophilic attack (by $BF_3$ or $CH_3CO^+$) on the negatively charged oxygen of the nitro-group is postulated as the initial step.

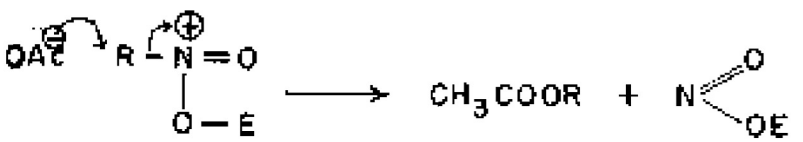


V

Where E = $BF_3$, or $CH_3CO^{\oplus}$

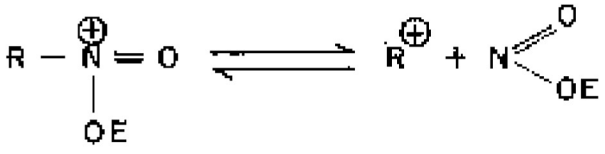
The intermediate (V) can then lead to the acetate by three different mechanisms.

(A) A bimolecular nucleophilic displacement ($S_N2$):

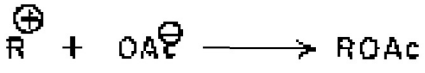


This will lead to an inversion of configuration.

(B) A unimolecular $S_N1$ type of reaction involving a carbonium ion:

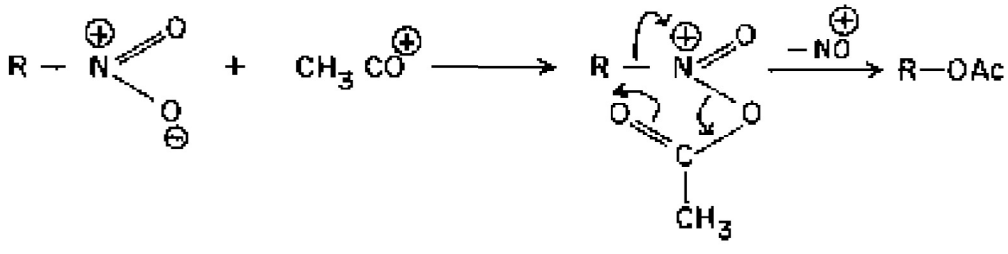


V



This mechanism is expected to proceed with racemisation with partial retention or inversion.

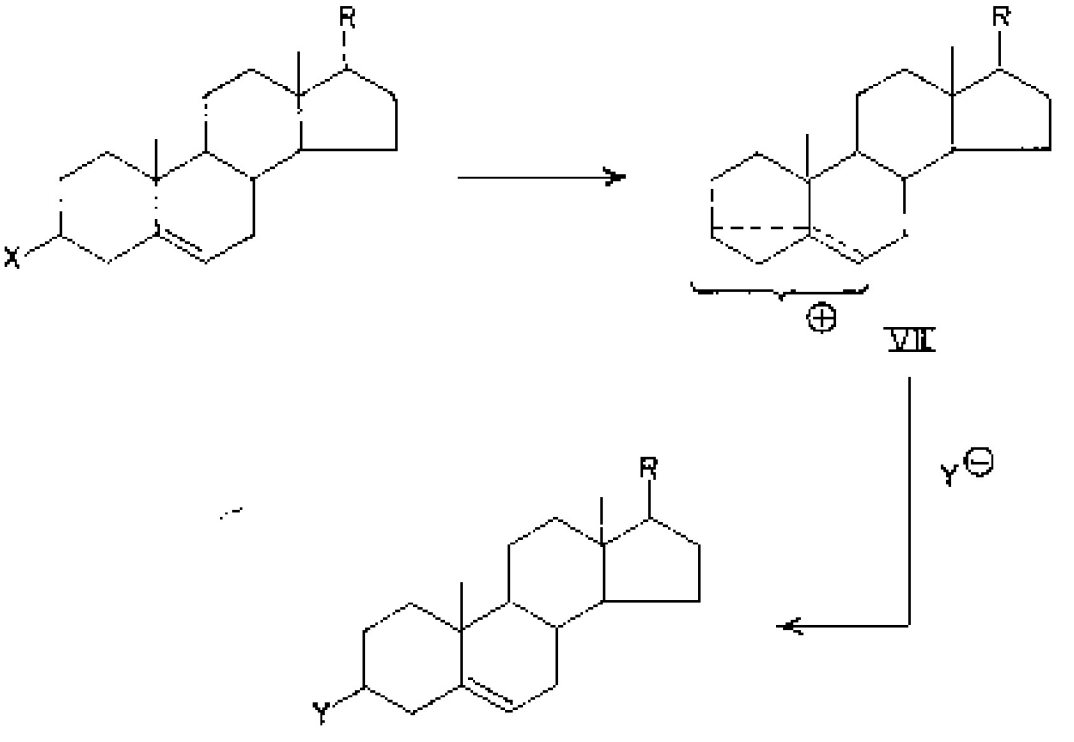
(C) An intramolecular $S_Ni$ type of reaction involving an acyl-nitro intermediate of the type VI.



VI

Normally one would expect a complete retention of configuration in this mechanism.

In the case of 3β-nitro- $\Delta^5$-steroid (I) reported by Mansa Ram et al., the 3β-acetoxy compound (II) was obtained exclusively with retention of configuration. This observation, unfortunately, cannot be regarded as a criterion for selecting the mechanism, as it is known that a nucleophilic displacement at position 3β in a $\Delta^5$-steroid in most cases proceed with retention of configuration involving the stabilised 3,5-cyclosteroid cation (VII).[2]



It was, therefore, necessary to prepare an optically active aliphatic nitro-compound to settle the question of mechanism. Both (+) and (-) 3-nitro-octane were prepared essentially according to procedure of Kornblum et al.[3], but using the technique of preparing the nitro-octane with sodium nitrite from the optically active octyl iodides in an aprotic solvent such as dimethylformamide.[4]

It was found that mostly the configuration was retained. However, in all experiments there was some racemisation reaching upto the extent of 35% depending on the conditions of the reaction. Some racemisation was also observed in the recovered nitro-compound from the reaction mixture.

These studies excluded the true bimolecular mechanism (A) as a possibility, but no distinction was possible between the mechanisms (B) and (C) on the basis of optical rotation of the products.

It was necessary to examine the reaction products to determine whether any nitrite ion could be detected in aqueous solution after decomposing the reaction mixture with aqueous bicarbonate. According to the mechanisms (A) and (B) the nitrogen from the nitro-compound is expected to be eliminated either as the anion as in ($BF_3, NO_2^-$) or

as a reactive acetyl nitrite, $CH_3CONO_2$, which should be hydrolysed to the $NO_3^-$ anion during the working up. It was however not possible to detect any nitrite ion in the aqueous phase despite of all attempts.

In mechanism (C) the nitrogen is expected to be eliminated as the nitrosyl cation ($NO^+$). Spectrophotometric determination of the nitrosyl cation which is reported to have a strong band at 250 $m\mu$[6] was not successful because of the absorption of the nitrocompounds in this region. Attempts to trap the $NO^+$ cation from the reaction mixture with the aid of a suitable nucleophile such as the dimethyl ether of resorcinol resulted in the recovery of only the $BF_3$ salt of resorcinol dimethyl ether as a crystalline product.

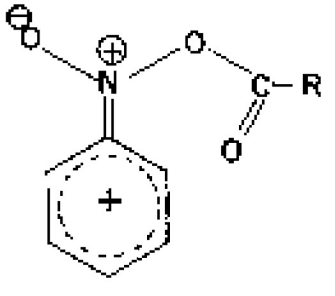
The reaction was then run with benzoyl chloride in place of acetic anhydride with continuous flushing of the reaction mixture with anhydrous carbon dioxide to remove any $NO^+$ formed as the gaseous nitrosyl chloride. The gases from the reaction mixture were bubbled through $\alpha$-pinene with the antipipation that any nitrosyl chloride will be trapped. There was no pinene-nitroso-chloride formation in the trap indicating that nitrosyl chloride was not released from the reaction mixture.

From the data it appears that the boron trifluoride-catalysed replacement of a nitro-group by an acetoxy group is a rather complex reaction. Although it has not been possible to establish the correct mechanism from the data, the absence of nitrite ion strongly indicates that the unimolecular mechanism (B) may not be of any major significance. The intramolecular mechanism (C) appears to be attractive, although no direct evidence could be obtained on the release of $NO^+$. The racemisation of the nitro-compound may be explained by an enolisation which may take place with ease under the reaction conditions.

Unfortunately, it has not been possible so far to prepare an optically active tertiary nitro-compound without the enolisable active α-hydrogen. The stereochemical behaviour of such a compound may establish the mechanism conclusively.

Anhydrous aluminium chloride was also found to catalyse the reaction. However, the yields were poor and the processing of the reaction mixture presented difficulties. A mixture of acetic anhydride and perchloric acid gave only a trace amount of methyl acetate from nitromethane. With higher nitro-compounds, however, no acetate esters could be isolated in appreciable amounts.

It is probable that the contribution to resonance in aromatic nitro-compounds by the canonical structure (IX) may be responsible for the failure of the reaction. The carbon nitrogen bond in (IX) has a more pronounced double bond character than in the case of aliphatic nitro-compounds and is more difficult to break.




IX

## EXPERIMENTAL

Nitromethane, 2-nitropropane, nitrobenzene, nitronaphthalene and 4-nitrobiphenyl were commercial samples which were dried and purified by redistillation or recrystallisation. Nitro-ethane was prepared from ethyl iodide and silver nitrite according to the classical method.[6] Nitrocyclohexane was obtained by reduction of 1-bromo-1-nitro-cyclohexane[7] which was obtained from cyclohexanone oxime according to the method of Iffland and Criner.[8]

All the reagents were freshly distilled. The reactions were carried out in glass stoppered flasks unless otherwise specified. To determine the yields, the reactions were run as follows:

Aliquots of 0.1 mole of the nitro compounds were added to an ice-cold mixture of 0.3 - 0.5 moles of acetic anhydride and 0.1 mole of boron-trifluoride. The reaction mixture was kept at room temp. for 30 min. and then poured into 200-600 ml. of ice cold 5% aqueous sodium bicarbonate with vigorous stirring. The mixture was then extracted with four 50 ml. portions of ether. The ethereal extracts were washed with water, dried over sodium sulphate and the ether removed carefully (through a fractionating column in case of nitromethane and nitroethane).

The residue was dried again on anhydrous magnesium sulphate, distilled and the quantity of the distillate measured. The IR spectrum of the distillate was taken and from the ratio of absorbance determined near 1740 $cm^{-1}$ (ester) and 1552 $cm^{-1}$ (nitro), the proportions of the acetate ester and the nitro compounds formed were calculated. Standard curves were also obtained for synthetic mixtures of the nitro compound and the acetate esters in proportions of 3:7, 4:6, 5:5, 6:4 and 7:3.

Examination of the distillate by vapour phase chromatography on a Perkin-Elmer Vapour Fractometer, Model 154 D on succinic acid polyester of diethylene glycol column revealed the presence of only two peaks with retention times corresponding to the acetate ester and the nitro compound. No other product could be detected in the vapour phase chromatograms. The relative proportion of the ester and the nitro compound as determined by the VPC method agreed within experimental limits with the IR spectra.

Table I summarises the results obtained with some aliphatic nitro compounds. In the experiments with aromatic nitro compounds, the conditions were similar. However, the reaction mixture was refluxed for 2-24 hours. In case of 1-nitro-naphthalene, the

IR spectral examination of the residue from the ether extracts revealed the existence of a small peak at 1751 $cm^{-1}$. However, it was not possible to isolate the compound in significant amounts.

For determination of stoichiometry, the conditions of the experiment were identical with that in case of aliphatic nitro compounds, only the amount of boron-trifluoride etherate was varied (Table II).

The (+) and (-)-2-nitro octane:

The (+) and (-) octyl iodides were prepared according to the method of Kornblum et al.[3,4] from (-) and (+)-2-octanols by passing anhydrous hydrogen iodide into the alcohols for 6 hours at 0°. The optically active alcohols were diluted with racemic 2-octanols.

The (+) alcohol employed had a specific rotation of $(\alpha)_D^{26}$ + 7.7° and the (-) alcohol, a rotation of $(\alpha)_D^{25}$ - 2.8°. They were converted into the corresponding nitro compounds through the iodides in the following manner:

An aliquot of 30 g. of the active iodide was added to a mixture of sodium nitrite (8 g) and urea(6 g) in dimethyl formamide (600 ml) at a temp. of 40°. The reaction mixture was stirred at 35-40° for 4 hours and

worked up according to the method of Kornblum[3,4] to yield 3-9 g. of the corresponding nitro compounds. The specific rotation of the nitro compound obtained from the dextro- and laevorotatory iodides were $(\alpha)_D^{25}$ - 2.8 and $(\alpha)_D^{25}$ + 6.0°.

These were subjected to the action of boron-trifluoride and acetic anhydride in the manner described earlier. The reaction with the laevo-rotatory nitro compound was run at 25° and that with the dextro at 0-5° for 30 minutes. The products were isolated in the manner described before and chromatographed over grade I alumina. Light petroleum ether (40-60°) eluted the ester and the unreacted nitro compound was eluted with mixtures of ether- and chloroform. The eluate portions were carefully evaporated, and the acetate fraction cooled. IR spectra of these fractions indicated that they were almost pure containing less than 5% of nitro compound. The products were purified by distillation.

Specific rotation of products from (-) 2-nitro-octane:-

(-) 2-octylacetate, $(\alpha)_D^{25}$ = - 0.83°;

recovered (-) 2-nitro-octane, $(\alpha)_D^{25}$ = - 2.6°.

Specific rotation of (-) 2-octylacetate obtained directly from (-) 2-octanol, $(\alpha)_D^{25}$ = - 1.4°.

Specific rotation of products from (+) 2-nitro-octane: -

(+) 2-octyl acetate, $(\alpha)_D^{25} = + 3.4^\circ$.

recovered (+) 2-nitro-octane, $(\alpha)_D^{25} = + 5.7^\circ$.

(+) 2-octylacetate prepared from (+) 2-octanol, $(\alpha)_D^{25} = + 3.9^\circ$.

Detection of nitrite anion ($NO_2^-$)

The aqueous bicarbonate from the reaction mixture was heated after acidification with sulphuric acid. There was no evolution of nitrous fumes indicating that nitrite was not present. The reaction mixture however gave feeble spot test for nitrite[9] but it was found that nitro compounds also give similar feeble spot tests.

Detection of the $NO^+$ cation

The reaction with 2-nitro propane was repeated in presence of 0.1 mole of dimethyl ether of resorcinol. An immediate violet brown colour was obtained and crystals started separating. After keeping the mixture overnight, the yellow crystals were filtered, and washed with ether, m.p. 300-302°.

Analysis: Found: C, 48.9; H, 4.23. Residue 5.3%.

Calc.for $BF_3 \cdot C_8H_{10}O_2$: C, 46.6; H, 4.8%. Residue 6.4%.

Nitrogen - absent.

In a model experiment without the addition of the compound, 2-nitropropane, similar yellow crystals, m.p. 300-302°, were formed. On recrystallisation from acetone, the salt yielded another product, m.p. 159° (residue 3.0%).

The reaction was run with nitromethane(0.1 mole) and benzoyl chloride (0.3 mole) and boron-trifluoride etherate (0.1 mole). Dry carbondioxide was bubbled through the reaction mixture and passed through a trap containing α-pinene kept in ice-salt mixture. After one hour, the α-pinene in the trap was recovered unchanged. No nitrosochloride formation could be detected in the IR spectra. The reaction mixture after working up contained methyl benzoate as revealed by IR bands, (1710, 1575, 825, 755 and 685 cm-1).

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# C H A P T E R III

## CHEMICAL INVESTIGATION OF OUGEINIA DALBERGIOIDES BENTH

# SUMMARY

Two pentacyclic triterpenes characterised as lupeol and betulin have been isolated from the petroleum ether and ether extracts respectively of the bark of *D. dalbergioides*. Besides these, a saponin has been obtained from the alcoholic extract of the bark which on hydrolysis produced another triterpene, identified as β-amyrin. The sugar moiety was found to consist of glucose and arabinose. The simultaneous occurrence of lupeol, betulin and β-amyrin, appears to be interesting from the view point of biogenesis.

Ougeinia dalbergioides Benth (N.O. Leguminosae; Hindi - Sandan; Marathi - Tiwas) is a moderate-sized deciduous tree found in many parts of India. Various medicinal properties have been ascribed to different parts of the tree.[1] The bark when incised, furnishes kino like exudation, which is useful in diarrhoea, dysentery and leprosy.

Very little attention has so far been given towards its chemical examination. While the present work with the bark of the plant was in progress, Balakrishna et al.[2] reported the isolation of homoferreirin (5,7-dihydroxy-2',4'-dimethoxy-isoflavanone) and ougenin, a new isoflavanone, from the acetone extract of the heartwood of O.dalbergioides. From the various degradative experiments ougenin was shown to be 5,2',4'-trihydroxy-7-methoxy-6-methyl-isoflavanone.

## The Present Work

The present study was undertaken with the bark of Ougeinia dalbergioides, made available from Khandesh, through the courtesy of the Forest Utilisation Officer, Poona (Maharashtra State). The dry powdered bark was extracted successively at room temperature with pet. ether (40-60°), ether and alcohol.

Examination of Pet.ether extract:
Isolation of Compound A (Lupeol)

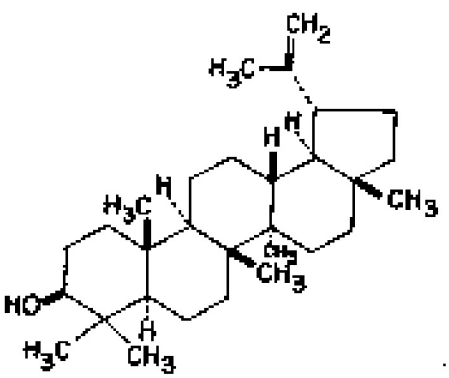
The total pet.ether extract on removal of the solvent was obtained as a light yellow semi-solid mass which could not be easily crystallised from any organic solvent. This was chromatographed through alumina twice and the product obtained from the benzene eluate, after removal of the solvent, crystallised from ether-methanol as colourless needles, m.p. 210-211°. This was designated as Compound A. The homogenity of the Compound A was ascertained from thin layer chromatography on silica gel with methylene chloride as the solvent and chlorosulphonic acid as developing reagent[3] when a single spot with $R_f$:0.66 was obtained. While there was no specific colour with $FeCl_3$, Compound A responded to positive Liebermann - Burchard test (violet). A chloroform solution of the substance produced a yellow colour with tetranitromethane, indicating the presence of unsaturation in the compound. With trichloroacetic acid, it gave a light-red colour and when warmed with acetyl chloride and zinc chloride, a pale reddish-purple colour was produced.

From the elementary analysis, the molecular formula was found to be $C_{30}H_{50}O$. It was optically active having $(\alpha)_D^{20}$ + 26° and contained one active hydrogen. It did not show any specific absorption in the UV region (220-400 mμ). Infrared spectrum of the compound taken in nujol mull showed strong peaks at 3600, 3420, 1026 $cm^{-1}$

(hydroxyl), 1640 $cm^{-1}$ and 887 $cm^{-1}$ (characteristic of a vinylidine group, $>=CH_2$).[4,5]

On acetylation with acetic anhydride and pyridine Compound A formed a monoacetyl derivative, colourless needles from ether - pet.ether, $C_{32}H_{52}O_2$, m.p. 214-216°; $(\alpha)_D^{30}$ + 46°. The acetate showed strong peaks at 1735, 1253 $cm^{-1}$ (acetate), 1640 and 886 $cm^{-1}$ (vinylidine group) (in $CHCl_3$) in the infrared spectrum. It easily formed a benzoate with benzoyl chloride and pyridine, crystallising from alcohol as colourless needles, $C_{37}H_{54}O_2$, m.p. 263-64°, $(\alpha)_D^{30}$ + 62°.

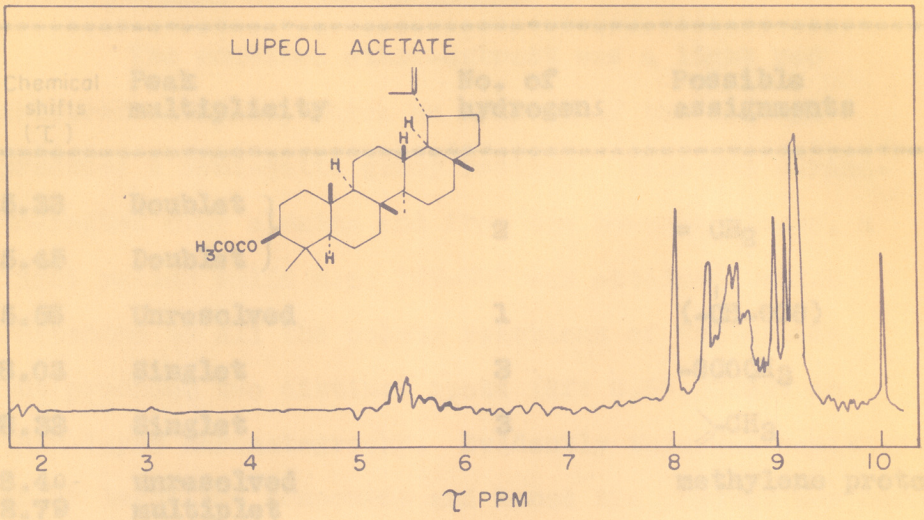
The above physical and chemical properties, strongly suggested the similarity of Compound A with lupeol (I), a pentacyclic triterpene.



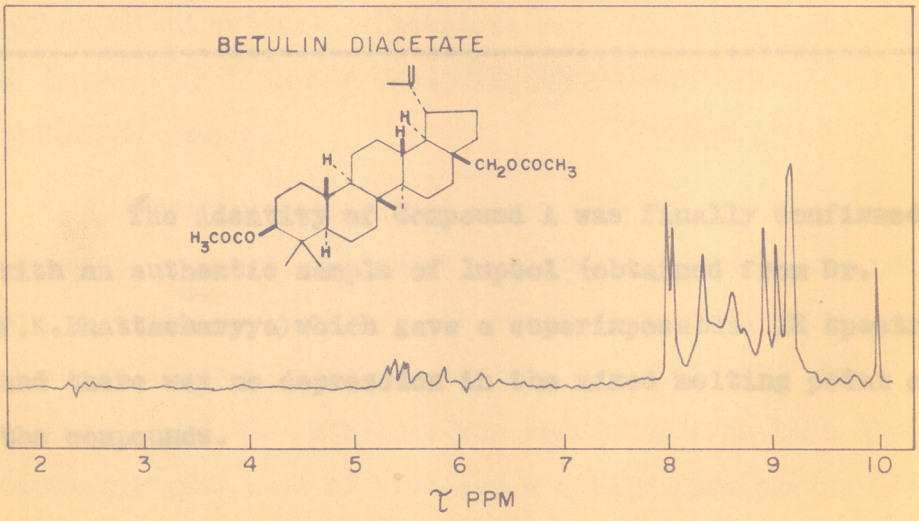

I

The nuclear magnetic resonance spectrum of Compound A - acetate in carbon tetrachloride was taken in a Varian A-60 Spectrophotometer using tetramethyl silane as the internal standard and the presence of 52 protons in the molecule (acetate) was confirmed* (Table I).

* Chemical shift values cited are on the $\tau$ scale. (Fig 31)


LUPEOL ACETATE
H
H
H
H3COCO
H
2
3
4
5
6
7
8
9
10
τ PPM

FIG·3·1


BETULIN DIACETATE
H
H
H
CH2OCOCH3
H3COCO
H
2
3
4
5
6
7
8
9
10
τ PPM

FIG·3·2

## TABLE I

| Chemical shifts (τ) | Peak multiplicity | No. of hydrogens | Possible assignments |
|---|---|---|---|
| 5.33 | Doublet | 2 | = $CH_2$ |
| 5.45 | Doublet | | |
| 5.55 | Unresolved | 1 | (-$\overset{|}{C}$H-OCO) |
| 8.03 | Singlet | 3 | -OCO$CH_3$ |
| 8.33 | Singlet | 3 | >$CH_3$ |
| 8.40-8.70 | unresolved multiplet | | methylene protons |
| 8.98 | singlet | 3 | tertiary methyl groups (6) |
| 9.06 | singlet | 3 | |
| 9.16 | unresolved quartet | 12 | |

The identity of Compound A was finally confirmed with an authentic sample of lupeol (obtained from Dr. P.K.Bhattacharyya) which gave a superimposable IR spectra and there was no depression in the mixed melting point of the compounds.

Examination of ether extract:
Isolation of Compound B (Betulin)

The combined ether-extract was a light red coloured solution, which on removal of the solvent produced a semi-solid gummy residue. This was chromatographed over alumina and from the benzene eluate, a light yellow solid m.p. 189-92° was obtained, which responded to all the colour reactions of triterpene, but produced two distinct spots (Rf: 0.66, 0.22) in thin-layer chromatography. Evidently this was a mixture of at least two compounds and hence the product was carefully rechromatographed over alumina, and the different eluates examined. From the pet.ether-benzene (95:5) fraction, a solid was obtained which gave a single spot in thin layer chromatography. It crystallised from ether-pet.ether as colourless needles, m.p. 210-211°, identified as lupeol (I) from its analysis, mixed m.p. and IR with an authentic sample.

From the benzene-pet.ether (80:20) and benzene fractions a second compounds was obtained, which was purified by rechromatography. It crystallised from ether-pet. ether as stout colourless needles, m.p. 252-53°. The uniformity of the substance was confirmed from thin layer chromatography when it produced a single spot having Rf:0.22. This was designated as Compound B. Like lupeol(Compound A), Compound B also responded to colour reactions of triterpenes.

With tetranitromethane, it gave a yellow colour indicating the presence of unsaturation in the molecule.

The molecular formula of Compound B was found from the elementary analysis as $C_{30}H_{50}O_2$, $(\alpha)_D^{20}$ + 18.6°. It contained two active hydrogens. Like Compound A, Compound B also did not show any specific absorption in the UV region (220-400 mμ). Infrared spectrum in nujol mull showed strong peaks at 3460, 1027 $cm^{-1}$ (hydroxyl), 1640 and 880 $cm^{-1}$ (vinylidine group).[4,5]

With acetic anhydride and pyridine, Compound B produced a diacetate, $C_{34}H_{54}O_4$, m.p. 218-19°, $(\alpha)_D^{20}$ + 24°. It showed strong absorption at 1736, 1240 $cm^{-1}$ (acetate), 1642 $cm^{-1}$ and 880 $cm^{-1}$ (vinylidine) in the IR spectrum taken in nujol mull. It easily formed a dibenzoate, $C_{44}H_{58}O_4$, m.p. 180-81°, $(\alpha)_D^{20}$ + 42°.

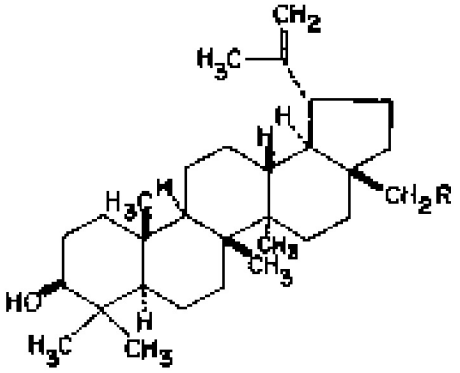
As in the previous case, the NMR spectrum of Compound B - acetate was taken in carbon tetrachloride solution which confirmed the total proton count in the molecule corresponding to 54, thus establishing the parent alcohol having 50 protons (Table II)(Fig 3-2)

It will be interesting to observe that the pattern of signals in the NMR of two spectra (Compound A and B - acetates) were very similar and a closer study would show that the Compound B - acetate had one less tertiary methyl group than Compound A-acetate. It had also two acetyl groups instead of one as in the case of Compound A-acetate,

TABLE II

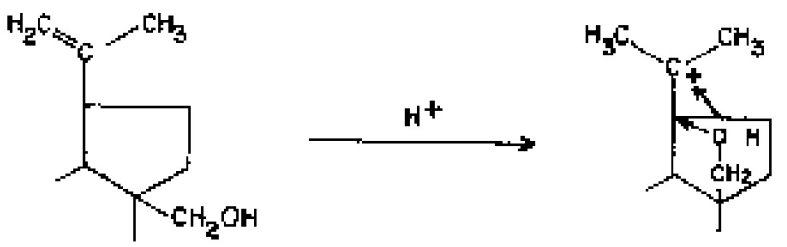
| Chemical shifts (τ) | Peak multiplicity | No. of hydrogens | Possible assignments |
|---|---|---|---|
| 5.33 | doublet | 2 | $=CH_2$ |
| 5.46 | doublet | | |
| 5.62 | unresolved | 1 | $-\underset{\vert}{C}H-OCO$ |
| 6.00 | quadruplet | 2 | $-CH_2-O$ |
| 8.00 | singlet | 3 | $-OCOCH_3$ |
| 8.03 | singlet | 3 | $-OCOCH_3$ |
| 8.32 | singlet | 3 | $>CH_3$ |
| 8.4-8.8 | unresolved multiplet | | methylene protons |
| 8.92 | singlet | 3 | tertiary methyls (6) |
| 9.05 | singlet | 3 | |
| 9.16 | unresolved | 9 | |

which are discernible in the NMR spectra. Further it had two protons on a carbon atom bound to oxygen at 6, which is absent in the case of Compound A-acetate. This observed difference would correspond to the replacement of a tertiary methyl group by a $-CH_2OH$ group in going from Compound A to Compound B. This indicated that the Compound B might be betulin (II) which differs from lupeol (I) in only having a $-CH_2OH$ at position 17 instead of a methyl group.



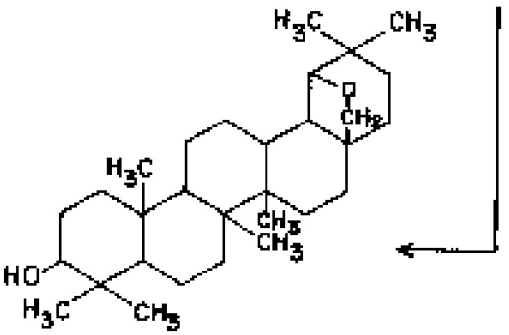
I R = H
II R = OH

The action of formic acid on Compound B was next studied. It is well-known that allo-betulin, an isomer of betulin is formed[6,7] when betulin is treated with formic acid, the mechanism of isomerisation being suggested by Davy et al.[8] as follows:



II

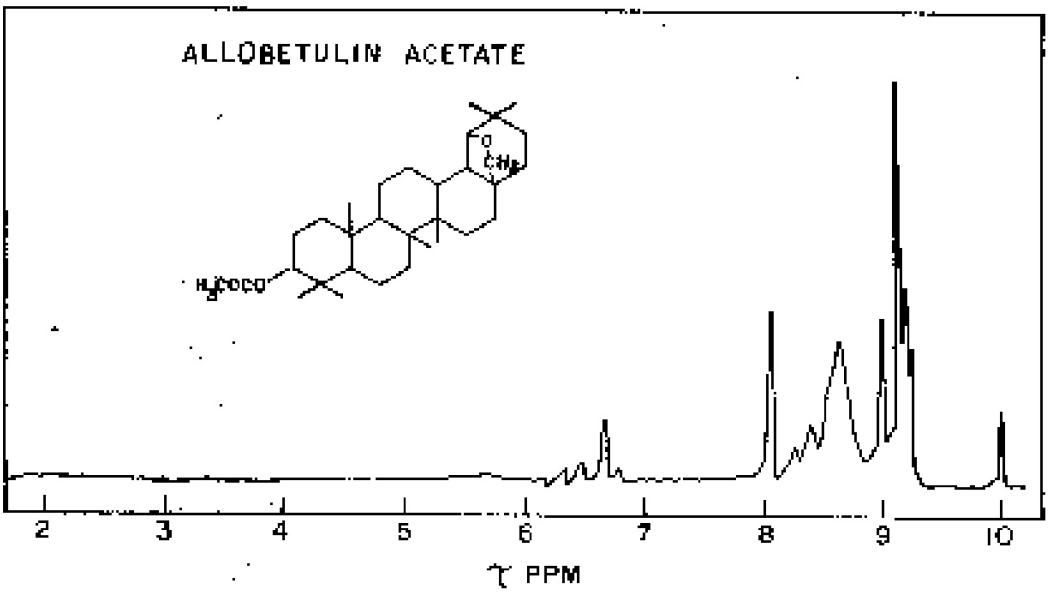
III



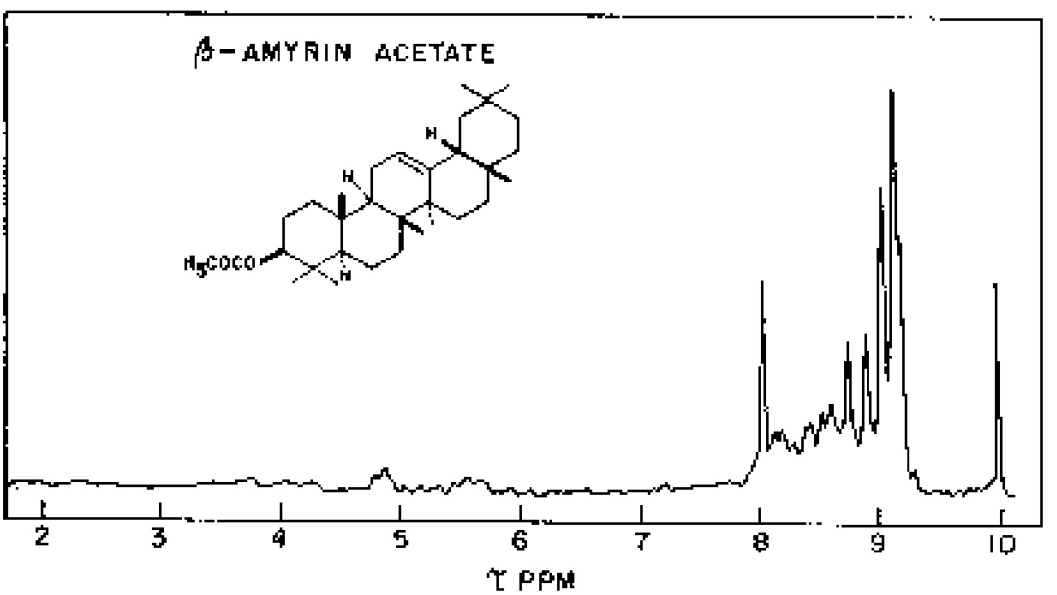
IV

In the present case the Compound B, on treatment with 93% formic acid for 2 hours, produced a formate, crystallising from benzene as colourless stout needles, m.p. 309-310°, analysing for $C_{31}H_{50}O_3$, $(\alpha)_D^{22}$ + 50°. The IR spectrum in nujol showed strong peaks at 1714 $cm^{-1}$ (formate), but no bands near 1640 $cm^{-1}$ and 880 $cm^{-1}$ indicating the absence of vinylidine group in the molecule. The formate on hydrolysis, produced an isomer of Compound B, crystallising from alcohol as stout needles, m.p. 260-61°, and analysing for $C_{30}H_{50}O_2$; $(\alpha)_D^{22}$ + 47°, which showed strong peaks in the IR at 3400, 1040 $cm^{-1}$ (hydroxyl), but no absorption near 1640 and 880 $cm^{-1}$. The acetate of this isomer had m.p. 278-79°, which showed in its spectrum strong bands at 1730 and 1260 $cm^{-1}$ (acetate) and had $(\alpha)_D^{30}$ + 60°.

The NMR spectra of this acetate (Table III) (Fig 3-3) & that of Compound B-acetate (Table II) showed many interesting characteristics. The former showed no signals for $=CH_2$ group in the 5.33-5.45 region as well as any signal at 8.32($>CH_3$). There were two acetate signals in the Compound B acetate which had been replaced by a single acetate signal in the spectrum of the isomerised product. Also the total number of tertiary methyl groups had increased from 6 to 7 in going from Compound B-acetate to the acetate of the isomerised product. The observed difference in the NMR spectra was consistent with the betulin-allobetulin transformation.


ALLOBETULIN ACETATE
2
3
4
5
6
7
8
9
10
τ PPM

FIG·3·3


β-AMYRIN ACETATE
2
3
4
5
6
7
8
9
10
τ PPM

FIG·3·4

TABLE III

| Chemical shifts (τ) | Peak multiplicity | No. of hydrogens | Possible assignments |
|---|---|---|---|
| 5.6 | broad | 1 | $-\overset{|}{C}H-OCO$ |
| 6.55 | quadruplet | 2 | $-CH_2-O$ |
| 6.68 | singlet | 1 | $-\overset{|}{C}H-O-\overset{|}{\underset{|}{C}}O-$ |
| 8.06 | singlet | 3 | $-OCOCH_3$ |
| 8.3-8.83 | unresolved multiplet | | methylene protons |
| 9.0 | singlet | 3 | tertiary methyls (7) |
| 9.13 | singlet | 3 | |
| 9.17 | singlet | 3 | |
| 9.2 | singlet | 3 | |
| 9.23 | singlet | 3 | |

Authentic samples of betulin were obtained through the courtesy of Prof. Djerassi and also from Prof. (Mrs) A. Chatterjee.

The Compound B showed superimposable IR spectrum with that of authentic betulin and there was no depression in the mixed melting point.

Examination of alcoholic extract:
Isolation of Compound C (β-amyrin) and
characterisation of the sugar moiety

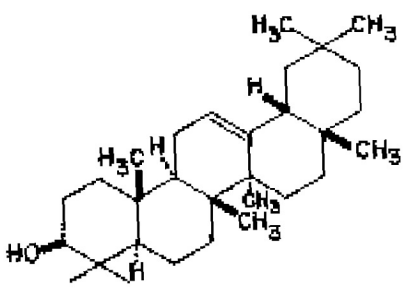
The alcoholic extract was obtained as a dark brown solution from which the solvent was removed under vacuum. During this process, considerable amount of frothing was observed and finally a bfownish resinous substance was obtained. The substance on shaking with water produced stable frothing having characteristic honeycomb structure, suggesting the presence of considerable amount of saponin. Inspite of various attempts, the saponin could not be obtained in a crystalline form, though a nearly colourless product could be prepared by charcoaling in alcohol and repeated precipitation with ether. The ethanolic solution of the saponin was, therefore, hydrolysed with 2% sulphuric acid when the aglycone was obtained as dark brown precipitate. It was filtered, washed free of acid, and dried. The filtrate was kept separately for the examination of the sugars. The dark coloured aglycone was extracted in a soxhlet with chloroform and the chloroform extract was chromatographed over alumina in the usual way. From the benzene eluate a colourless substance was obtained which was further purified by rechromatography and finally by crystallisation. It crystallised from ether-methanol mixture as colourless needles, m.p. 194-196° and was designated as Compound C.

Like Compounds A and B, the Compound C also responded to characteristic colour reactions of triterpenes.

From elementary analysis, the molecular formula was found to be $C_{30}H_{50}O$, $(\alpha)_D^{20}$ + 83°. It was optically active and showed no absorption in the UV (220-400 mμ) spectrum. The infrared spectrum of Compound C showed strong peaks at 3620, 1029 $cm^{-1}$ (hydroxyl) but no peaks near 1640 and 880 $cm^{-1}$ indicating the absence of any vinylidine group in the molecule. However, the presence of unsaturation in the molecule was indicated from the tetranitromethane reaction, in which a yellow colour was produced.

By acetylation with acetic anhydride and pyridine, a mono acetate was formed, crystallising from ether-pet. ether as shining colourless needles, $C_{32}H_{52}O_2$, m.p.237-38°, $(\alpha)_D^{20}$ + 82°. In the IR spectrum, it showed strong characteristic bands at 1730 $cm^{-1}$ , 1247 $cm^{-1}$ for acetate grouping. Compound C also produced a monobenzoate, $C_{37}H_{54}O_2$, m.p. 228-29°, $(\alpha)_D^{20}$ + 80°.

For the various physical and chemical properties, the substance was believed to be identical with β-amyrin(V).




V

The NMR spectrum of Compound C-acetate in carbon tetrachloride solution (Table IV) was also in good agreement with β-amyrin acetate (Fig 3-4)

TABLE IV

| Chemical shifts (τ) | Peak multiplicity | No. of hydrogens | Possible assignments |
|---|---|---|---|
| 4.85 | triplet | 1 | -CH= |
| 5.5 | triplet | 1 | -CH-OCO |
| 8.04 | singlet | 3 | $-COCH_3$ |
| 8.1-8.67 | multiplet | | methylene protons |
| 8.75 | singlet | 3 | tertiary methyls (8) |
| 8.89 | singlet | 3 | |
| 9.04 | singlet | 6 | |
| 9.12 | broad singlet | 12 | |

An authentic sample of β-amyrin was available through the courtesy of Dr. S.C. Bhattacharyya, which showed superimposable IR spectra with Compound C and there was no depression in the mixed melting point.

Characterisation of the sugar moiety

The acidic filtrate from the saponin hydrolysis was deep brownish red in colour. An aliquot part of this solution was neutralised with $BaCO_3$ and filtered. The

brown-red filtrate was charcoaled when a colourless solution was obtained which was concentrated by freeze drying. This gave positive Molisch's test for sugar and reduced Fehling's solution. The pure sugar fraction could also be separated from the saponin hydrolysate by treatment with a chloroform solution of methyl di-n-octyl amine,[9] the inorganic impurities forming acid salt with the amine which remained in the chloroform layer whereas the aqueous solution contained the sugar moiety. In the present study, the qualitative characterisation of the sugars was carried by circular paper chromatography according to the method of Giri et al.[10,11] and also by descending paper chromatography.[12] In the former case, the system n-butanol-acetone-water (30:70:10) was made use of, while aniline-diphenylamine-phosphoric acid was used as the spraying agent. In the case of descending chromatography, the solvent system used was n-butanol-water-ethanol (5:1:4), while aniline hydrogen phthalate or silver nitrate was used for identification of the sugar spots. In both the cases, two sugars were detected which corresponded to glucose and arabinose, the former predominating and were confirmed by parallel spotting of the authentic sugar solutions.

## EXPERIMENTAL

Neutral alumina of different grades standardised according to Brockmann's method[13,14] was used in chromatography. In thin-layer chromatography, methylene chloride was used as the solvent system and the development was done with chlorosulphonic acid. Other physical measurements were taken essentially as described in Chapter I.

### Isolation of Compound A (Lupeol)

The dried, powdered bark (1.5 kg) of *Guagnina dalbergioides* Benth was extracted with pet.ether (b.p. 40-60°) at room temperature. Extraction was repeated until the pet.ether solution became colourless. The solvent was distilled off under vacuum. The resulting gummy solid (yield 7.5 g., 0.5%) was taken in pet.ether (b.p. 40-60°) and passed through a column of alumina (Grade II, 300 g). The column was first washed with 400 ml of pet.ether (b.p. 40-60°) when a negligible amount of a yellow oily eluate was obtained. Elution was continued with a mixture of pet.ether-benzene (1:1, 200 ml) and finally with pure benzene (500 ml). The benzene fraction on removal of the solvent gave a colourless solid, m.p. 184-90° (yield 2.5 g). It was further purified by rechromatography over a second column of alumina (Grade II, 46 g) in benzene solution and finally

crystallised from a mixture of ether-methanol, when it was obtained as colourless needles, m.p. 210-11°; $(\alpha)_D$ + 26° (c, 2.01); [ lit.[15] m.p. 212-213°, $(\alpha)_D$ + 29° (TLC Rf =0.66)] It gave a violet colour with Liebermann-Burchard reagent and a yellow colour with tetranitromethane, and responded to colour reactions of triterpene.

Infrared spectrum: 3600, 3420, 1640, 1196, 1110, 1070, 1047, 1025, 987, 977, 923 and 887 $cm^{-1}$.

Analysis: Found: C, 83.8; H, 11.7.
$C_{30}H_{50}O$ requires C, 84.4; H, 11.8%.

Acetylation of Compound 'A' (Lupeol acetate)

Compound A (300 mg) was warmed with acetic anhydride (10 ml) and pyridine (1 ml) on a water bath for 2 hrs. After 24 hrs. the reaction mixture was poured into ice-water and the shining solid which separated was filtered, washed with water, dried (370 mg) and crystallised from a mixture of ether-pet.ether (b.p. 40-60°), when glistening colourless needles were obtained, m.p. 214-15°; $(\alpha)_D^{30}$ + 46° (c, 2.2) [lit.[15] m.p. 211-13°; $(\alpha)_D$ + 48°] (TLC Rf =0.88).

Infrared spectrum: (in chloroform), 2950, 1725, 1640, 1253, 1200, 1150, 1104, 1013, 979, and 886 $cm^{-1}$.

Analysis: Found; C, 81.8; H,11.3;$COCH_3$ 9.3,MW 445.
$C_{32}H_{52}O_2$ requires: C, 81.9; H,11.1;$COCH_3$ 9.1%,MW 468.

Benzoylation of Compound A (Lupeol benzoate)

A mixture of Compound A (90 mg), dry pyridine(.3 ml) and benzoyl chloride (1 ml) was heated on a water bath for

one hour. The solution was cooled and poured into crushed ice. The white oily solid was taken in ether and the ether solution washed well with aqueous sodium carbonate (5%;50 ml) and then again with water. The ether layer was dried over anhydrous sodium sulphate, filtered and ether removed. The residual solid (93 mg) was crystallised from ethanol, when colourless needles were obtained, m.p. 363-64°, $(\alpha)_D^{20}$ + 62°, (c, 1.8) [lit.[15] m.p. 267-68°; $(\alpha)_D$ + 68°] .

Analysis: Found; C, 83.4; H,10.1.
$C_{37}H_{54}O_2$ requires: C, 83.7; H,10.2%.

Isolation of Compound β (Betulin)

After extraction with pet.ether the bark of O.dalbergioides was exhaustively extracted with ether at room temperature. The brown red ether extract, on removal of the solvent afforded a semi-solid gummy residue; yield 8.75 g. (0.6%). The substance (4.5 g) was taken in benzene and chromatographed over alumina (Grade II, 150 g).

From the benzene eluate, a light yellow solid, m.p. 189-92° (yield 3.6 g) was obtained which showed two spots on thin layer chromatography. It was taken in a mixture of benzene and pet.ether (5 ml) and passed over a column of alumina (100 g) in pet.ether (40-60°). Elution with pet. ether was followed by pet.ether-benzene mixture (95:5, 90:10, 80:20, 50:50, 20:80), 40 x 10 ml of each fraction was collected and examined separately. Finally the column was eluted with pure benzene (500 ml).

The pet. ether-benzene (95:5) fraction afforded a colourless solid (.8 g) which gave a single spot(Rf:0.66). It was crystallised from ether-pet.ether as colourless needles, m.p. 210-11°; $(\alpha)_D^{20}$ + 26° (c, 1.92).

Analysis: Found: C, 84.6; H, 11.7.

$C_{30}H_{50}O$ requires C, 84.4; H, 11.8%.

It was identified as lupeol isolated from the pet. ether extract.

From the pet.ether-benzene (20:80) and benzene fractions, a colourless solid (2.6 g) was obtained on removal of the solvent. It gave a single spot on thin layer chromatography (Rf0.23). It crystallised from a mixture of ether-pet.ether as stout colourless needles, m.p. 252-53°, $(\alpha)_D^{27}$ + 18.6°, [lit.[16] m.p. 250-51°, $(\alpha)_D$ + 17.8°; (c, 2.01)]. It gave a violet colour with Liebermann Burchard reagent and a yellow colour with tetranitromethane.

Infrared spectrum: 3460, 3380, 3200, 1640, 1336, 1396, 1340, 1188, 1168, 1140, 1106, 1080, 1037, 1010, 985, 973, 945, 933, 915, 890 $cm^{-1}$.

Analysis: Found: C, 81.2; H, 11.3; MW 449.

$C_{30}H_{50}O_2$ requires: C, 81.3; H, 11.3%; MW 442.

Acetylation of Compound B (Betulin diacetate)

A mixture of Compound B (200 mg), acetic anhydride (8 ml) and pyridine (1 ml) was heated on a water-bath for 3 hrs. The reaction mixture was kept overnight and the shining needles which separated were filtered, washed with

water, dried (305 mg), crystallised from a mixture of ether-methanol, when it was obtained as colourless needles, m.p. 218-19°, $(\alpha)_D^{20}$ + 84° (c, 3.0) [lit.[16] m.p. 217-18°, $(\alpha)_D$ + 86°].

Infrared spectrum: 1735, 1642, 1310, 1240, 1150, 1109, 1080, 1030, 1018, 983, 947, 918, 903, and 880 $cm^{-1}$.

Analysis: Found: C, 77.8; H, 10.5; $-COCH_3$ 16.05%.

$C_{34}H_{54}O_4$ requires: C, 77.8; H, 10.3; $-COCH_3$ 16.36%.

Benzoylation of Compound B (Betulin dibenzoate)

A mixture of Compound B (96 g), pyridine (8 ml) and benzoyl chloride (2 ml) heated on a water bath for 2 hrs. After 24 hrs., the reaction mixture was poured into crushed ice. The separated oily solid was successively washed with sodium carbonate solution (5%) and water, dried (100 mg) and crystallised from ethanol as colourless needles, m.p. 180-81°; $(\alpha)_D^{20}$ + 42° (c, 2.01) [lit.[7] m.p. 181°, $(\alpha)_D$ + 43.28°].

Analysis: Found: C, 81.0; H, 8.9.

$C_{44}H_{58}O_4$ requires: C, 81.1; H, 8.9%.

Action of formic acid on Compound B (Allobetulin formate)

Compound B (400 mg) was refluxed with formic acid (99%, 15 ml) for 90 minutes on an oil bath at 150°. The reaction mixture was cooled and poured into crushed ice. The white solid (395 mg) was filtered and crystallised from benzene, when stout needles were obtained, m.p. 309-310°;

$(\alpha)_D^{23}$ + 80° (c, 2.02), [lit.[6] m.p.311-12°, $(\alpha)_D^{16}$ + 81.8°] .

Infrared spectrum: 1780, 1714, 1388, 1219, 1180, 1149, 1120, 1040, 1028, 1013, 983, 955, 949, 931, 912, and 897 $cm^{-1}$.

Analysis: Found: C, 78.8; H, 10.8

Calc.for $C_{31}H_{50}O_3$. C, 79.1; H, 10.7%.

Preparation of allobetulin from allo-betulin formate

The formate (300 mg) obtained in the previous experiments was taken in alcoholic potassium hydroxide (8%, 18 ml) solution and the reaction mixture was heated on a water bath. Benzene was slowly added until a clear solution was obtained. After 30 minutes, the benzene and alcohol were distilled off until a colourless solid started separating out. The flask was cooled and the product collected at the filter (188 mg). Recrystallisation from alcohol afforded stout colourless needles, m.p. 260-61°, $(\alpha)_D^{22}$ + 47° (c, 2.03) [lit.[6] m.p. 260-61°; $(\alpha)_D$ + 48.25° (TLC Rf: 0.28)].

Infrared spectrum: 3400, 1300, 1382, 1205, 1140, 1079, 1040, 1010, 998, 978, 949, 932, 910, and 773 $cm^{-1}$.

Analysis: Found: C, 81.3; H, 11.2.

$C_{30}H_{50}O_2$ requires: C, 81.3; H, 11.3%.

Acetylation of the isomerised product of Compound B (Allo-betulin acetate)

The above mentioned alcohol (100 mg) was heated with acetic anhydride (5 ml) and pyridine (1 ml) on a

water bath for 2 hrs. The reaction mixture was cooled and poured into crushed ice. The white solid was collected and dried (105 mg). It crystallised from ether as fine needles, m.p. 275-79°; $(\alpha)_D^{20}$ + 60° (c, 2.12), [lit.[6] m.p. 277-78°; $(\alpha)_D$ + 64.16°].

Infrared spectrum: 1730, 1250, 1203, 1142, 1038, 1028, 979, 935, 905, and 895 $cm^{-1}$.

Analysis: Found: C, 79.1; H, 10.7 $-COCH_3$ 8.2.
$C_{32}H_{50}O_3$ requires: C, 79.2; H, 10.8 $-COCH_3$ 8.8%.

Isolation of Compound C (β-amyrin)

After the extraction with ether, the bark was finally extracted with rectified spirit. The dark brown solution was concentrated 'in vacuo' (40 mm) when a residous substance (12.5 g) was obtained. It produced frothing on shaking with water, but the saponin could not be isolated in pure form. The substance (10 g) was taken in ethanol (300 ml) and 2N $H_2SO_4$ (200 ml) was added to it. After heating on a water bath for 8 hrs., the dark brown solid which separated was filtered, washed free of water, and dried (8 g). The filtrate was tested separately for the sugars.

The dark brown solid was successively extracted (soxhlet) with n-hexane, ether, benzene, and chloroform. Extraction with the first three solvents afforded very little solid. The dark red chloroform solution was

concentrated and passed through a column of alumina (Grade II, 150 g). It was eluted with benzene, when a colourless substance (1.5 g) was obtained. This was further purified by rechromatography which finally crystallised from a mixture of ether-methanol as colourless needles, m.p. 194-96°; $(\alpha)_D^{20}$ + 83° (c, 3.1) [lit.[16] m.p. 196-98°, $(\alpha)_D$ + 86°] (TLC $R_f$:0.68).

Infrared spectrum: 3630, 1300, 1125, 1140, 1096, 1029, 987, 882, 826, 815, and 798 $cm^{-1}$.

Analysis: Found: C, 83.9; H, 11.7.

$C_{30}H_{50}O$ requires: C, 84.4; H, 11.8%.

Acetylation of Compound C

Compound C (200 mg) was heated with acetic anhydride (15 ml) and pyridine (1 ml) on a water bath for 3 hrs. The reaction mixture was cooled and poured into ice water. The colourless solid was collected, washed with water and dried (195 mg). It crystallised from a mixture of ether-pet. ether as shining colourless needles, m.p.237-38°; $(\alpha)_D^{20}$ + 82° (c, 2.6), [ lit.[15] m.p. 238-39°; $(\alpha)_D$ + 81°].

Analysis: Found: C, 81.7; H,11.2; $COCH_3$, 8.9.

$C_{32}H_{52}O_2$ requires: C, 81.9; H,11.1; $COCH_3$ 9.1%.

Benzoylation of Compound C

Compound C (100 mg) was benzoylated by heating with benzoyl chloride (2 ml) and pyridine (0.5 ml) on a water-bath for 1 hour. The product (102 mg)was crystallised

from ether-pet.ether mixture (1:1) as glistening needles, m.p. 232-29°, $(\alpha)_D^{21}$ + 90° (c, 2.3) [ lit.[15] m.p.232-35°, $(\alpha)_D$ + 96° ].

Analysis: Found: C, 83.6; H, 10.4.

$C_{37}H_{64}O_2$ requires: C, 83.7; H, 10.3%.

Separation and identification of the sugars

A part of the filtrate from the acid hydrolysis product of the ethanol extract was carefully neutralised with barium carbonate and filtered. The brown red filtrate was charcoaled till colourless. The aqueous filtrate was concentrated by freeze drying.

An improved technique was used in subsequent experiments whereby the inorganic impurities were removed by treating the filtrate from the hydrolysis product with a chloroform solution of methyl di-n-octylamine, obtained through the courtesy of Dr. J.L.Bose. The neutral aqueous layer was decolourised by charcoal and concentrated by freeze drying.

Two methods, namely circular paper chromatography and descending paper chromatography were used for the detection and identification of the sugars.

Circular paper chromatography

The method of Giri et al. (loc.cit) was used with a little modification in the apparatus. In a pneumatic trough (30 cm diameter) covered with a glass plate, a petri dish (15 cm diameter) was kept as the container

for the developing solvent and as the support for the paper (18 cm diameter). The inside of the chamber was kept saturated with the solvent vapour, by keeping a layer of the solvent in a small beaker.

Circular filter paper (Whatman No.1) 18 cm. in diameter was used. The solution for chromatography was prepared by dissolving 10-12 mg. of the sugar mixture (isolated as above) in 1 ml. of water. Standard solution of known sugars, glucose, xylose, arabinose and galactose, were prepared by dissolving 10 mg of each sugar in 1 ml water. The solvent mixture used was n-butanol-acetone-water (20:10:80).

The solvent was allowed to run a distance of about 12 cm from the centre through a wick during 1 hr. The paper was removed and dried at room temperature. For better resolution, the irrigation was repeated once more. After the paper was dried in air, the positions of sugars were rendered visible by applying aniline-diphenylamine-phosphoric acid reagent and heating at 100-105° for a few minutes.

Glucose (Rf 0.53) and arabinose (Rf 0.63) were detected by this method. This was confirmed by running the chromatography along with authentic samples of the two sugars.

Descending paper chromatography

This was carried on a Whatman filter paper No.1, 56.5 cm. long and 18 cm. wide. The apparatus used was same as that described in Hough and Jones (loc. cit). The solvent system, n-butanol-water-ethanol (5:1:4) was used. The spraying reagent used as (i) aniline hydrogen phthalate or (ii) silver nitrate reagent.

The presence of glucose and arabinose in the hydrolysis product were confirmed by parallel spotting of authentic glucose and arabinose solutions.

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