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EXPERIMENTS ON SOME NATURALLY OCCURRING ANTHRAQUINONE CARBOXYLIC ACIDS

A

THESIS

SUBMITTED BY

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CONTENTS

PART I

NATURALLY OCCURRING ANTHRAQUINONE CARBOXYLIC ACIDS

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Among the many naturally occurring colouring matters derived from vegetable and animal origin, hydroxy-anthraquinone carboxylic acids form an important class of pigments. These acids have been isolated from plants, lichen, fungi and insects. The natural durability of teak to wood-destroying fungi and termites may be partly attributed to the presence of anthraquinone-2-carboxylic acid. The three important insect pigments (kermesic acid, laccaic acid and carminic acid) were at one time extensively used for dyeing wool and silk. Carminic acid dyes wool and silk a magnificient scarlet on a tin mordant[1] and was especially favoured for ceremonial uniforms. Minor uses included the colouring of food, cosmetics, water colours and biological tissues. Kermesic acid was extensively used for dyeing of shades of red on an aluminium mordant. Laccaic acid, the colouring matter of stick lac was used for dyeing wool a fine scarlet on tin mordant, but with the advent of synthetic dyes, its importance rapidly declined. These acids provide a convenient illustration of the manner in which colour is dependent not only on the number of auxochromes (O) present, but also on their positions in the molecule.

A considerable amount of research has been carried out during half a century on the elucidation of the structures of these pigments, which provided a direct impetus for synthesis in the laboratory and subsequent introduction in the market of a large number of dyes which could compete with and excel the natural substances in colour, dyeing properties and fastness. A well known example is furnished by synthetic alizarin, which was made available in the market within years of the isolation of alizarin from madder root, with the result that madder cultivation declined in favour of synthetic alizarin, for the latter was one fourth in cost and of much more uniform and dependable quality.

Cathartic action is one of the important physiological properties of rhein. Rhein was identified as the active principle of the extract of the leaves of *Cassia reticulata*[2], reported to be active (*in vitro*) against *Bacillus mycoides*, *Bacillus subtilis*, *Staphylococcus aureus*, etc. An antibacterial substance isolated from the seeds of *Kniphofia uvaria* was found to be rhein.[4] Very recently Chinese workers have shown that rhein inhibited melanoma growth by 73% in mice.[5]

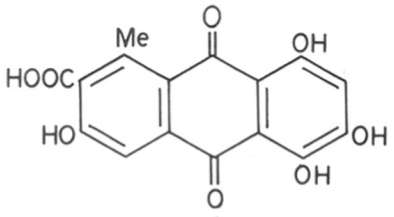
Although some attempts have been made to establish a relationship between the anthraquinone content as measured by the Borntrager[6,12] test and the cathartic activity, no satisfactory correlation has been found to exist. The activity depends to a great extent upon the oxidation stage. Fairbairn[6] has shown that anthracene derivatives are most effective as anthrone glycosides, less as free anthrones, and still less as free anthraquinones. The free hydroxyanthraquinones are susceptible to metabolic destruction when orally administered. The glycosides on the other hand are unaffected because of the protective action of the sugar residue.

OCCURRENCE

So far eighteen anthraquinone carboxylic acids have been isolated from natural sources. They occur either in the free state or as glycosides. According to T. Robinson[7] "the problem of the form in which these anthraquinones actually exist in plants remains a knotty one, and there are apparently several possibilities. Since the native precursors generally break down readily under the influence of enzymes or extraction

procedures, reports of the appearance of free anthraquinones must be regarded cautiously".

Out of the 18 acids, four have been isolated from plants, five from fungi and lichens, and the remaining nine from insects. Rhein has been found to occur as a glycoside with a sugar residue linked through one of the phenolic hydroxyl groups. The fungal and lichen acids are closely related in their chemical constitution. The fungal and lichen acids (except boletol) contain two α-hydroxyl groups in the 1,8-positions, a C-methyl and a carboxyl group, both in β-positions.[7] The acids [(kermesic (1), carminic (2), cero-albolinic (3)] from three genera of the insect family Coccidae, show very close resemblance in their structures. The pattern of substitution in the A ring of the anthraquinone nucleus is the same for the three acids; and carminic acid is merely the 2-C-glucoside of kermesic acid.



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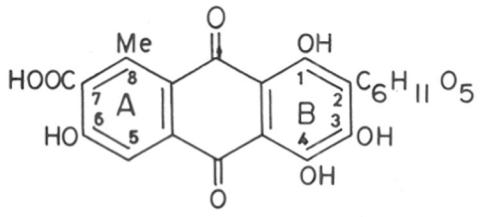

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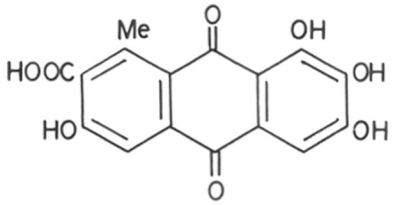


(3)

In contrast to the fungal and lichen acids (endocrocin, clavorubin), the insect acids have a C-methyl group in the α-position. Four laccaic acids show the same pattern of substitution.

ISOLATION

Chromatography is a valuable addition to the older methods for the isolation of plant constituents, such as extraction with solvents of increasing polarity, fractionation from different solvents and crystallisation. The water-soluble acids, carminic and laccaic acids, can be extracted from cochineal and stick lac with water. The homogeneity of the isolated acids can be followed by chromatography on silica gel impregnated with oxalic acid. The isolation of closely related compounds can only be effected by carefully chosen chromatographic techniques. Pandhare[10] and Shaikh[9] have successfully separated five different laccaic acids by making use of different adsorbents and solvent systems. Thus from the crude mixture of laccaic acids, laccaic acids A and B were separated by column chromatography on polycaprolactam powder of a solution of lac dye in n-butanol saturated with 0.3N

hydrochloric acid solution. Laccaic acid D[11] was isolated by chromatography of an acetone solution of lac dye on a column of silica gel. Chromatography on a cellulose column and butanol-acetic acid-water as solvent separated laccaic acids C and E.[9]

COLOUR REACTIONS

These acids being derivatives of hydroxyanthraquinones, give distinct colour reactions with alkali and concentrated sulphuric acid, which can be used to get an idea of the arrangement of substituents in the anthraquinone nucleus. The Borntrager test[6,12] is a useful test for detecting the hydroxy anthraquinones in plant extracts. In this test the crude extraction is treated with dilute mineral acid to hydrolyse the glycosides and the liberated aglycones are extracted with organic solvents such as benzene. The benzene extract is shaken with aqueous alkali, when a beautiful rose pink to cherry red colour is produced in the aqueous layer. Ferric colouration[8] is not characteristic of hydroxyanthraquinone carboxylic acids. According to Shibata[8] hydroxyanthraquinones having at least one α-hydroxyl groups give a characteristic colouration with magnesium acetate in

alcoholic solution. The acids having two adjacent hydroxyl groups (like alizarin) give a bluish violet colouration in acetone solution with zirconium nitrate.[13] Anthraquinones having hydroxyls in 1:4 positions show a fluoroscence in acetic acid.[8] A solution of ceroalbolinic acid[35] (anthragallol type) in aqueous sodium hydroxide rapidly changes colour from green to yellow on exposure to air. These derivatives also give a flocculent greenish precipitate with sodium amalgam in ethanolic solution (Bargellini test) which shows the presence of hydroxyl groups in 1,2,3-positions.[14] With the exception of purpurin derivatives all acids having hydroxyl groups in the para positions are oxidised by lead tetraacetate to coloured diquinonoid compounds. In the case of purpurin derivatives, which may be thus detected, the colour disappears rapidly as the hydroxylated ring undergoes degradation.[15]

CONSTITUTION

Zinc dust distillation of hydroxyanthraquinone carboxylic acids gives anthracene or the corresponding alkyl-anthracene. Hydroxyl groups in the α-position do not undergo methylation easily with ethereal diazomethane

solution, but can be methylated with dimethyl sulphate and alkali, whereas methylation of β-hydroxyls can be easily carried out. Acids containing a purpurin moiety undergo, with sodium dithionite and sodium hydroxide, reduction to xanthopurpurin, an α-hydroxyl group being eliminated. By a careful study of these reactions, together with the colour reactions mentioned above and infrared and ultraviolet spectra, the position of the substituents in these anthraquinones can usually be determined. The presence of the purpurin moiety in the laccaic acids was proved by Bhide et al.[15], mainly by a comparative study of the vat spectra in comparison with those of a few hydroxyanthraquinones. Infrared spectroscopy helps in finding out the nature (or environment) of the quinone carbonyls, as also in determining the presence of carboxyl groups. NMR spectra provided the main evidence for the elucidation of the structures of the laccaic acids,[9-11] and for modifying the earlier structures of carminic acid[34] and kermesic acid.[17]

The naturally occurring anthraquinone carboxylic acids, which have been isolated so far, are listed in Table 1.

TABLE 1

No.	Name	Substitution in anthraquinone	Occurrence	References	
				Isolation	Synthesis
1.	Anthraquinone β-carboxylic acid	2-COOH	Tectona grandis	18	19
2.	Munjistin	1,3-$(OH)_2$-2-COOH	Rubia sp.	1,3	20
3.	Rhein	4,5-$(OH)_2$-2-COOH	Rheum officinale	1,3	21
4.	Pseudopurpurin	1,2,4-$(OH)_3$-3-COOH	Rubia tinctorum	1	1
5.	Boletol	1,2,4-$(OH)_3$-5- or 8-COOH	Boletus Sp.	23	23
6.	Emodic acid	4,5,7-$(OH)_3$-2-COOH	Penicillium cyclopium	1	22
7.	Endocrocin	1,6,8-$(OH)_3$-3-Me-2-COOH	Nephromopsis endocrocea; Aspergillus amstelodami	8	25
8.	Ptilometric acid	1,6,8-$(OH)_3$ 3-C_3H_7-2-COOH	Ptilometra australis	26	-
9.	Laccaic acid D	1,3,6-$(OH)_3$-8-Me-7-COOH	Coccus laccae	11	-
10.	Clavorubin	1,5,6,8-(OH)3 3-Me-2-COOH	Claviceps purpurea	27	27
11.	Rhodocladonic acid	1,3,6,8-$(OH)_4$-2-CH_2OH-7-COOMe	Cladonia Sp.	8	-

Table I contd.

No.	Name	Substitution in anthraquinone	Occurrence	References	
				Isolation.	Synthesis.
12	Kermesic acid	1,3,4,6-(OH)$_4$-8-Me-7-COOH	Coccus ilici	1	Part II
13.	Ceroalbolinic acid	1,2,3,6-(OH)$_4$-8-Me-7-COOH	Ceroplastes albolineatus	35	-
14.	Carminic acid	1,3,4,6-(OH)$_4$-2-$C_6H_{11}O_5$-8-Me-7-COOH	Coccus cacti	31	-
15.	Laccaic acid C	1,3,4,6-(OH)$_4$-2-(2'-OH-5'-β-amino-β-carboxyethyl) phenyl-7,8-(COOH)$_2$	Coccus laccae	9	-
16.	Laccaic acid E.	1,3,4,6-(OH)$_4$-2-(2'-OH-5'-β-aminoethyl) phenyl-7,8-(COOH)$_2$	""	9	-
17	Laccaic acid A	1,3,4,6-(OH)$_4$-2-(2'-OH-5'-β-N-acetylaminoethyl) phenyl-7,8-(COOH)$_2$	""	10	-
18	Laccaic acid B	1,3,4,6-(OH)$_4$-2-(2'-OH-5'-β-hydroxyethyl) phenyl-7,8-(COOH)$_2$	""	10	-

The simplest anthraquinone-2-carboxylic acid was isolated by Rudman[18] by the Craig countercurrent separation of the extractives of teak (Tectona grandis). This was synthesised by the oxidation of 2-methyl anthraquinone with potassium permanganate in conc. sulphuric acid.[19]

The first unambiguous and practicable synthesis of munjistin[20] was by the oxidation of lucidin with silver oxide in aqueous caustic soda.

Rhein, 1,8-dihydroxyanthraquinone-3-carboxylic acid, was assigned this structure since it could be obtained by the oxidation of chrysophanol and aloe-emodin. Starting from the common dye intermediate 1-amino-5-chloroanthraquinone, the synthesis of rhein has recently been reported.[21]

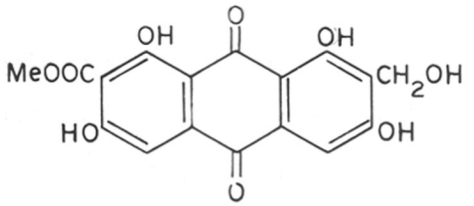
The structure of emodic acid was confirmed to be 1,6,8-trihydroxyanthraquinone-3-carboxylic acid by the oxidation of triacetyl-emodin and deacetylation.[1,22]

Pseudopurpurin gave purpurin on decarboxylation, and munjistin on vatting and reoxidation , and was therefore 1,2,4-trihydroxyanthraquinone-3-carboxylic acid.[1]

Boletol[23] was found to be 1,2,4-trihydroxy-anthraquinone-5 or 8-carboxylic acid, since it gave purpurin on decarboxylation, and hemimellitic acid on oxidation with alkaline hydrogen peroxide. Kogl and Deijs[23] attempted the synthesis by two routes: (1) Hemimellitic anhydride was condensed with 1,2,4-trimethoxy benzene, from the products boletol was isolated. (2) Condensation of hemimellitic anhydride with hydroquinone gave quinizarin-5-carboxylic acid. Oxidation of this with lead tetraacetate yielded the diquinone, which on Thiele acetylation and hydrolysis gave a mixture of 1,2,4-trihydroxyanthraquinone-5 and 8-carboxylic acids. The two were separated chromatographically, and one of them was identical with the natural pigment. Thus the structure of boletol is uncertain, so far as the position of the hydroxyl group is concerned.

The ruby-red rhodocladonic acid[8] was isolated by Zopf from the lichen Cladonia fimbriata; Koller and Hamburg showed that it was probably an anthraquinone containing four hydroxyl groups, a hydroxymethyl, and a carbomethoxy group. Shibata consequently observed that rhodocladonic acid was similar in its properties and

absorption spectrum to 1,3,6,8-tetrahydroxyanthraquinone, which he prepared from emodin by a series of reactions. In view of the usual orientation of methyl, carbinol and carboxyl groups in β-positions in naturally occurring anthraquinones the structure (4) was suggested for rhodocladonic acid.[3,8]



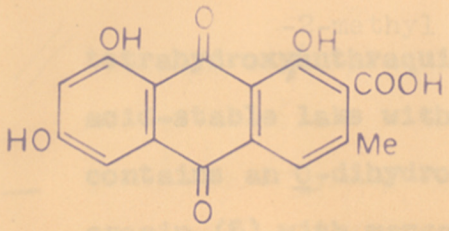
(4)

Endocrocin, the copper-red pigment of the Japanese lichen Nephromopsis endocrocea, was assigned the structure (5) by Asahina and Fuzikawa.[8] As Raistrick[24] has suggested, endocrocin may be synthesised on paper by the condensation of 6-methylsalicylic acid (a metabolite of Penicillium griseo-fulvum) and 3,5-dihydroxyphthalic acid (a metabolite of Penicillium brevicompactum). Endocrocin gave emodin on thermal decomposition, and the ultraviolet and visible spectra of both emodin and endocrocin overlapped each other. Oxidation of the fully methylated endocrocin yielded the methyl ether

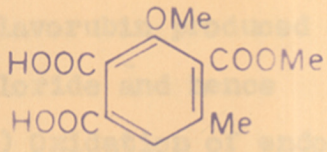
of the monomethyl ester[6] of isocochenillic acid. The fact that in the carbonyl region the IR spectrum of endocrocin has a band at 1718 cm^{-1}, which is too high a frequency for an aromatic carboxylic acid, led Ramanathan et al.[25] to consider (7) as an alternate structure. Ramanathan synthesised (7), but found that its tetramethyl ether-ester was different from the ether-ester of endocrocin. Ultimately the ether-ester of (5) was synthesised by two methods and found to be identical with endocrocin ether-ester.[25]

Powell and Sutherland[26] recently isolated ptilometric acid from a crinoid Ptilometra australis and assigned to it structure (8) a homologue of endocrocin. They compared the NMR spectra of the tetramethyl ether-esters of ptilometric acid and of (5), and observed that the agreement was satisfactory.

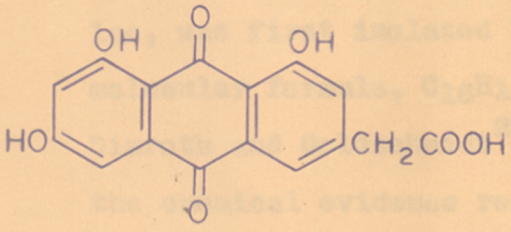
Clavorubin, the red pigment from the lichen Claviceps purpurea, was assigned the structure (9) by Frank and Zimmer[27] on the basis of the following evidence. (a) The ultraviolet and IR spectra showed that it was an anthraquinone nucleus with three α-hydroxy groups, a C-methyl group and a carboxyl group. (b) The decarboxylated product was not identical with catenarin (1,4,5,7-


O
OH
OH
COOH
HO
Me
O

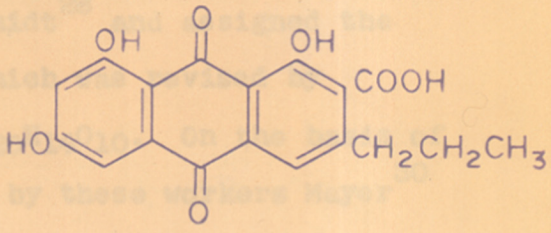
(5)


OMe
HOOC
COOMe
HOOC
Me

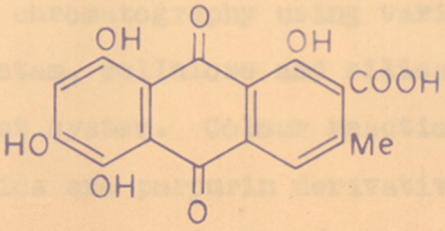
(6)


O
OH
OH
HO
CH2COOH
O

(7)


O
OH
OH
COOH
HO
CH2CH2CH3
O

(8)

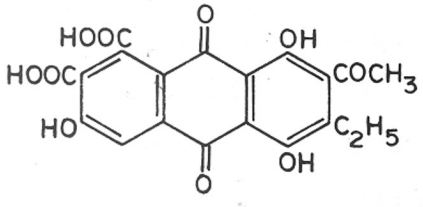

O
OH
OH
COOH
HO
Me
OH
O

(9)

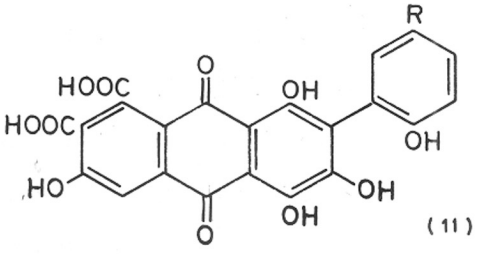
-2-methyl
tetrahydroxyanthraquinone). (c) Clavorubin produced an acid-stable lake with zirconyl chloride and hence contains an o-dihydroxy group. (d) Oxidation of endocrocin (5) with manganous dioxide and sulphuric acid gave a product which was found to be identical with clavorubin in all respects.[27]

Laccaic acid, the colouring matter of stick lac, was first isolated by Schmidt[28] and assigned the molecular formula, $C_{16}H_{12}O_8$, which was revised by Dimroth and Goldschmidt[29] to $C_{20}H_{14}O_{10}$. On the basis of the chemical evidence recorded by these workers Mayer[30] suggested structure (10) for laccaic acid. Recently the constitution of laccaic acid has been re-examined in our laboratory and it was found[9,10,11] that laccaic acid is a complex mixture of five compounds which could be separated by chromatography using various adsorbents (polycaprolactam, cellulose and silica gel) and appropriate solvent system. Colour reactions showed that all these acids are purpurin derivatives (except LA-D) and there was no evidence for C-acetyl and C-ethyl groups as in (10). Isolation of phenol-2,3,4,5-tetracarboxylic acid as an oxidation product of lac dye showed the presence of a 3-hydroxyanthraquinone-1,2-

dicarboxylic acid group. The presence of a phenyl ring in the 3-position of the purpurin and a secondary amino group was determined by NMR spectral data and mass spectral analysis. Important features of the NMR spectra of all the methylated laccaic acids are the absence of ethyl and C-acetyl groups, the presence of a group of the type $ArCH_2CH_2O$- and the presence of four aromatic protons. Based on the mass spectral molecular weights,



(10)



(11)

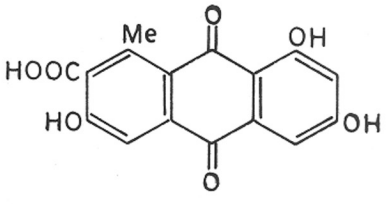
Laccaic acid A; R = $CH_2CH_2NHCOCH_3$

" " B; R = CH_2CH_2OH

" " C; R = $CH-\underset{NH_2}{CH}COOH$

" " E; R = $CH_2CH_2NH_2$

UV, IR and NMR data and chemical evidence, Pandhare[10] and Shaikh[9] assigned the structures (11) to laccaic acids A, B, C and E. Laccaic acid D (12) proved to be identical with xanthokermesic acid.[17]



(12)

The red colouring matter of cochineal, carminic acid, was first isolated by Dimroth.[31] Based on the colour reactions and oxidative degradation studies, Dimroth assigned structure (2; COOH in the 5 position) to carminic acid. The evidence in favour of the proposed structure is extensive, but by no means conclusive. Recently Ali and Haynes[32] have proved that carminic acid is a C-glycoside in which the sugar is D-glucose. The recent synthesis of 5-methoxytoluene-2,3,6-tricarboxylic acid, by Van der Kerk[33] and Bhatia,[34] which is identical with the methyl ether of the phenolic acid obtained by oxidation of carminic acid, has shown the position of the carboxyl group to be β (as in 2). This is also confirmed by the NMR spectrum of carminic acid[34] in dimethyl sulphoxide; a single aromatic proton appears at 2.33 which must therefore be an α- and not a β-proton in the anthraquinone nucleus.

The other two naturally occurring hydroxy-anthraquinone carboxylic acids, kermesic acid and cero-albolinic acid, have been dealt in the thesis.

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P A R T II

KERMESIC ACID

(a) SYNTHESIS OF PHENOL-2,3,4,5-TETRACARBOXYLIC ACID

(b) STRUCTURE AND SYNTHESIS OF KERMESIC ACID.

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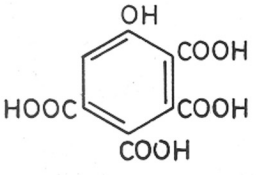
Synthesis of phenol-2,3,4,5-tetracarboxylic acid

INTRODUCTION

In the course of a programme of work on the anthraquinonoid insect pigments, a phenol tetracarboxylic acid was obtained by the oxidation of laccaic acid with alkaline hydrogen peroxide.[1] There are three possible structures (1), (2) and (3) for this acid. The acid (2) was



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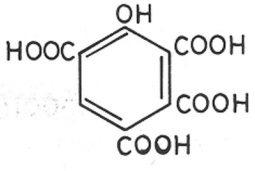

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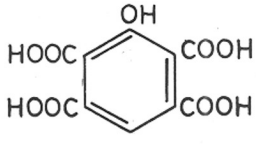
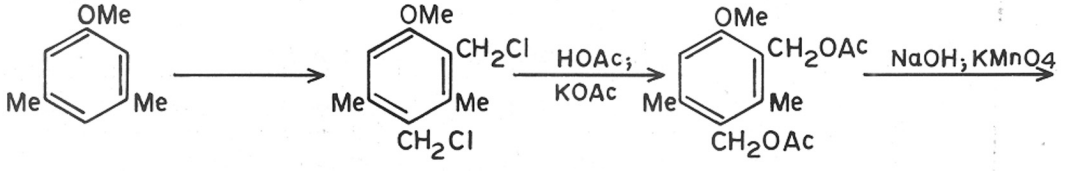


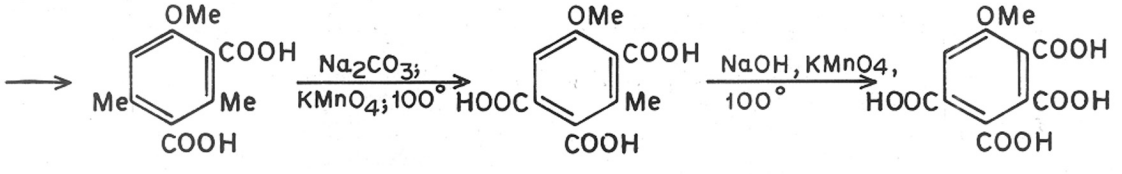
(3)

synthesised by Grove[2] from nitromesitylene and also by Charlesworth[3] et al.[3] from mesitylene. The acid (3) was synthesised by Roberts from m-5-xylenol by a series of reactions.[4] The methyl ether-ester of the phenol tetracarboxylic acid obtained from laccaic acid was found to be different[5] from the ether-esters of the acids (2) and (3). In order to obtain an authentic sample of tetramethyl anisole-2,3,4,5-tetracarboxylate (pentamethyl ether-ester of 1), Bhatia[5] used the following route. Chloromethylation of 3,5-dimethylanisole (4) by

paraformaldehyde and hydrochloric acid in carbon tetrachloride gave the 2,4-bis-chloromethyl derivative (5), which was converted to the bis-acetoxymethyl derivative (6). Oxidation of (6) with alkaline permanganate at room temperature gave the dicarboxylic acid (7). Further oxidation with potassium permanganate and aqueous sodium carbonate at 100° gave the tricarboxylic acid (8), the sandwiched methyl group resisting oxidation under these conditions. The methyl ester of (8) (m.p. 110-111°) was found to be identical (mixed melting point and superposable IR spectra) with the product of methylation of

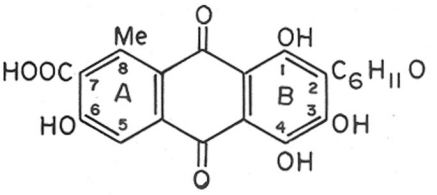


(4) (5) (6)



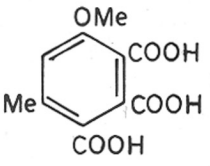
(7) (8) (9)

the phenolic acid obtained by oxidation of carminic acid[6] by alkaline hydrogen peroxide. The Dimroth[8] structure for carminic acid (10; COOH in the 5-position) was revised to (10) by Bhatia and Venkataraman,[6] and was confirmed by the NMR spectrum of carminic acid in DMSO; a single aromatic proton at 2.33 τ, indicating an α- and not a β-proton in the anthraquinone nucleus.



(10)

Overeem and van der Kerk,[7] in connection with the structure of mollusin, a naturally occurring chlorine-containing quinone synthesised 4-methoxy-6-methylhemimellitic acid (11) and its isomers 3-methoxy-5-methyltrimellitic (12) and 5-methoxy-3-methyltrimellitic acid (8). 4-Methoxy-6-methylhemimellitic acid (11) was found



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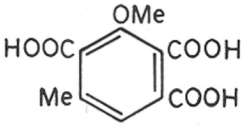
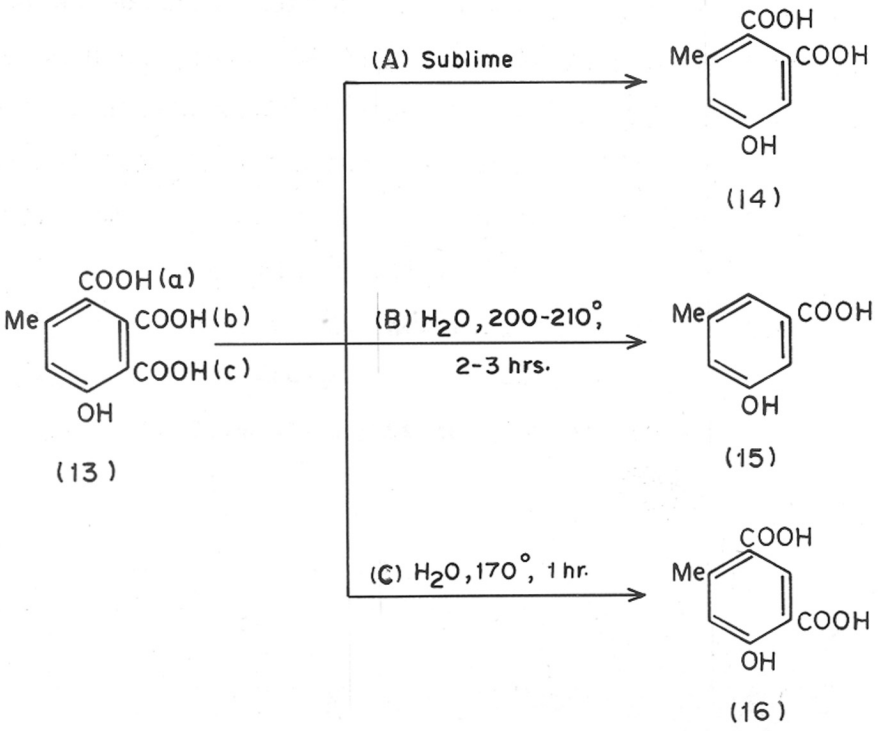
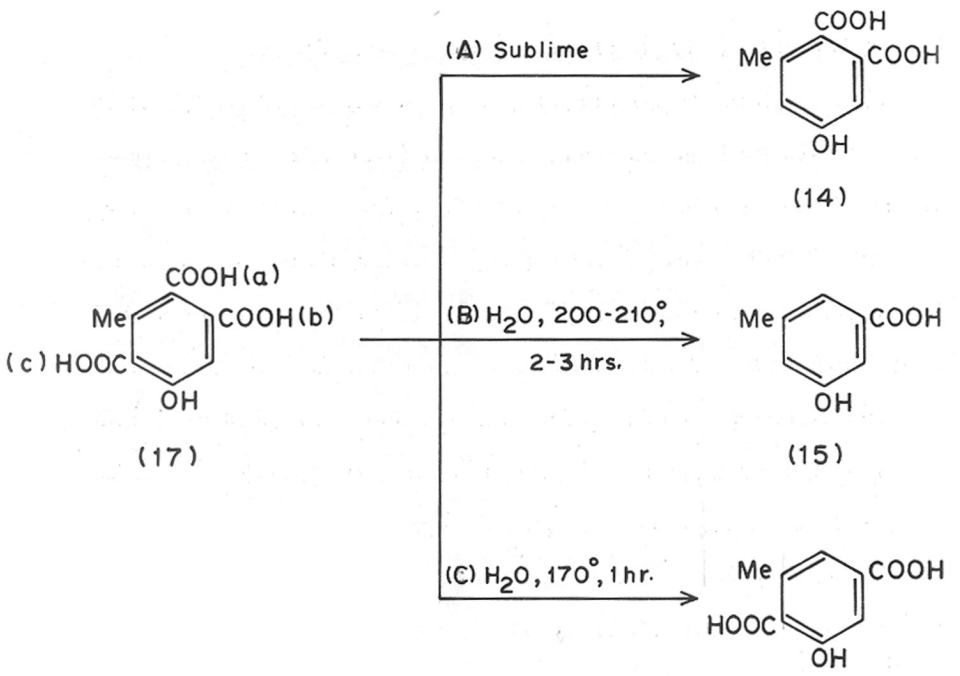


(12)

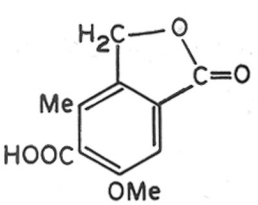
to be identical with the degradation product of mollusin methyl ether, while 5-methoxy-3-methyltrimellitic acid (8) and its trimethyl ester were found to be identical with the methyl ether and methyl ether-ester of the acid obtained by oxidation of carminic acid.[7] On the basis of these findings Overeem and van der Kerk independantly revised the structure for cochenillic acid. Dimroth[8] assigned the structure (10; COOH in the 5-position) for carminic acid on the basis of the structure (13) assigned for the cochenillic acid by Liebermann and Voswinckel.[9] The structural proof for cochenillic acid given by these authors[9] is presented in the following scheme.



The structures of products (14) and (15) were rigidly established. The product of reaction (C), α-coccinic acid (16) was claimed to be identical with m-hydroxyuvitic acid,[9] first prepared from ethyl acetoacetate and chloroform by Oppenheim and Pfaff[10] and later confirmed by Claisen.[11] A close examination of the paper of Liebermann and Voswinekel[9] revealed that the identity of α-coccinic acid with Oppenheim and Pfaffs[10] "m-hydroxyuvitic acid" was mainly based on the comparison of properties, and no mixed melting point was reported. Later Meldrum and co-workers[12] reported the synthesis of both (16) and (13) and stated that the synthetic compound of structure (13) was identical with cochenillic acid in m.p. and mixed m.p., the mixed melting point of the two methyl ethers was not reforded, and it is difficult to explain Meldrum's results. Overeem and van der Kerk[7] thought that the structure (17) (5-hydroxy-3-methyltrimellitic acid) was more rational than (13) on the basis of the decarboxylation of cochenillic acid. In the reaction (B) the decarboxylation of the groups (a) and (c) (ortho- and para to the OH group) takes place, while in the reaction (C), under less stringent conditions, only the carboxyl



group (a) (para to the OH group) was eliminated. The revised structure (17) for the acid was confirmed by synthesis of its methyl ether. 2-Hydroxy-6-methyl-terephthalic acid (18) was methylated and chloromethylated to give the phthalide for which two structures (19 and 20) had to be considered; the former was proved



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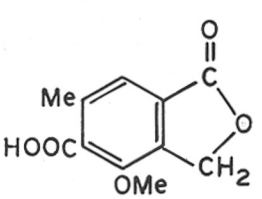

(20)

to be correct because (20) would have given on oxidation isocochenillic acid methyl ether (12), which was prepared for comparison according to Muhlemann.[13]

Further oxidation of 5-methoxy-3-methyltrimellitic acid (8) with potassium permanganate and aqueous sodium hydroxide solution gave anisole-2,3,4,5-tetracarboxylic acid (9). The product obtained by diazomethane methylation of (9) had a m.p. 105°, while the anisoletetracarboxylic acid tetramethyl ester, obtained from laccaic acid A methyl ether-ester by nitric acid oxidation, followed by diazomethane methylation had the m.p. 130°.[14] Because of the discrepancy in the m.p., the present work was undertaken.

PRESENT WORK

The objects were (a) to obtain pure samples of anisole-2,3,4,5-tetracarboxylic acid and its tetramethyl ester for comparison with the product obtained from methylated laccaic acid A;[14] and (b) to synthesise the unknown phenol-2,3,4,5-tetracarboxylic acid (1).

Anisole-2,3,4,5-tetracarboxylic acid (9) has been synthesised by the procedure of Bhatia.[5] When 6-methoxy-2,4-dimethylisophthalic acid (7) was oxidised, under the conditions employed by Bhatia,[5] with potassium permanganate in aqueous sodium carbonate (2N) at steam-bath temperature for 3 hr., and the product treated with diazomethane, the resultant ester showed two spots on TLC [silica gel and benzene-acetone (9:1) as solvent]. The fast moving minor fraction was found to be identical with the ester of the acid (7) (R_f 0.57), while the slow moving major fraction (R_f 0.45) was characterised as the trimethyl ester of 5-methoxy-3-methyltrimellitic acid (8). However, the acid (8) could be obtained in pure form by repeated crystallisation from water. The acid (8) softens at 205° and melts at 224-226°. The lower m.p. reported by Bhatia[5] (213°) is probably because

of the presence of a trace of the isophthalic acid (7). Further oxidation of 5-methoxy-3-methyltrimellitic acid (8) (using Bhatia's procedure) with potassium permanganate in aqueous sodium hydroxide at steam-bath temperature for 6 hr. gave an acid, the methyl ester of which prepared by diazomethane methylation, showed two spots, on TLC (silica gel and benzene:acetone, 9:1). The fast moving minor spot (Rf 0.45) corresponded to the ester of (8), while the slow moving major spot (Rf 0.31) was characterised as the ester of anisole-2,3,4,5-tetracarboxylic acid. However, prolonged oxidation under identical conditions for 12 hr. (instead of 6 hr) gave the acid (9), m.p. 232-234°, which yielded an ester (m.p. 129-130°) free from any impurity as shown by its chromatographic behaviour. The m.p. of the ester of (9) remained undepressed when mixed with the methylation product of the acid obtained by oxidation of laccaic acid A methyl ether-ester with nitric acid. Demethylation of tetramethyl anisole-2,3,4,5-tetracarboxylate in a melt of aluminium chloride-sodium chloride gave phenol-2,3,4,5-tetracarboxylic acid (1). It gave a violet colour with alcoholic ferric chloride solution. The product, crystallised from water, melted at 238-240°.

After completion of this work Maximov[15] in connection with work on the chromatographic behaviour of benzene carboxylic acids and hydroxybenzene carboxylic acids, reported the synthesis of phenol-2,3,4,5-tetracarboxylic acid, but details of the synthesis are not available. A sample of phenol-2,3,4,5-tetracarboxylic acid obtained from Maximor was found to be identical with the synthetic sample obtained from anisole-tetracarboxylic acid (m.p., mixed m.p. and chromatographic behaviour). Recently Tsuji,[16] in connection with the structure of julimycin B-II isolated from the metabolites of Streptomyces shiodaensis, synthesised anisole-2,3,4,5-tetracarboxylic acid (m.p. of ester 128-128.5°), starting from 1,2,3,4-tetramethyl benzene.

STRUCTURE AND SYNTHESIS OF KERMESIC ACID

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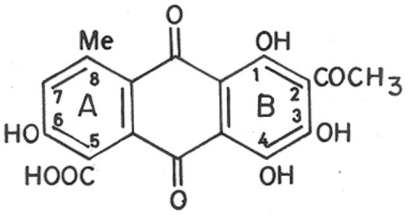
INTRODUCTION

According to Thomson, "kermes is a dyestuff of very great antiquity and is probably the earliest of which we have records. It appears to have been used by Phoenicians and is mentioned in the scriptures. Indeed the Hebrew words translated as scarlet in the Old Testament probably signified a red colour dyed with kermes." The colouring matter was obtained from the insect Kermococcus ilicis Linn. belonging to the same family Coccidae, as the lac & cochineal insects. found on the kermes-oak (Quercus coccifera Linn.). The female insects were collected, killed and sun dried. It was cultivated in France, Spain, Portugal and Morocco, and was used extensively for dyeing shades of red on an aluminium mordant.

The colouring principle, kermesic acid (m.p. 250° decomp.) was first obtained by Heise and was later examined by Dimroth and co-workers, who assigned the structure (I). The red pigment was obtained by extracting the insects (after removal of wax)



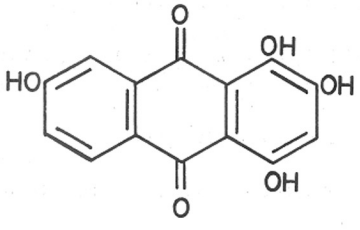
(21)

with ethereal hydrochloric acid. Based on the elemental analysis, Dimroth[21] assigned the molecular formula, $C_{18}H_{12}O_9$ to kermesic acid. It formed a tetraacetate (m.p. 245°) by refluxing with acetic anhydride and sodium acetate, a trimethyl ether ester (m.p. 310°) on methylation of its potassium salt with methyl sulphate in toluene, and gave 1-methyl anthracene on zinc dust distillation. Presence of carboxyl group was indicated by the solubility of (21) in sodium bicarbonate and on heating in water at 150° it lost carbon dioxide to form decarboxy kermesic acid which was insoluble in aqueous bicarbonate, while the absence of methoxyl group was indicated by Zeisel's method. Of these six substituents [Me, $(OH)_4$, COOH] three were found to have the same orientation as in the A ring of carminic acid (10; COOH in 5 position) on the basis of oxidative degradation experiments. Alkaline permanganate oxidation of kermesic acid methyl ester dimethyl ether yielded methyl cochinellate methyl ether. Saponification of the ether ester followed by demethylation with hydroidic acid gave cochenillic acid[21], which was also obtained from carminic acid (10; COOH in 5 position) by treatment with potassium persulphate.[9] Isolation of cochenillic acid led Dimroth to assign the pattern of substitution

of the three groups (Me, OH, COOH) as in the ring A of carminic acid (10; COOH in the 5 position). Of the remaining three hydroxyl groups, one hydroxyl group was assigned to the β-position and the other two at the 1 and 4 positions of the B ring of anthraquinone nucleus, since it resembles purpurin rather than anthragallol in its dyeing properties.

Bromination of kermesic acid in glacial acetic acid gave monobromococcin,[22] $C_{16}H_9BrO_8$, which like the parent compound formed a tetra-acetate and yielded cochenillic acid, on oxidation with hydrogen peroxide in presence of manganous salt. This confirmed that the ring A is unaffected and that only the ring B undergoes bromination. In the conversion of kermesic acid to monobromococcin, Dimroth[22] observed that the group $-C_2H_3O$ was displaced by bromine, although there was no specific reason for its displacement. However, the elimination of an acetyl group by bromine is not without analogy. In the case of purpurin carboxylic acid, the carboxyl group can be eliminated by boiling with water. The group $-C_2H_3O$ could be either $-CH_2-C{=}O{-}H$ or $-C{=}O{-}CH_3$ and since no aldehyde properties could be detected Dimroth and Scheurer[22] regarded the substitution at 2-position as $-C{=}O{-}CH_3$.

The assignment of the three hydroxyl groups, in kermesic acid, was further made by comparison with hydroxyanthrapurpurin (22) and hydroxyflavopurpurin (23) which could be distinguished in their dyeing properties and absorption spectra in the visible region. These



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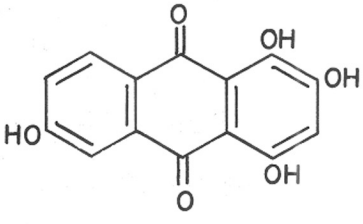
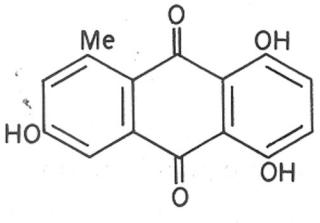


(23)

two isomeric compounds (22 and 23) were obtained by oxidation of anthrapurpurin [1,2,7-$(OH)_3$-anthraquinone] and flavopurpurin [1,2,6-$(OH)_3$-anthraquinone] respectively. Hydroxyanthrapurpurin is distinguished from its isomer sharply by the absorption spectrum and the bluish shade on mordanted substances. Dimroth and Fick[23] noted also that the absorption spectra of kermesic acid, decorboxy-kermesic acid, carminic acid and hydroxyanthrapurpurin in alkali and conc. sulphuric acid solution showed a close similarity which confirmed their opinion that kermesic acid has the structure (21). This conclusion was also supported by the fact that the compound obtained

by bromination of hydroxyanthrapurpurin and tribromo-coccin had similar properties.

Kermesic acid on reduction with zinc and acetic acid, gave a compound (24). Dimroth[23] noticed that during reduction kermesic acid lost besides the β-hydroxyl group also the acetyl group in the purpurin residue and the carboxyl group. The structure of this



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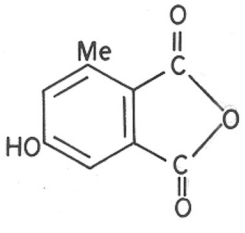

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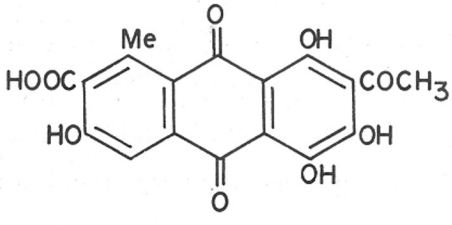
methyltrihydroxyanthraquinone (24) was confirmed by synthesis. It was obtained by condensing β-coccin acid anhydride (25) and hydroquinone diacetate with boric acid and benzoic acid at 220°, conditions which will be regarded as abnormal at the present time.

On the basis of the above observation, Dimroth assigned the structure (21) to kermesic acid closely related to carminic acid (10; COOH in 5 position) and

in fact differing only in the nature of the substituent at C-2. The acetate hypothesis[24] also favoured the structure (21).

Since both carminic and kermesic acids yield cochenillic acid (17) by oxidation, Overeem and van der Kerk[7] concluded that kermesic acid is constituted as (26).



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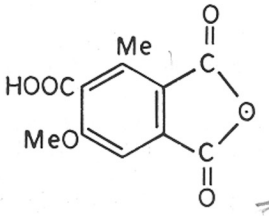
PRESENT WORK

Stuff. We have undertaken The synthesis of kermesic acid (I) was undertaken with the object of proving its structure. This normally involves the Friedel-Crafts acylation reaction of the corresponding substituted phthalic anhydride with the appropriate phenol. Many condensing agents for Friedel-Crafts acylation have been described[25] and of all aluminium chloride is by far the most frequently used condensing agent, except perhaps when an acid is used as an acylating agent. The condensation can be step-wise in which the first step is the formation of an o-aroylbenzoic acid, which can be cyclized under suitable conditions to the corresponding anthraquinone[26] or under vigorous conditions such as condensing the two products in a melt of aluminium chloride and sodium chloride mixture resulting in the formation of anthraquinone derivatives. The appropriate phthalic anhydride required for the synthesis of kermesic acid will be 3-methyl-4-carboxy-5-methoxyphthalic anhydride (IV). (A) This was prepared by refluxing the acid (V) with acetic anhydride for 30 min. Overeem and van der Kerk[7] converted cochenillic acid methyl ether to (IV) by sublimation at 200-210° and 0.1 mm.



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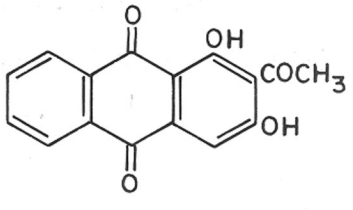
Next step in the synthesis of kermesic acid involves the condensation of the anhydride (27) with a suitable phenol which ultimately can give the desired product. In order to achieve this, the acetyl group, either has to be introduced after building the anthraquinone or condensation has to be carried out under forcible conditions employing the appropriate acetophenone. It is well known that acylation of an aromatic ring by dibasic acid derivative is hindered by a carbonyl group directly attached to the ring. Sometimes the effect is so powerful that either one or more activating groups, or in some cases forcing conditions one required to make acylation possible. In order to arrive for a suitable method, the synthesis of 2-acetylxanthopurpurin (28) was



(28)

undertaken. Introduction of nuclear acetyl group by prolonged refluxing xanthopurpurin with boron fluoride acetic acid complex met with failure. However, phthalic anhydride and monomethyl ether of 2-acetylresorcinol on condensation in a melt of aluminium chloride-sodium chloride mixture at 180° for 15 minutes yielded 2-acetylxanthopurpurin along with certain amount of corresponding phthaleins. 2-Acetylxanthopurpurin (28) was obtained by chromatography of the crude reaction product over silica gel column using benzene for development and elution. Methylation of (28) with dimethyl sulphate, potassium carbonate in boiling acetone gave dimethyl ether. The NMR (Fig.1) and mass spectrum are in complete agreement with the structure.

Encouraged by the formation of 2-acetylxanthopurpurin in reasonable yields, first attempt was directed in condensing the anhydride (27) with 4-chloro-2-acetylresorcinol. To obtain this product 2-acetylresorcinol was treated with sulphuryl chloride in dry ether at room temperature and the resulting product was found to be a dichloro-derivative (29). However, monomethyl ether of 2-acetylresorcinol under similar conditions gave the monochloro derivative (30). Condensation of (30) with

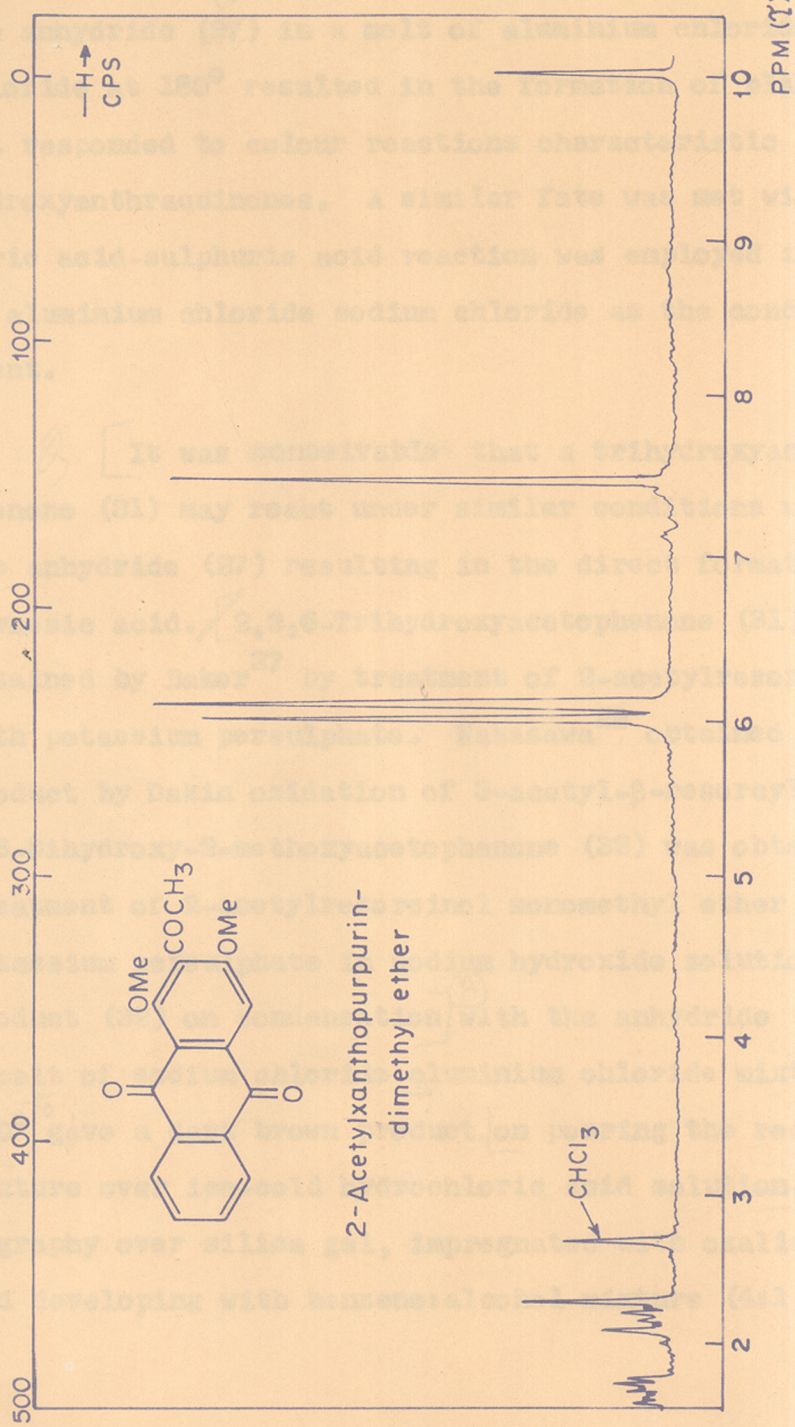
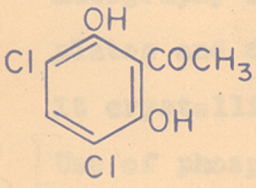

500
400
300
200
100
0
H
CPS
2
3
4
5
6
7
8
9
10
PPM (τ)
CHCl3
OMe
COCH3
OMe
O
O
2-Acetylxanthopurpurin-
dimethyl ether

Fig.1

the anhydride (27) in a melt of aluminium chloride-sodium chloride at 180° resulted in the formation of black mass not responded to colour reactions characteristic for hydroxyanthraquinones. A similar fate was met with when boric acid-sulphuric acid reaction was employed in place of aluminium chloride sodium chloride as the condensing agent.

It was conceivable that a trihydroxyacetophenone (31) may react under similar conditions with the anhydride (27) resulting in the direct formation of kermesic acid. 2,3,6-Trihydroxyacetophenone (31) was obtained by Baker[27] by treatment of 2-acetylresorcinol with potassium persulphate. Nakazawa[28] obtained this product by Dakin oxidation of 3-acetyl-β-resorcylaldehyde. 3,6-Dihydroxy-2-methoxyacetophenone (32) was obtained by treatment of 2-acetylresorcinol monomethyl ether with potassium persulphate in sodium hydroxide solution. This product (32) on condensation with the anhydride (27) in a melt of sodium chloride-aluminium chloride mixture at 180° gave a dark brown product on pouring the reaction mixture over ice-cold hydrochloric acid solution. Chromatography over silica gel, impregnated with oxalic acid and developing with benzene:alcohol mixture (4:1), it



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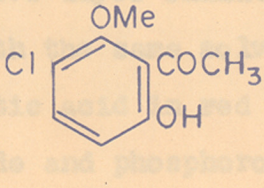

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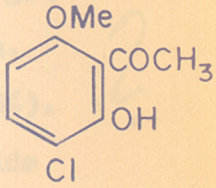
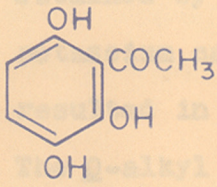
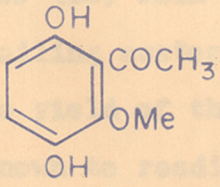


(30)



(31)

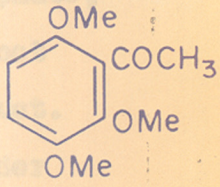


VIII

(32)

known?

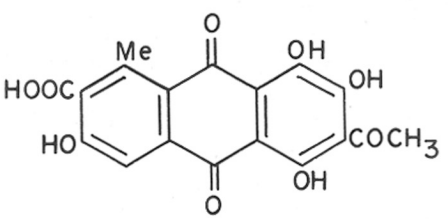
Ref?



(33)

showed one major spot along with two to three minor fractions. The major product was collected by chromatography over preparative layer oxalated silica gel plates and developing with the same solvent system. It crystallized from acetic acid in red needles (5%).

Use of phosphoryl chloride and phosphorous pentoxide in Friedel-Crafts condensation of dibasic acid and phenols, resulting into quinonoid structure has been recently reported by Core and Kirch.[29] Using these conditions the condensation of phenol (32) and the anhydride (27) resulted in the formation of resinous products. Using 2,3,6-trimethoxyacetophenone (33), obtained by methylation of (32) with dimethyl sulphate, potassium carbonate in boiling acetone, has also not resulted in improving the yield of the final product. The O-alkyl linkage is known to readily cleave under such drastic conditions and as such two products (26 and 34) are expected to form during the reaction. The resultant product gave an orange-red colour in sodium bicarbonate, reddish violet in sodium hydroxide, which



(34)

changed to reddish brown colour after addition of sodium dithionite, cherry red in conc. sulphuric acid and gave a brown colouration with alcoholic ferric chloride solution. All the above reactions were similar to those described by Dimroth[21] for kermesic acid.

Colour reactions

	Kermesic acid (Dimroth)	Synthetic product
Colour	Red	Red
Water solubility	Sparingly soluble	Sparingly soluble
Aq. NaOH	Red-violet	Violet red
Conc. H_2SO_4	Red-violet	Violet red
$FeCl_3$ (alc.)	Dark brown	Dark brown

The NMR spectrum of the condensation product in dimethyl sulphoxide shows a singlet absorption at 2.57 (chemical shifts on the τ scale) which can be assigned to the 5-H of the ring A. The spectrum in NaOD-D_2O (three singlets of intensity 1:3:3, the 3-proton singlets appearing at 4.46 and 4.65 ppm higher than the aromatic proton signal) confirmed the structure (26) or (34). As the tetra-acetate and trimethyl ether described

by Dimroth[21] were found to be difficult to prepare, it was clear that the direct comparison with the natural kermesic acid was necessary before any conclusion could be drawn. After an extensive search, we were fortunate in obtaining an authentic sample from Professor Karl Dimroth, a sample of O. Dimroth's kermesic acid. Except for a minor impurity readily removed by chromatography on oxalated silica gel, the product more than fifty years old was remarkably pure. The synthetic sample showed a different behaviour on oxalated silica gel plate, using benzene:alcohol (4:1) as a solvent. (R_f for natural product = 0.33 and synthetic sample = 0.47).

The electronic spectrum of the kermesic acid (Fig. 2) in sulphuric acid shows bands at 295 mμ (log ε 4.77), 340 (4.27), 390 (3.74), 470 (3.98), 500 (4.18) and 540 (4.08) and is in complete agreement with the reported spectral data by Dimroth.[23]

The NMR spectrum of Dimroth's kermesic acid in dimethyl sulphoxide (Fig. 3) shows two single proton signals at 2.34 and 3.40. The former corresponds to the α-proton in the A ring, but the signal at 3.4 can be only assigned to a β-proton in an anthraquinone flanked by hydroxyl groups as in purpurin (1,2,4-trihydroxyanthraquinone).

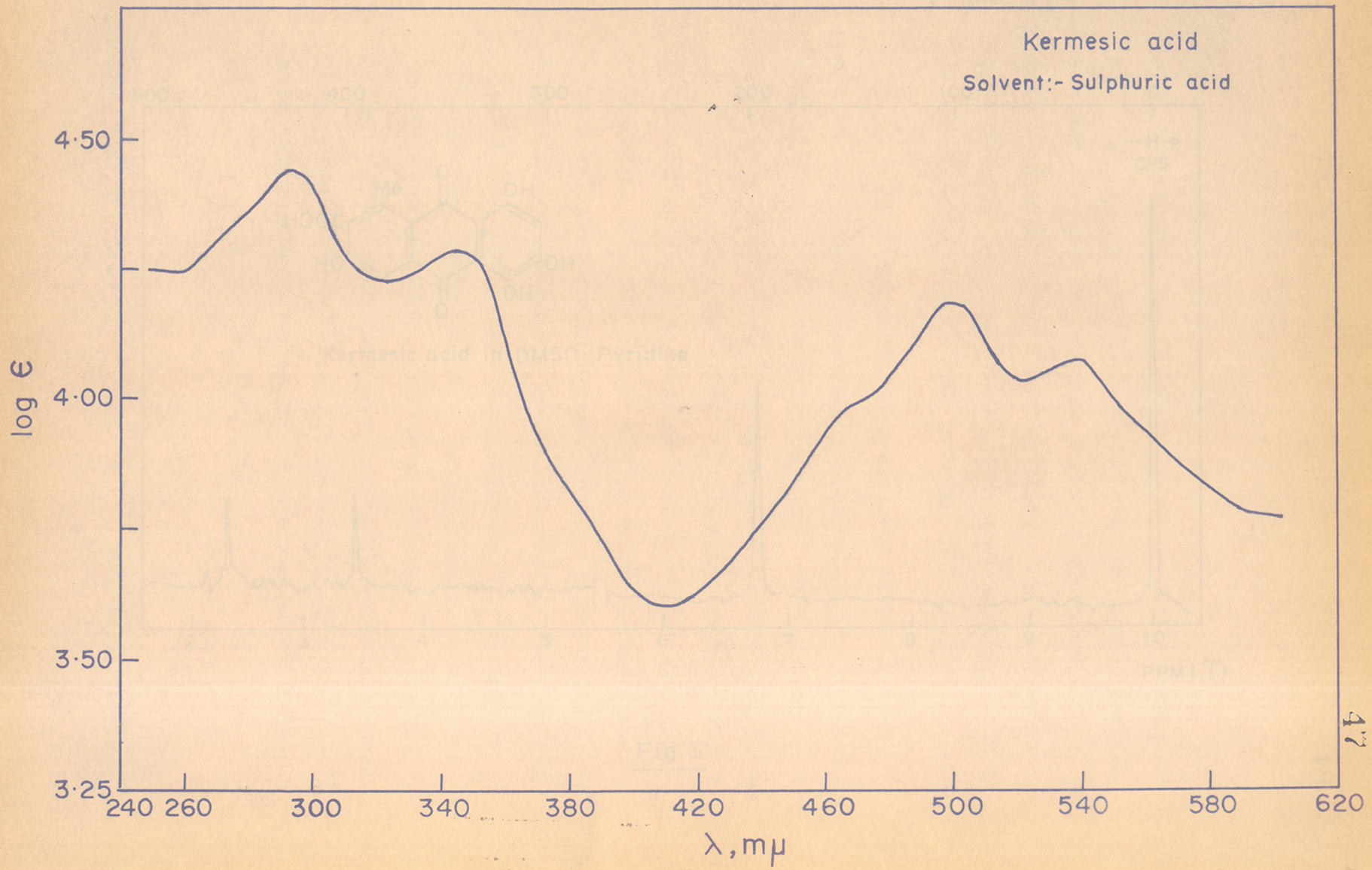

Kermesic acid
Solvent:- Sulphuric acid
log ε
4·50
4·00
3·50
3·25
240 260 300 340 380 420 460 500 540 580 620
λ, mμ

Fig. 2

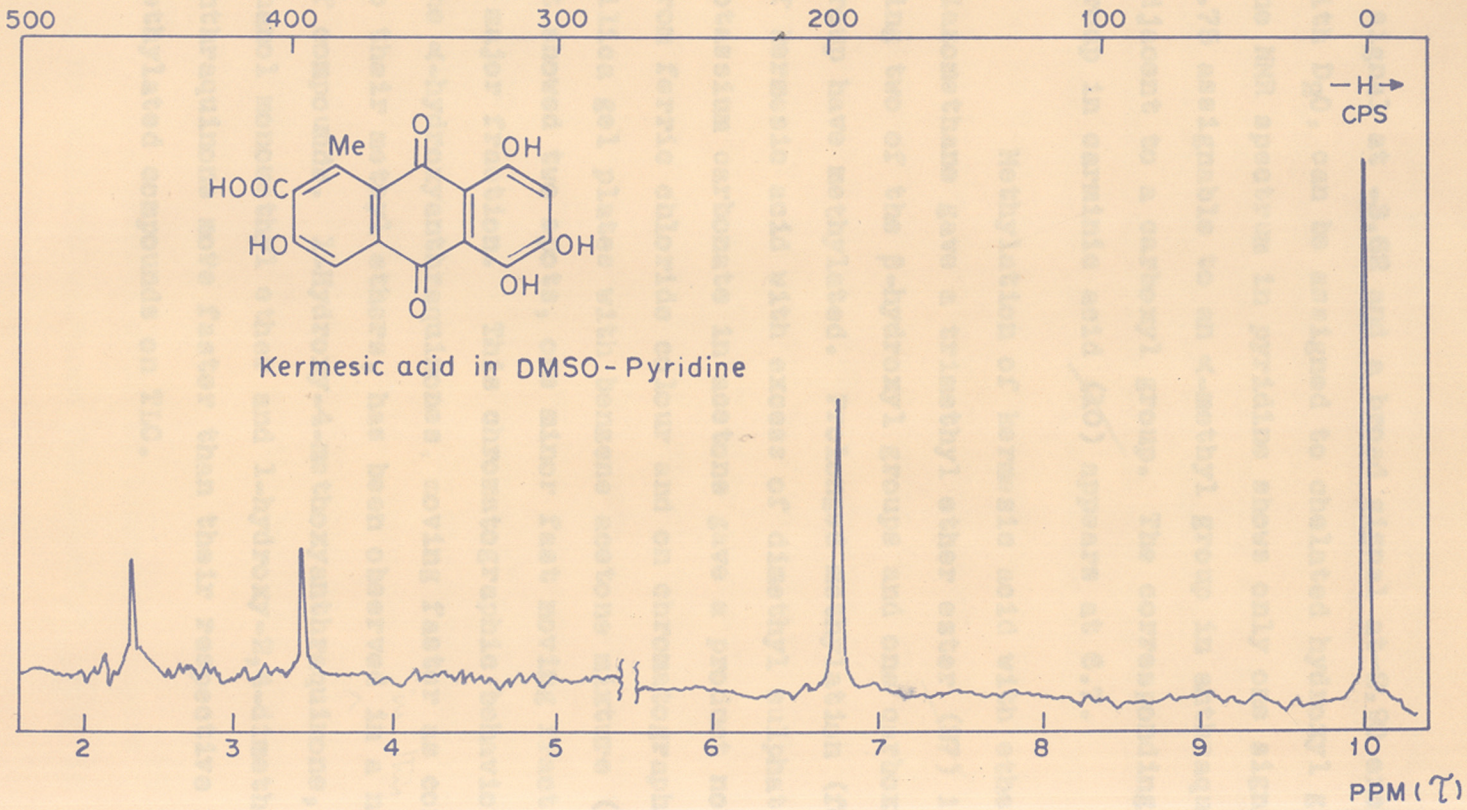

500
400
300
200
100
0
—H→
CPS
Me
O
OH
HOOC
HO
OH
OH
O
Kermesic acid in DMSO-Pyridine
2
3
4
5
6
7
8
9
10
PPM (τ)

Fig.3

A signal at -3.62 and a broad signal at-2.90 exchange with D_2O, can be assigned to chelated hydroxyl groups. The NMR spectrum in pyridine shows only one signal at 6.75 assignable to an ∝-methyl group in anthraquinone adjacent to a carboxyl group. The corresponding methyl group in carminic acid (10) appears at 6.7.

Methylation of kermesic acid with ethereal diazomethane gave a trimethyl ether ester (37) indicating two of the β-hydroxyl groups and one carboxyl group have methylated. Prolonged methylation (for 3 days) of kermesic acid with excess of dimethyl sulphate and potassium carbonate in acetone gave a product not free from ferric chloride colour and on chromatography over silica gel plates with benzene acetone mixture (4:1) it showed two spots, one minor fast moving fraction and a major fraction. This chromatographic behaviour of the ∝-hydroxyanthraquinones, moving faster as compared to their methyl ethers, has been observed in a number of compounds. 1-Hydroxy-4-methoxyanthraquinone, chrysophanol monomethyl ether and 1-hydroxy-2,4-dimethoxyanthraquinone move faster than their respective fully methylated compounds on TLC.

The methylated product was separated into two fractions by preparative chromatography over silica gel plates using the same solvent. The minor fraction (m.p. 243-244°) gave a dark brown colour with alcoholic ferric chloride solution while the other major product [(m.p.196°; mass spectral molecular weight 400 (Fig.4)] in its NMR spectrum (Fig. 5) in $CDCl_3$ shows two single proton singlets at 2.45 and 3.19 corresponding to the α- and β-protons in the pentamethyl ether ester (38). The latter being comparable to the β-proton in purpurin moiety. Two singlets at 6.01 and 6.04 integrating for 6 and 9 protons respectively, correspond to five methoxyl groups and a three proton singlet at 7.35 to an α-methyl group. The NMR spectrum of the minor product (m.p. 244°) compared with the major product (38) contained one methoxyl group less, but instead it shows the presence of a sharp singlet at -3.70 indicating the presence of a strongly bonded hydroxyl group and is probably the tetramethyl ether ester (39) of kermesic acid. From this it is clear that kermesic acid has the constitution as (35) or (36), but the former is preferred on the basis of biogenesis from acetate units.

A careful study of Dimroth's papers[3-5,21-23] shows that no positive evidence for C-acetyl group in the

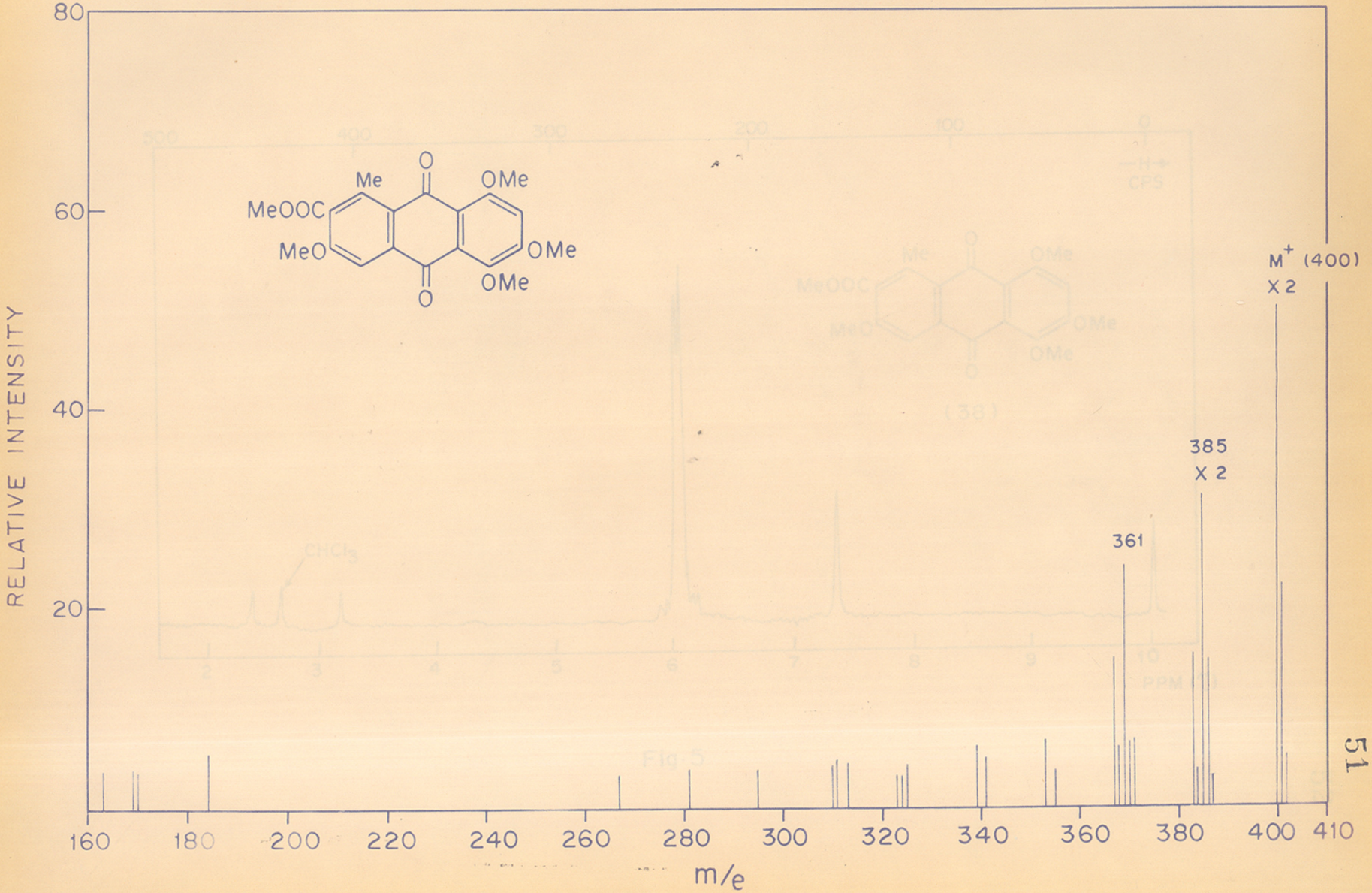
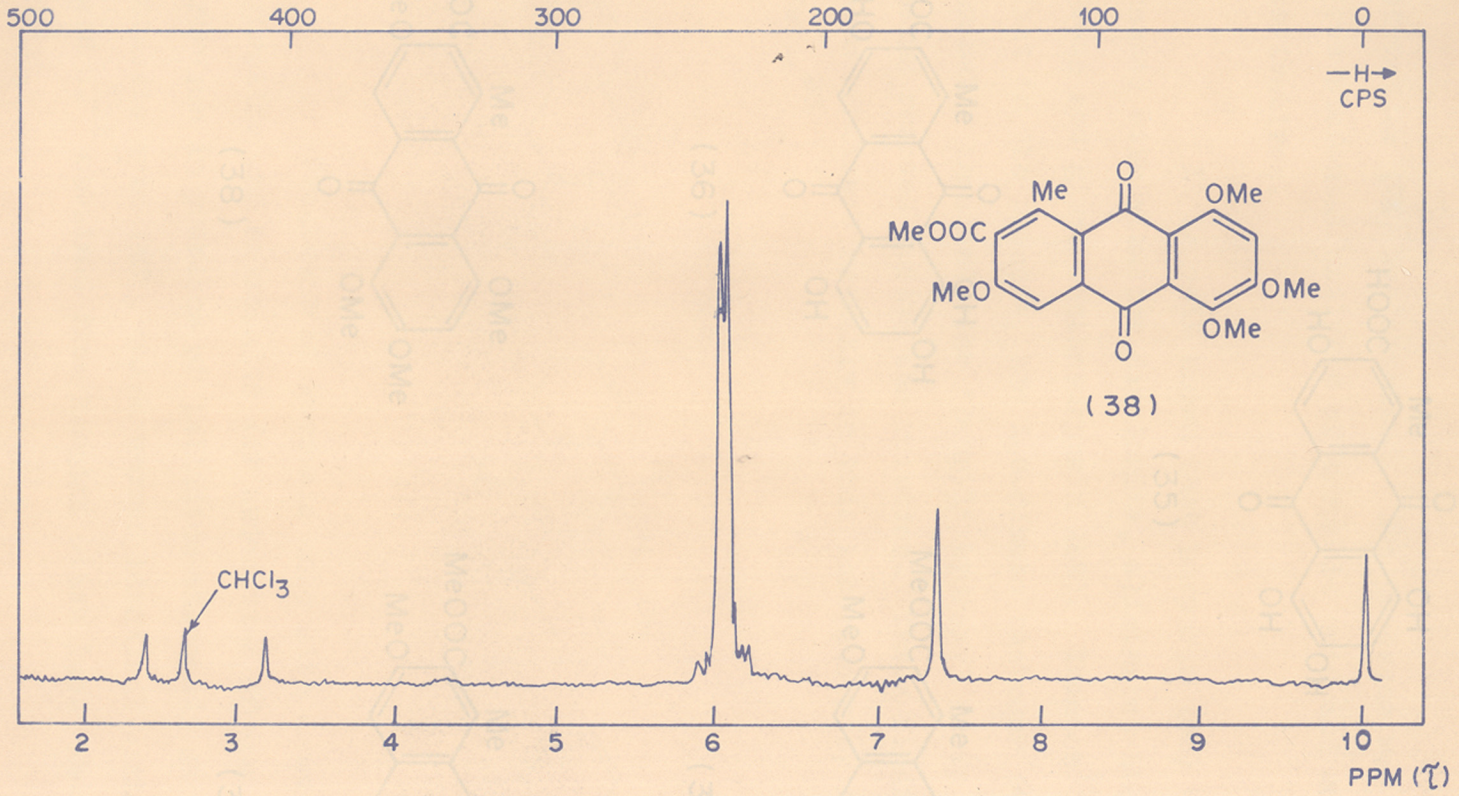
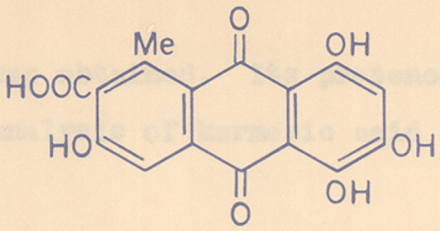

80
60
40
20
RELATIVE INTENSITY
Me
OMe
MeOOC
MeO
OMe
OMe
O
O
M+ (400)
X 2
385
X 2
361
160
180
200
220
240
260
280
300
320
340
360
380
400
410
m/e

Fig. 4

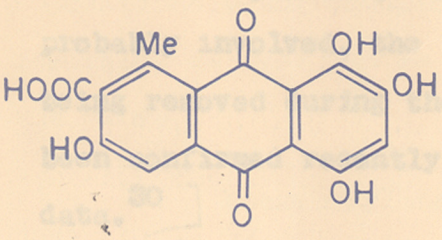

500
400
300
200
100
0
—H→
CPS
Me
OMe
MeOOC
MeO
OMe
OMe
O
O
O
CHCl3
2
3
4
5
6
7
8
9
10
PPM (τ)

(38)

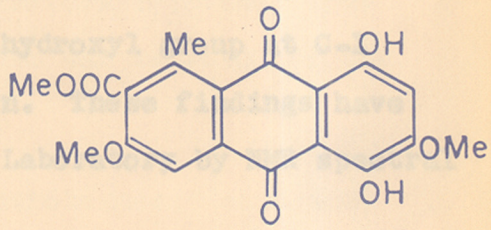
Fig. 5


Me
O
OH
HOOC
HO
OH
OH
O

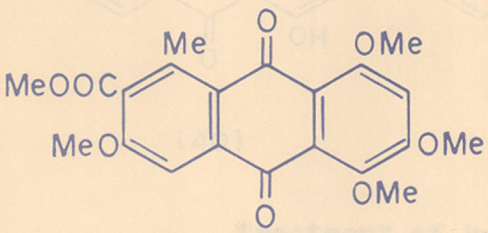
(35)


Me
O
OH
HOOC
OH
HO
OH
O

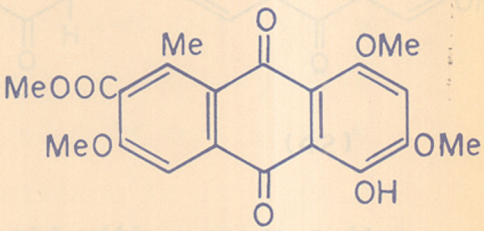
(36)


Me
O
OH
MeOOC
MeO
OMe
OH
O

(37)


Me
O
OMe
MeOOC
MeO
OMe
OMe
O

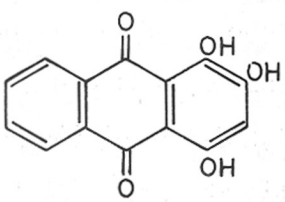
(38)


Me
O
OMe
MeOOC
MeO
OMe
OH
O

(39)

structure (21) was obtained. Its presence was surmised from elemental analysis of kermesic acid and some of its derivatives.

It is well known that purpurin (40) when heated with alkaline dithionite, eliminates the 1-hydroxyl group and gives xanthopurpurin (42). Thomson[17] suggests that in the process, the 1,3-diketo tautomer (41) is probably involved, the 'benzyl' hydroxyl group at C-1 being removed during the reaction. These findings have been confirmed recently in this Laboratory by NMR spectral data.[30]



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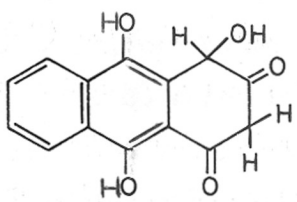

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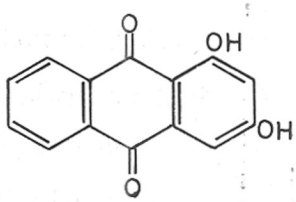
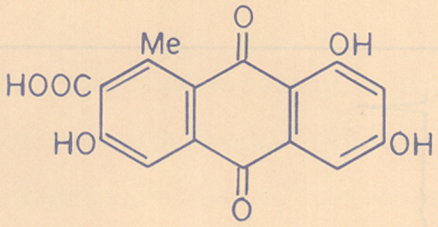


(42)

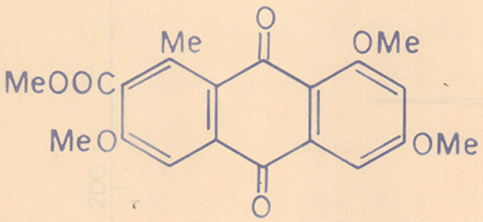
Treatment of kermesic acid with aqueous sodium hydroxide and dithionite (or absorption of two mole of hydrogen in presence of palladium-charcoal) gave xanthokermesic acid (43), which proved identical with laccaic acid D,[31] obtained from Rangini stick lac. Methylation

of xanthokermesic acid by dimethyl sulphate and potassium carbonate in boiling acetone gave tetramethyl ether ester (44), m.p. 226°. The NMR spectrum in $CDCl_3$ shows three aromatic protons, four methoxyl groups (at 6.0) and a C-methyl group. Two doublets at 2.69 and 3.23 with the same coupling constant (J= 3 cps) correspond to two aromatic protons in one ring of anthraquinone, the two positions being occupied by two methoxyl groups. The singlet at 2.37 can be assigned to the α-proton of the A ring.

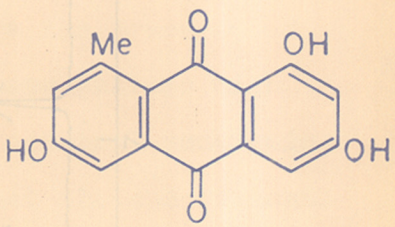
Decarboxylation of xanthokermesic acid (43) by refluxing with diethylaniline for 2 hr., followed by methylation with dimethyl sulphate, potassium carbonate and acetone, gave a product, proved to be (47) by its NMR spectrum in $CDCl_3$ (Fig. 6) shows three methoxyl groups at 6.03, 6.08 and 6.09 and an α-methyl group at 7.23. In the aromatic region there are four protons, two doublets (J= 3 cps) at 3.26 and 2.68 showing that two methoxy groups are in 1,3-positions in one ring, two other doublets (J = 3 cps) at 2.47 and 3.0 correspond to α- and β- protons of the second ring of anthraquinone, the latter signal being in the right position if it is flanked by a methyl and a methoxyl group. This product



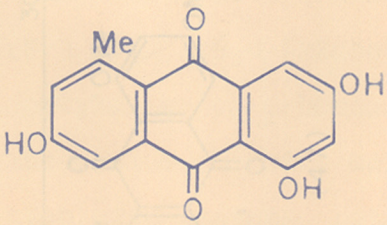
(43)



(44)



(45)



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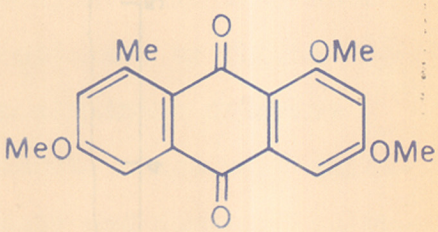
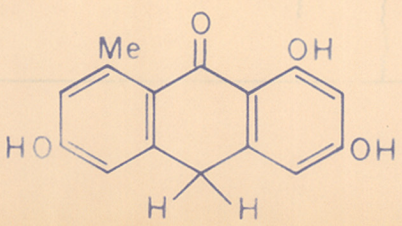


(47)



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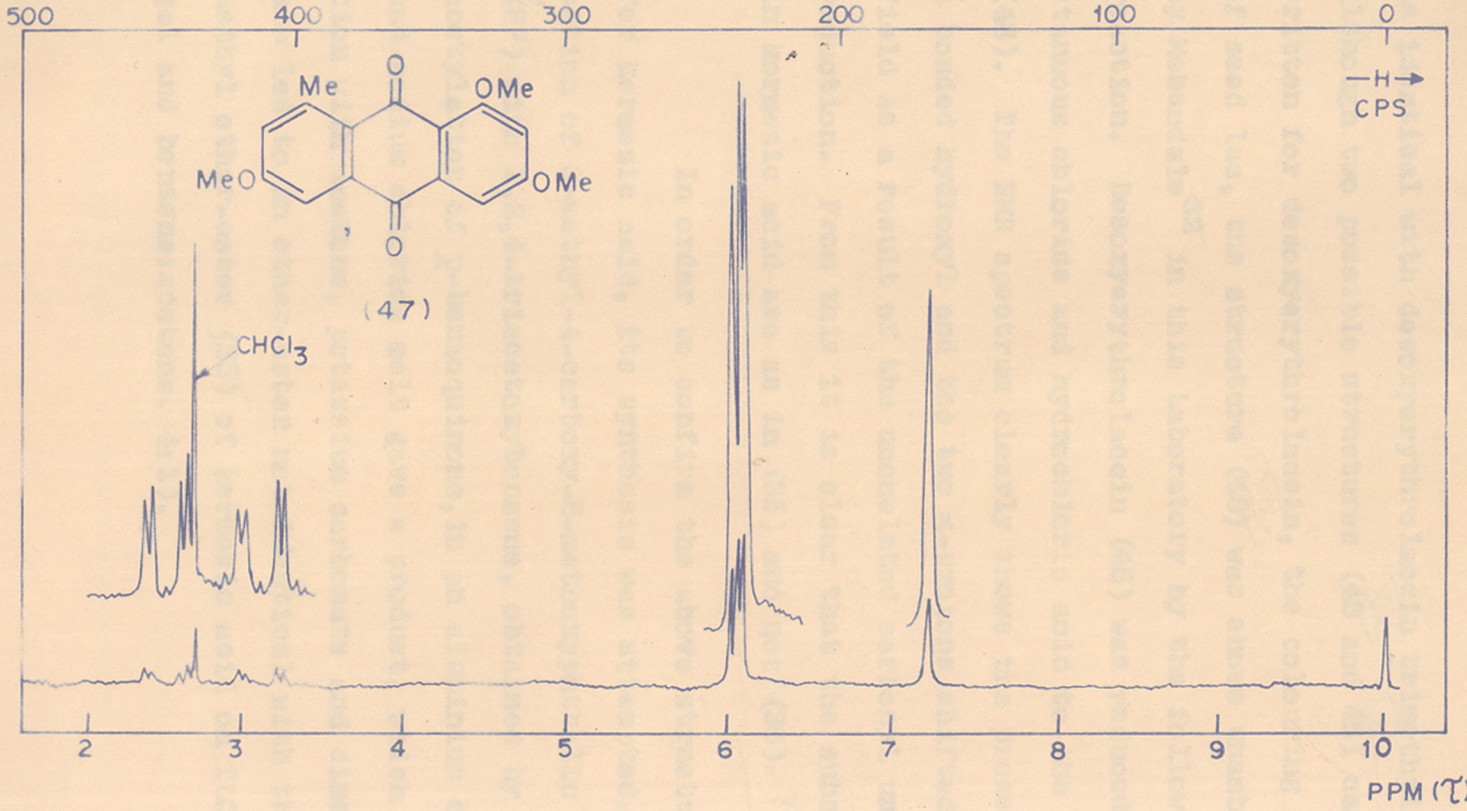

500
400
300
200
100
0
—H→
CPS
Me
OMe
MeO
OMe
O
O
(47)
CHCl3
2
3
4
5
6
7
8
9
10
PPM (τ)

Fig. 6

is identical with desoxyerythrolaccin trimethyl ether.[31] Although two possible structures (45 and 46) can be written for desoxyerythrolaccin, the colouring matter of seed lac, the structure (45) was shown unambiguously by Mehandale[32] in this Laboratory by the following reaction. Desoxyerythrolaccin (45) was reduced with stannous chloride and hydrochloric acid to the anthrone (48). The NMR spectrum clearly shows the presence of a bonded hydroxyl and the two α-protons shifted high field as a result of the unchelated carbonyl undergoing reduction. From this it is clear that the substituents in kermesic acid are as in (35) and not (36).

In order to confirm the above structure (35) for kermesic acid, its synthesis was attempted. Condensation of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (27) with 1,2,4-triacetoxybenzene, obtained by Thiele acetylation of p-benzoquinone, in an aluminium chloride and sodium chloride melt gave a product, which on methylation with acetone, potassium carbonate and dimethyl sulphate led to an ether-ester not identical with the pentamethyl ether-ester (38) of kermesic acid on TLC (silica gel and benzene:acetone, 4:1).

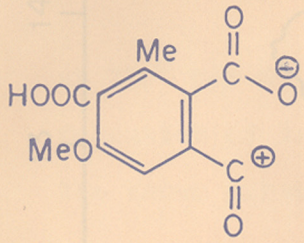
It has been found in the course of this work that hydroquinones or substituted hydroquinones condense smoothly with phthalic anhydride in presence of boron fluoride etherate at steam-bath temperature to form hydroxyanthraquinones. Although a large number of condensing agents have been used in the synthesis of hydroxyanthraquinones, the use of boron fluoride is rare in acylation of di- and polycarboxylic acid derivatives. Its use in the condensation of phenol and resorcinol with phthalic anhydride resulted in the formation of phthaleins[11-33] in quantitative yield. However, when hydroquinone was condensed with phthalic anhydride in presence of boron fluoride etherate at 100° for 4 hrs. it gave quinizarin in 40% yield. Similarly 2-methoxy-hydroquinone under identical condition gave 2-methyl ether of purpurin in 50% yield (Table 1). This method has no definite advantage particularly when phthalic anhydride is condensed with various phenols. Quinizarin is obtained in 74% yield by condensing phthalic anhydride with p-chlorophenol using a mixture of sulphuric acid and boric acid.[12-34] But when substituted phthalic anhydrides are employed, the products are obtained either in very poor yield or gave tarry products. The use of boron trifluoride etherate as a condensing agent has definite

advantage because the products though obtained in poor yield are not accompanied by undesirable tarry by-products.

TABLE 1

No.	Anhydride	Phenol	Product (Yield %)
1	Phthalic	Hydroquinone	Quinizarin (40)
2.	Phthalic	2-Methoxy-hydroquinone	Purpurin-2-methyl ether (50)
3.	Phthalic	4-Methyl-catechol mono-methyl ether.	1-Hydroxy-2-methoxy-4-methylanthraquinone (10)
4.	3-Chlorophthalic	Hydroquinone	5-Chloroquinizarin (10)
5.	3-Methyl-4-carboxy-5-methoxyphthalic	2-Methoxyhydroquinone	1,4-Dihydroxy-2,6-dimethoxy-8-methylanthraquinone-7-carboxylic acid (15) + negligible amount of its isomer.

2-Methoxyhydroquinone (51), obtained by Dakin oxidation of vanillin with hydrogen peroxide in sodium hydroxide, and the anhydride (27) were condensed in presence of boron fluoride etherate at 100° for 4 hrs. The resultant red product on methylation with dimethyl sulphate and potassium carbonate in acetone gave yellow ether-ester, which showed on silica gel TLC and benzene: acetone (4:1) two spots having very close Rf values, the minor spot corresponding to the pentamethyl ether-ester (38) of kermesic acid. The major product crystallised from methanol to yield a pure chromatographically homogeneous compound (m.p. 211°). The NMR spectrum of this compound in $CDCl_3$ showed five methoxyl groups (6.00 and 6.05), a nuclear C-methyl group (7.35), and two uncoupled aromatic protons (2.40 and 3.27); and closely resembles the spectrum of methyl tetramethyl kermesate (38); but the IR spectra of the synthetic (Fig. 7) and natural products in chloroform are not superposable, particularly in the finger-print region. The synthetic ether-ester therefore has the structure (54), and is isomeric with methyl O-tetramethyl kermesate (38). The same ether-ester (54) was obtained by condensation of 2-methoxyhydroquinone with the anhydride (27) in an aluminium chloride and sodium chloride melt



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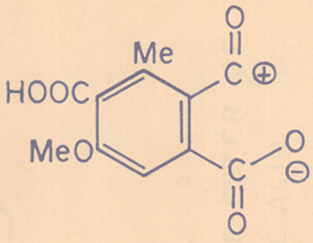

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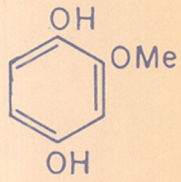

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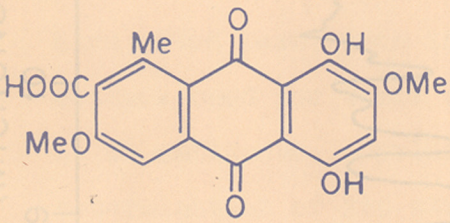

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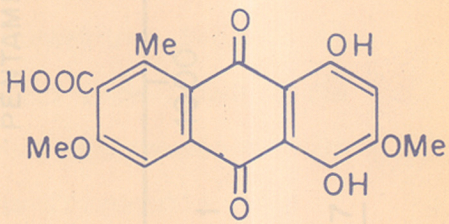

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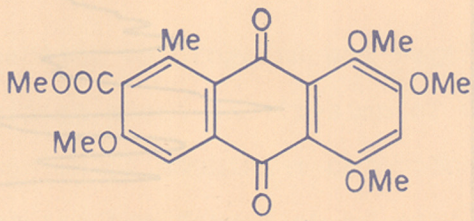
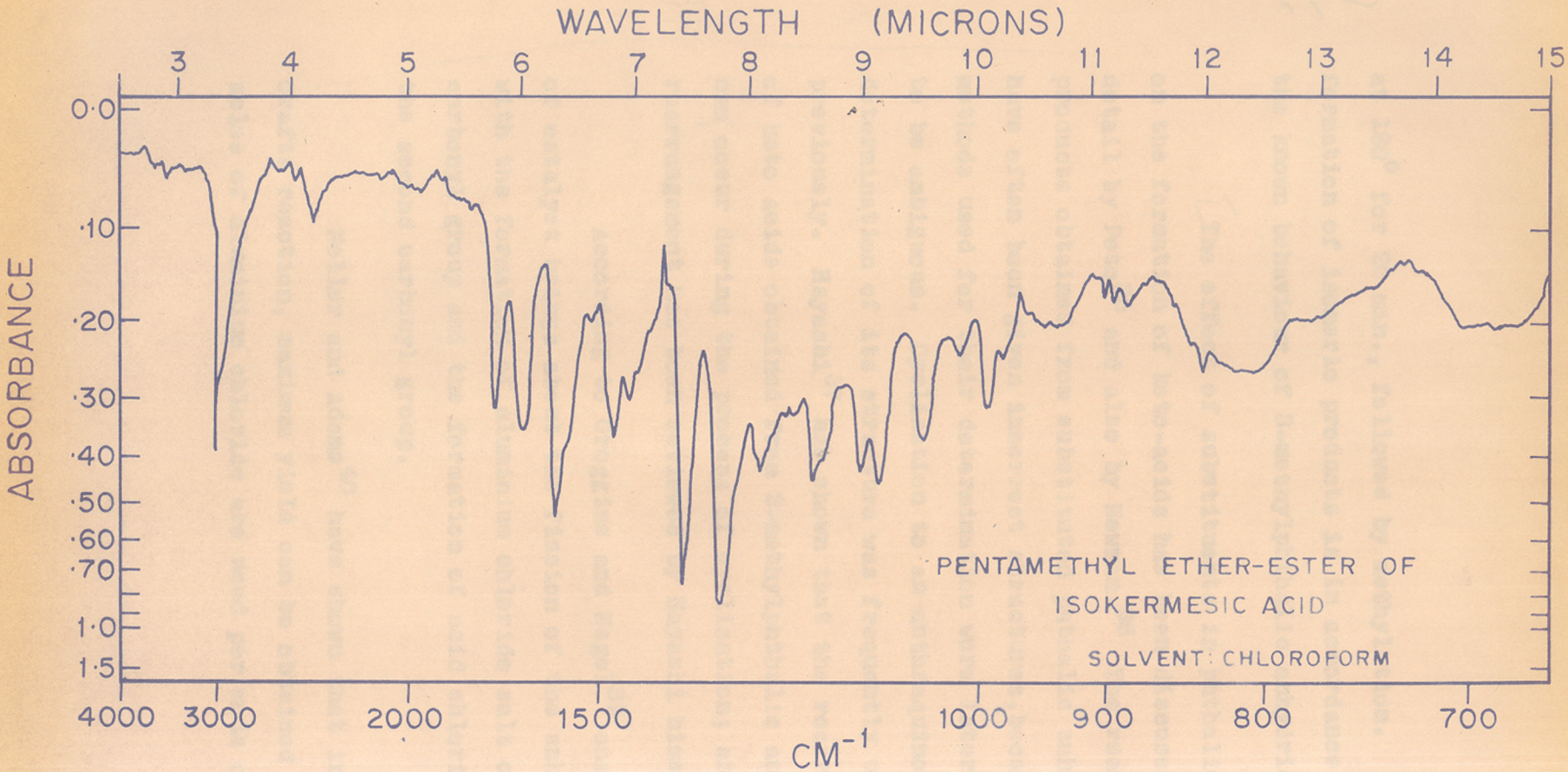


(54)


WAVELENGTH (MICRONS)
3
4
5
6
7
8
9
10
11
12
13
14
15
ABSORBANCE
0·0
·10
·20
·30
·40
·50
·60
·70
1·0
1·5
PENTAMETHYL ETHER-ESTER OF
ISOKERMESIC ACID
SOLVENT: CHLOROFORM
4000
3000
2000
1500
1000
900
800
700
CM⁻¹

Fig·7

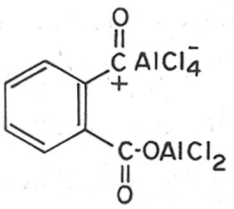
at 180° for 20 min., followed by methylation. The formation of isomeric products is in accordance with the known behaviour of 3-methylphthalic anhydride.[35,36]

The effect of substituents in phthalic anhydride on the formation of keto-acids has been discussed in detail by Peto[35] and also by Newman.[36] The reaction products obtained from substituted phthalic anhydrides have often been given incorrect structures, because the methods used for their determination were later found to be ambiguous. Cyclization to an anthraquinone and determination of its structure was frequently used previously. Hayashi[37] has shown that the rearrangement of keto acids obtained from 3-methylphthalic anhydride can occur during the process of cyclization; and the rearrangement has been reviewed by Hayashi himself.[38]

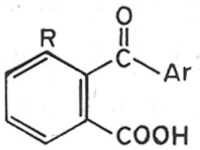
According to Groggins and Nagel[39] one mole of catalyst brings about the fission of the anhydride with the formation of aluminium chloride salt of one carbonyl group and the formation of acid chloride from the second carbonyl group.

Noller and Adams[40] have shown that in Friedel-Crafts reaction, maximum yield can be obtained when two moles of aluminium chloride are used per mole of anhydride.

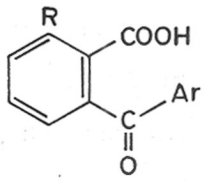
The reaction proceeds through a complex (55), which attacks the aromatic ring by virtue of the electrophilic centre on the acyl ion.



(55)



(TYPE A)



(TYPE B)

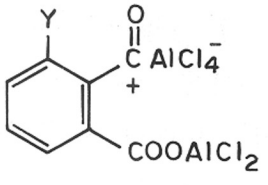
R = CH_3, Cl, NO_2, Br

A large number of Friedel-Crafts condensations of 3-substituted phthalic anhydrides (substitution by nitro,[41] bromo,[42] and acetamido[43]) with various reactants are reported to yield exclusively or predominantly the corresponding 3-substituted 2-aroylbenzoic acids (Type A). But if the 3-position is substituted by a methyl group, the normal tendancy is for the 6-methyl-2-aroylbenzoic acid[44-46] to be obtained preferentially (Type B). Newman[46] compared the reactions of 3-methyl and 3-chlorophthalic anhydride and his results are summarised in Table 2.

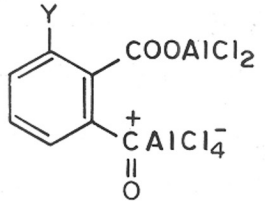
TABLE 2

Hydrocarbon	Phthalic anhydride			
	3-Methyl		3-Chloro-	
	Unhindered Type B %	Hindered Type A %	Unhindered Type B %	Hindered Type A %
Benzene	38	37	0	96
m-Xylene	42	32	0	89
Mesitylene	68	16	18	81

When 3-chlorophthalic anhydride reacts with benzene or m-xylene the product formed is 2-aryl-3-chlorobenzoic acid involving the condensation of (Type C) and explicable by assuming that either (Type C) is formed exclusively or that (Types C & D) are in mobile equilibrium



(Type C)

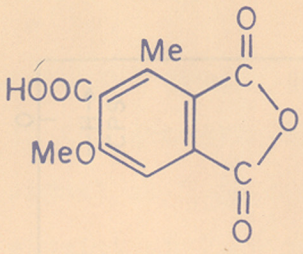


(Type D)

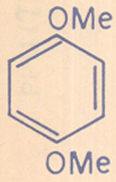
and (Type C) is more reactive. The former explanation seems unlikely because an 18% yield of product, resulting from (Type D) was obtained when mesitylene was employed as the reactant. If the second alternative is correct, there should be some explanation as to why the reaction proceeds from (Type C) when Y=chlorine; and yet, when $Y=CH_3$ there is little preference in the case of benzene and m-xylene. These reasons are not clear, but a combination of steric and electronic effects is probably involved. The reactions of 3-methylphthalic anhydride[35] are exceptional in the formation of less hindered ketones as the major product, but accompanied by substantial (and with benzene very nearly equal) amounts of the isomer. According to Newman and Scheurer[46] "the non-specificity of 3-methylphthalic anhydride in the Friedel-Crafts reaction seems unusual".

In the case of the anhydride (27) and 2-methoxy-hydroquinone (51), it appears that the tendency to form the acylium ion (49) in preference to (50) results in the formation of (52) in larger amount as compared to (53). The carbonyl group adjacent to the methyl in (27) may have some significant effect in the formation of (52) in large amounts.

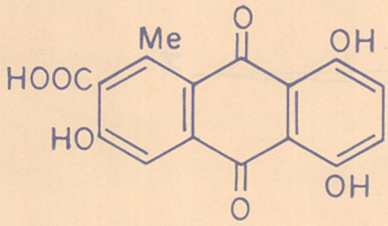
Finally the synthesis of kermesic acid (35) was achieved by the following method. Hydroquinone dimethyl ether (56) and 3-methyl-4-carboxy-5-methoxy phthalic anhydride (27) were condensed in a melt of aluminium chloride and sodium chloride at 180° for 15 min. The resultant red product (57) dissolved in aqueous sodium bicarbonate with a pink colour, and in sodium hydroxide with a violet-red colour, turning to yellow on the addition of dithionite. It gave a purple solution in conc. sulphuric acid and a brown colour with alcoholic ferric chloride. On methylation with ethereal diazomethane (57) gave (58). The same product was obtained by treating kermesic acid trimethyl ether-ester (37) in aqueous sodium hydroxide with sodium dithionite. Ayyangar et al[30] have shown that purpurin 2-methylether can be converted to quinizarin in quantitative yield by treating with sodium dithionite in alkaline solution. The mixed m.p. of the synthetic and natural products remained undepressed and the IR spectra also were superposable. Methylation of (57) with dimethyl sulphate and potassium carbonate in boiling acetone gave a product (59), which crystallised from methanol in yellow needles. The NMR spectrum of (59) in $CDCl_3$ (Fig. 8) showed three


Me
HOOC
MeO
O
C
O
C
O

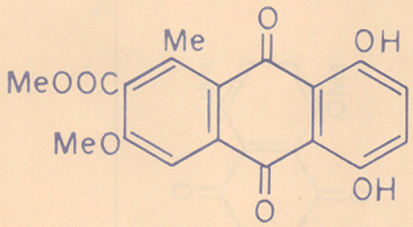
(27)


OMe
OMe

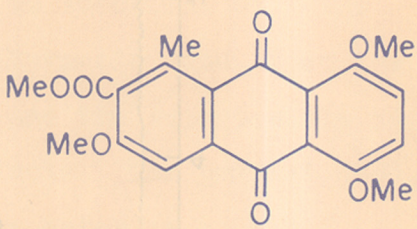
(56)


Me
OH
HOOC
HO
O
O
OH

(57)


Me
OH
MeOOC
MeO
O
O
OH

(58)


Me
OMe
MeOOC
MeO
O
O
OMe

(59)

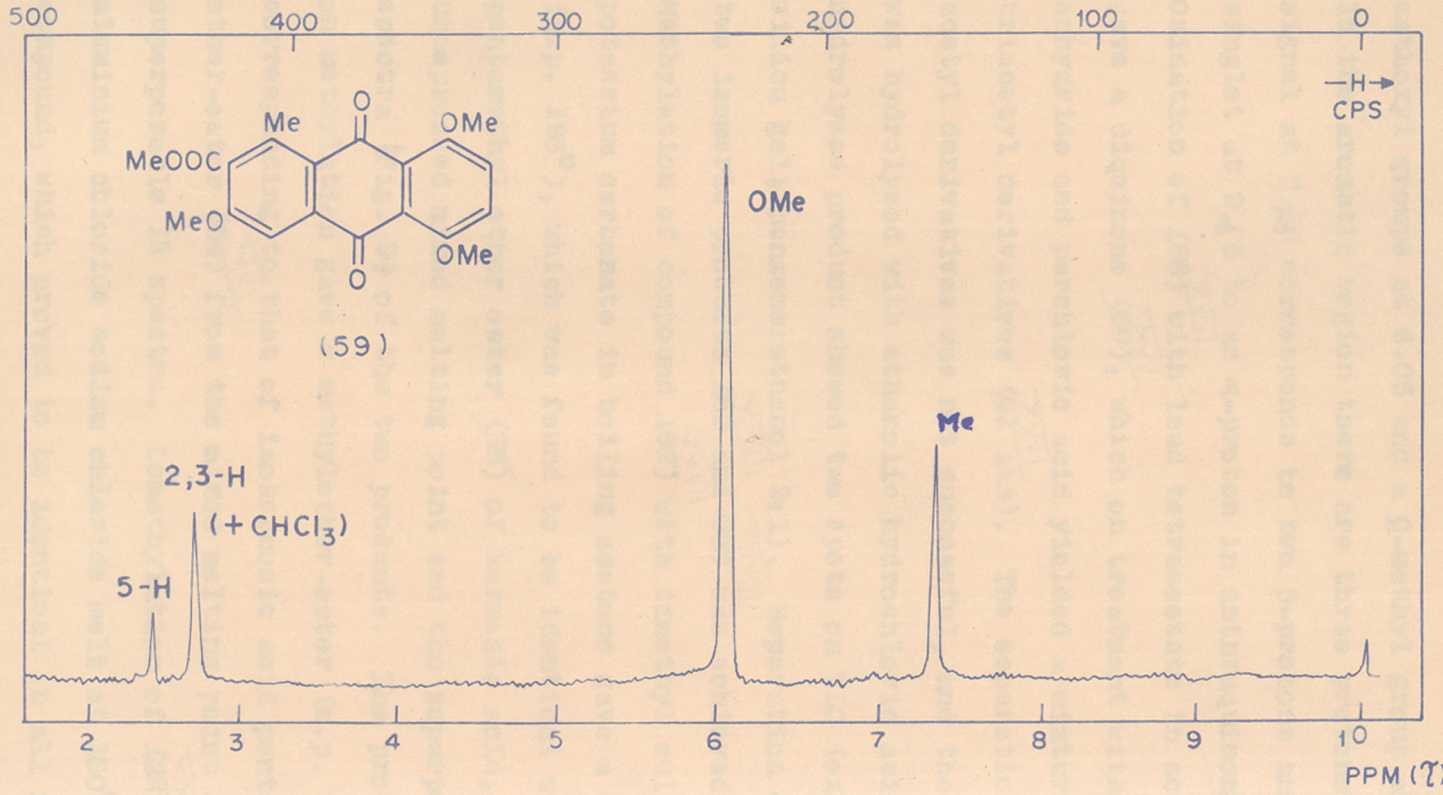
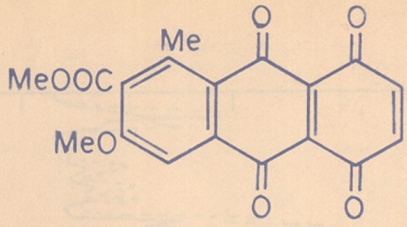

500
400
300
200
100
0
—H→
CPS
Me
OMe
MeOOC
MeO
OMe
O
O
O
(59)
OMe
Me
2,3-H
(+CHCl3)
5-H
2
3
4
5
6
7
8
9
10
PPM (τ)

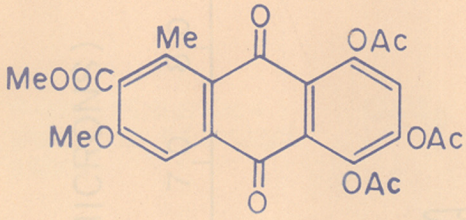
Fig. 8

methoxyl groups at 6.05 and a C-methyl group at 7.35. In the aromatic region there are three protons. A signal at 2.73 corresponds to two β-protons and a singlet at 2.45 to an α-proton in anthraquinone.

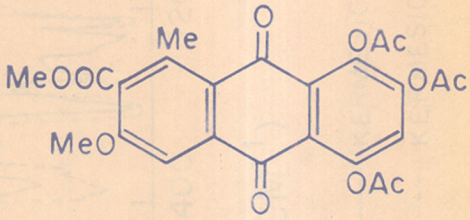
Oxidation of (58) with lead tetraacetate in acetic acid gave a diquinone (60), which on treatment with acetic anhydride and perchloric acid yielded a mixture of triacetyl derivatives (61 A&B). The separation of two acetyl derivatives was not successful, and the mixture was hydrolysed with ethanolic hydrochloric acid. The hydrolysed product showed two spots on TLC (oxalated silica gel; benzene:ethanol 9:1). Separation of the two isomeric products (62 and 63) was achieved by PLC. Methylation of compound (62) with dimethyl sulphate and potassium carbonate in boiling acetone gave a compound (m.p. 196°), which was found to be identical with the pentamethyl ether ester (38) of kermesic acid, from the undepressed mixed melting point and the superposable IR spectra (Fig. 9) of the two products. The product (63), on methylation gave a methylether-ester (m.p. 211°), corresponding to that of isokermesic acid pentamethyl ether-ester (54) from the mixed melting point and the superposable IR spectra. Demethylation of (38) using aluminium chloride sodium chloride melt at 160°, gave a compound, which proved to be identical in all respects with the natural kermesic acid.



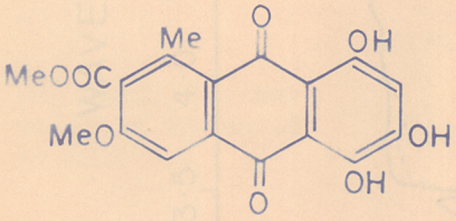
(60)



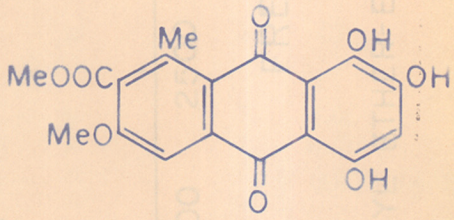
(61 A)



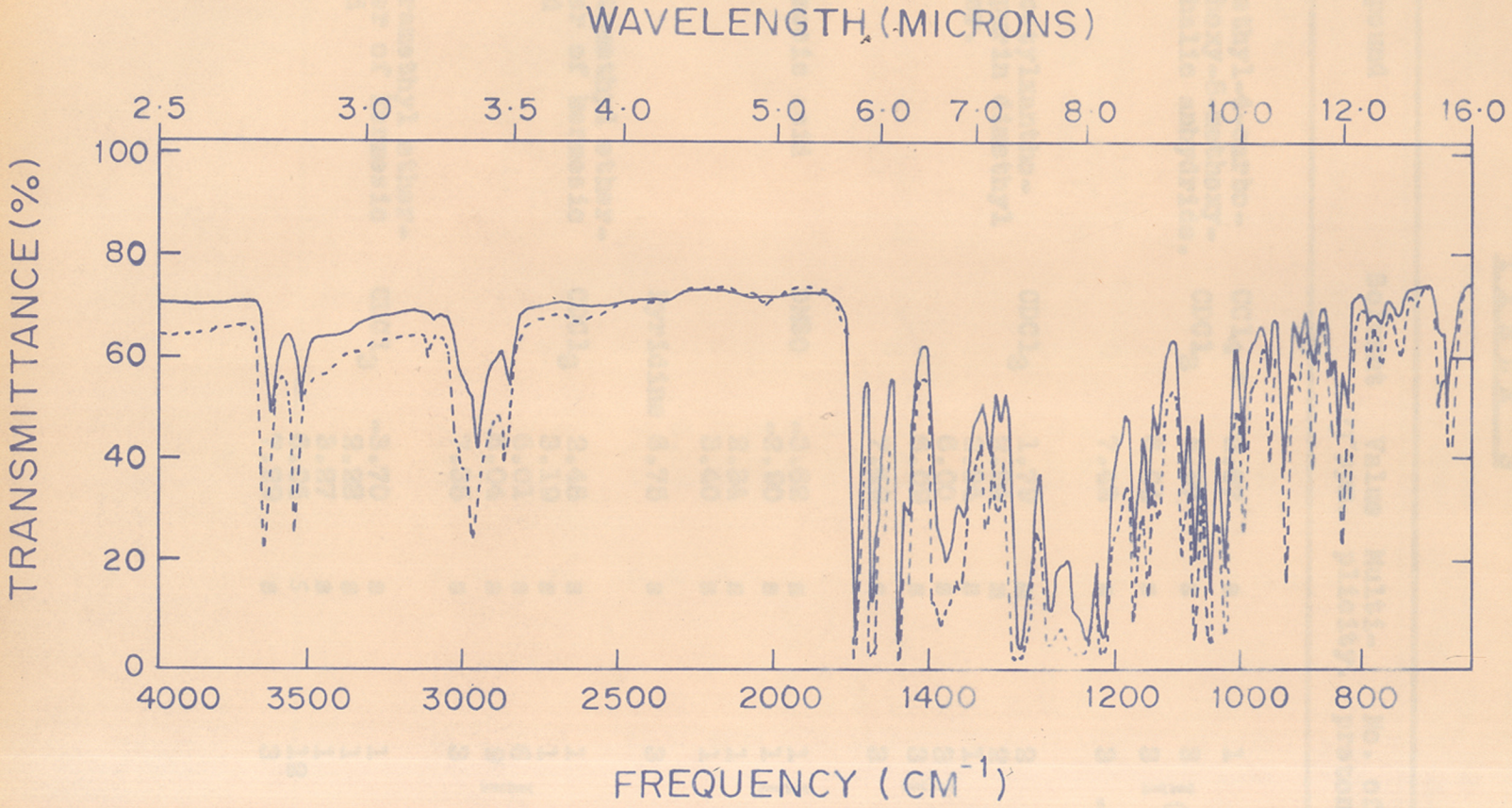
(61 B)



(62)



(63)


WAVELENGTH (MICRONS)
2·5
3·0
3·5
4·0
5·0
6·0
7·0
8·0
10·0
12·0
16·0
TRANSMITTANCE (%)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1400
1200
1000
800
FREQUENCY (CM⁻¹)
PENTAMETHYL ETHER-ESTER OF:
KERMESIC ACID (NATURAL)
KERMESIC ACID (Synthetic)

Fig·9

TABLE 3

No.	Compound	Solvent	Value τ ppm.	Multiplicity.	No. of protons	Assignment
1.	3-Methyl-4-carbomethoxy-5-methoxyphthalic anhydride.	CCl_4 + $CDCl_3$	2.67	s	1	6-H
			5.97	s	3	$(OCH_3)_2$ groups
			6.04	s	3	
			7.42	s	3	$-CH_3$ group
2.	2-Acetylxanthopurpurin dimethyl ether.	$CDCl_3$	1.79	m	2	5 and 8-H
			2.24	m	2	6 and 7-H
			2.34	s	1	4-H
			6.00	s	3	$(OCH_3)_2$ groups
			6.09	s	3	
			7.49	s	3	$-COCH_3$ group
3.	Kermesic acid	DMSO	-3.62	s	1	OH
			-2.90	s	1	
			2.34	s	1	5-H
			3.40	s	1	2-H
		Pyridine	6.75	s	3	$-CH_3$
4.	Pentamethyl ether-ester of kermesic acid	$CDCl_3$	2.45	s	1	5-H
			3.19	s	1	2-H
			6.01	s	6	$(OCH_3)_5$ groups
			6.04	s	9	
			7.35	s	3	$-CH_3$ group
5.	Tetramethyl ether-ester of kermesic acid	$CDCl_3$	-3.70	s	1	OH
			2.28	s	1	5-H
			3.27	s	1	2-H
			6.05	s	12	$(OCH_3)_4$ groups
			7.29	s	3	$-CH_3$ group.

Table 3 contd.

No.	Compound	Solvent	Value τ ppm.	Multiplicity.	No. of protons.	Assignment
6.	Pentamethyl ether-ester of isokermesic acid	$CDCl_3$	2.40	s	1	5-H
			3.27	s	1	3-H
			6.00	s	6	$(OCH_3)_5$ groups
			6.05	s	9	
			7.35	s	3	CH_3 group
7.	Tetramethyl ether-ester of xanthokermesic acid	$CDCl_3$	2.37	s	1	5-H
			2.69	d;J=3 cps	1	4-H
			3.23	d;J=3 cps	1	2-H
			6.00	s	12	$(OCH_3)_4$ groups
			7.30	s	3	$-CH_3$ group
8.	Desoxyerythrolaccin trimethyl ether	$CDCl_3$	2.47	d;J=3 cps	1	5-H
			2.68	d;J=3 cps	1	4-H
			3.00	d;J=3 cps	1	7-H
			3.26	d;J=3 cps	1	2-H
			6.03	s	3	$(OCH3)_3$ groups
			6.08	s	3	
			6.09	s	3	
			7.23	s	3	$-CH_3$ group
9.	1,4,6-Trimethoxy-7-carbomethoxy-8-methylanthraquinone	$CDCl_3$	2.45	s	1	5-H
			2.73	s	2	2 and 3-H
			6.05	s	12	$(OCH_3)_4$ groups
			7.35	s	3	$-CH_3$ group

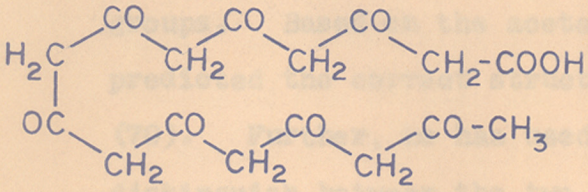
The biogenesis of kermesic acid and related anthraquinonoid insect pigments were presented in our earlier communication.

Biogenesis

Anthraquinones and some of the related anthrones are common metabolic products of higher plants, lichens, micro-organisms and insects. The acetate theory of biosynthesis which postulates that the head to tail union of acetic acid molecules is involved in the biogenesis of many phenols, was originally formulated in a simple manner by Collie[47] in 1907. Robinson[48] had shown that many naturally occurring quinones owe their origin to polyacetic acid precursors. For example, eight acetic acid units can build the heptaketopalmitic acid (64), which further cyclizes to anthrone (65) from which endocrocin (66), a component from lichen Nephromopsis endocrocea[49] and emodin (67) can be obtained.

Some structural alterations by oxidation of the side chains and removal of nuclear hydroxyl groups probably before cyclisation might have involved in the formation of compounds such as chrysophanol (68) and rhein (69).

The acetate hypothesis was further extended by Birch[50] by making suitable allowances for nuclear oxidation and reduction reactions and introduction of



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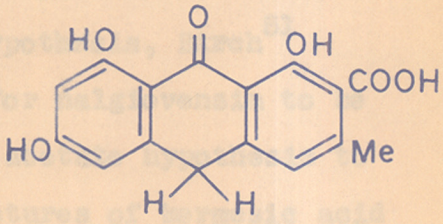
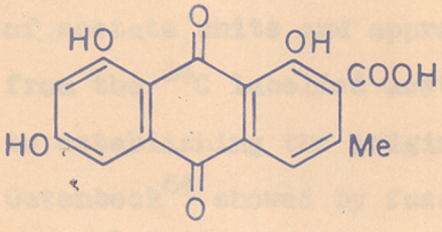
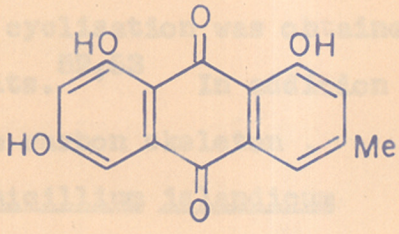


(65)



(66)



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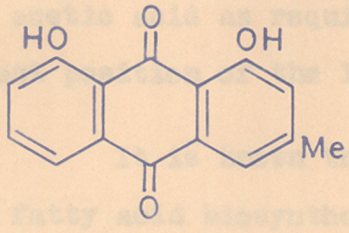

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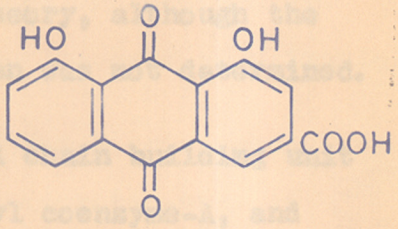
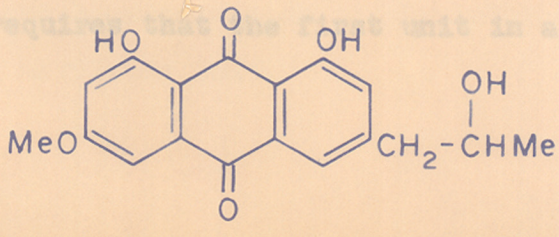


(69)



(70)

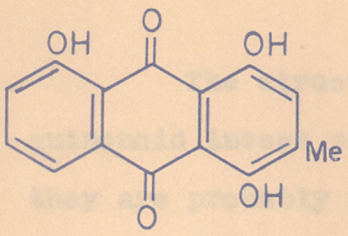
substituents such as methyl and isopentenyl or related groups. Based on the acetate hypothesis, Birch[51] predicted the correct structure for nalgiovensin to be (70). Further, he has used the acetate hypothesis to distinguish between the two structures of kermesic acid (21 and its isomer) and favoured (21) for the same.[24] The proof for the formation of anthraquinone nucleus (from fungal metabolites) from head to tail linkage of acetate units and appropriate cyclisation was obtained from the ^{14}C labelled acetate units.[52,53] In addition to establishing the origin of the carbon skeleton , Gatenbeck[54] showed by feeding Penicillium islandicum with (^{14}C-^{18}O)-acetate, that three oxygen functions in islandicin (71) and four in emodin (67) were labelled with ^{18}O and hence were derived from the carbonyl group of acetic acid as required by the theory, although the exact position of the labelled oxygen was not determined.

It is known that the actual chain building unit in fatty acid biosynthesis is malonyl coenzyme-A, and recent work with micro-organisms has established that (2-^{14}C)-malonate is incorporated into several "acetate-derived" phenolic compounds.[55] This biosynthetic modification requires that the first unit in a β-polyketide

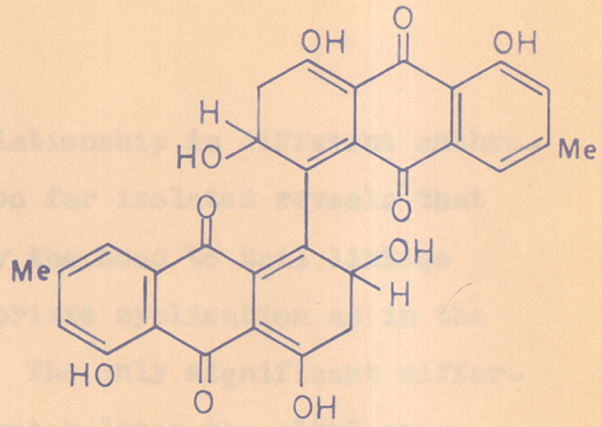
chain is supplied by acetic acid; for example orsenillic acid[56] and 6-methylsalicyclic acid[57] are formed by condensation of one acetate and three malonate units with loss of carbon dioxide. A further consequence is that in labelling experiments using acetate, higher incorporation of ^{14}C is to be expected at the first unit (in anthraquinones the β-methyl group and the neighbouring carbon atom). Shibata and Ikekawa[58] have shown that rugulosin (72) is biosynthesised by this route.

Some of the anthraquinones such as alizarin (73) and rubiadin (74) cannot be so simply explained from a polyketo methylene acid. But Leister and Zenk[59] have shown that in higher plants or at least Rubiaceae have developed an entirely different pathway for the biosynthesis of anthraquinones than fungi and acetate was incorporated via shikimic acid as in the case of alizarin.

The occurrence of anthraquinones and naphthaquinones together in the teak and in some species of Rubiaceae provide an evidence for the formation of anthraquinones in these plants from naphthalenic precursors. The experiments of Burnett and Thomson[60] provide evidence for formation of one benzenoid ring in the anthraquinone molecule from mevalonic acid.


OH
O
OH
Me
OH
O

(71)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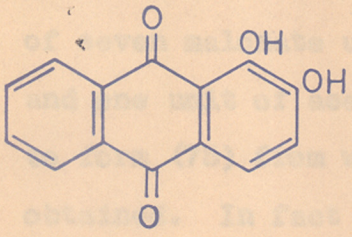
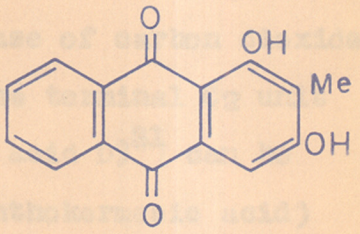
O
OH
H
HO
Me
O
Me
OH
H
HO
O
OH

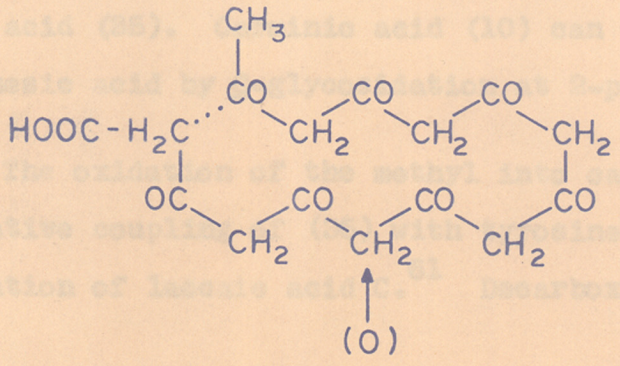
(72)


O
OH
OH
O

(73)


O
OH
Me
OH
O

(74)

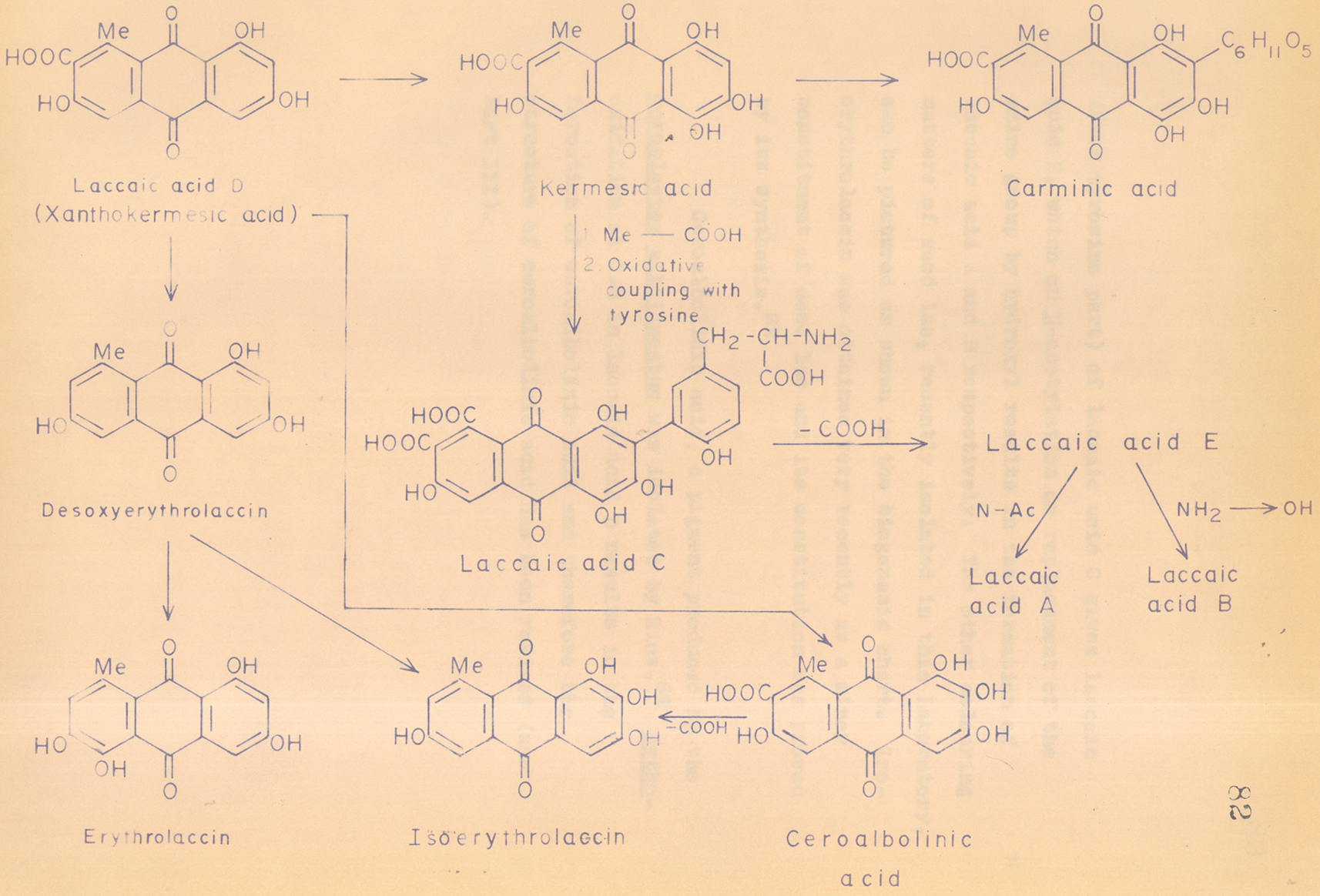

CH3
CO
CO
CO
HOOC-H2C
CH2
CH2
CH2
OC
CO
CO
CO
CH2
CH2
CH2
(O)

(75)

The structural relationship in different anthraquinonoid insect pigments so far isolated reveals that they are probably formed by the head to tail linkage of acetate units and appropriate cyclisation as in the case of mould metabolites. The only significant difference is that in the mould metabolites the alkyl group is at β-position of the anthraquinone molecule; while in most of the insect pigments it is at α-position. From this it appears that the anthraquinonoid insect pigments are biosynthesised by head to tail condensation of seven malonate units with the release of carbon dioxide and one unit of acetate which forms the terminal C_2 unit to form (75) from which (43) (laccaic acid D)[31] can be obtained. In fact laccaic acid D (xanthokermesic acid) appears to play a crucial role in the biosynthesis of other insect pigments. The introduction of hydroxyl group by para-oxidation will result in the formation of kermesic acid (35). Carminic acid (10) can be obtained from kermesic acid by C-glycosidation at 2-position.

The oxidation of the methyl into carboxyl group and oxidative coupling of (35) with tyrosine results in the formation of laccaic acid C.[61] Decarboxylation

Biosynthesis of some insect pigments derived from anthraquinone



(from tyrosine part) of laccaic acid C gives laccaic acid E, which on N-acetylation or replacement of the amino group by hydroxyl results in the formation of laccaic acid A and B respectively. The other colouring matters of seed lac, recently isolated in this Laboratory, can be pictured as shown in the biogenesis chart. Iso-erythrolaccin was obtained very recently as a minor constituent of seed lac and its constitution was proved by its synthesis.[32]

Ceroalbolinic acid, a pigment produced by the Ceroplastes albolineatus was isolated by Rios.[62] Ortho-oxidation at C-2 in laccaic acid D results in the formation of ceroalbolinic acid and therefore the structure of ceroalbolinic acid has been revised (see Part III).

EXPERIMENTAL

6-Methoxy-2,4-dimethylisophthalic acid (7)

It was prepared according to the method described by Bhatia and Venkataraman.[6]

5-Methoxytoluene-2,3,6-tricarboxylic acid (8)

To the above acid (1.7 g) in sodium carbonate (2 N; 15 ml) was added a warm solution of potassium permanganate (4.8 g) in water (100 ml) and the mixture heated on a steam bath for 3 hr. Excess permanganate was destroyed by the addition of ethanol, the solution was filtered and washed with hot water. The filtrate was concentrated to 25 ml., acidified with conc. hydrochloric acid and left in a refrigerator overnight, when colourless crystals separated. Repeated crystallisation from acidulated water gave colourless needles (0.8 g), which softened at 200°, and melted at 224-226° (Overeem and van der Kerk[7]. The acid softened at 200 and sublimed, finally melting at 228-230°, Bhatia and Venkataraman[6] 213°) (Found: C, 52.2; H, 4.2. $C_{11}H_{10}O_7$ requires: C, 52.0; H, 4.0%).

The methyl ester, prepared by treatment with ethereal diazomethane, crystallised from benzene-hexane

in colourless needles, m.p. 110-111° (lit.[6,7] m.p. 110°) (Found: C, 56.5; H, 5.5. $C_{14}H_{16}O_7$ requires: C, 56.8; H, 5.4%).

Anisole-2,3,4,5-tetracarboxylic acid (9)

To the above acid (5 g) in sodium hydroxide solution (2 N; 40 ml) was added potassium permanganate (10 g) in water (100 ml) and heated on a water bath for 12 hr. Excess of permanganate was destroyed by the addition of ethanol; the solution was filtered and washed with hot water. The filtrate, after concentrating to 40 ml. and acidification with conc. hydrochloric acid, was extracted with methyl ethyl ketone. Concentration of the solvent gave a solid (3.6 g), which was crystallised from acidulated water in colourless needles (1.6 g), m.p. 232-234° (Found: C, 46.2; H, 2.9. $C_{11}H_8O_9$ requires: C, 46.5; H, 2.8%).

The methyl ester, prepared by treatment with ethereal diazomethane, crystallised from methanol in colourless needles, m.p. 129-130° (Tsuji[16] 128-128.5°) (Found: C, 53.1; H, 4.7. $C_{15}H_{16}O_9$ requires: C, 52.9; H, 4.7%). The mixed m.p. with methylation product of the acid obtained by the oxidation of laccaic acid A methyl ether-ester remained undepressed.

Phenol-2,3,4,5-tetracarboxylic acid (1)

To a melt prepared from anhydrous aluminium chloride (2.5 g) and dry sodium chloride (0.5 g), tetramethyl anisole-2,3,4,5-tetracarboxylate (0.5 g) was added and the mixture stirred at 150° for 10 min. It was cooled and poured over ice cold hydrochloric acid (2%). The solution was extracted with methyl ethyl ketone which on concentration gave a dark brown compound (0.15 g). Repeated crystallisation from acidulated water gave colourless needles, m.p. 238-240° (lit.[15] 241-243°) (Found: C, 44.5; H, 2.3. $C_{10}H_6O_9$ requires: C, 44.4; H, 2.2%). It gave a violet colouration with alcoholic ferric chloride solution. Mixed m.p. with the sample, obtained from Professor Maximov remained undepressed.

3-Methyl-4-carboxy-5-methoxyphthalic anhydride (27)

A mixture of 5-methoxy-3-methyltrimellitic acid (8) (1.5 g) and freshly distilled acetic anhydride (3 ml), was refluxed for ½ hr. On cooling, colourless needles separated which were filtered, washed with water and dried (0.75g). Crystallisation from dry benzene gave colourless needles, m.p. 233-234° (Overeem and van der Kerk[7]

234-235°) (Found: C, 55.5; H, 3.6. $C_{11}H_8O_6$ requires: C, 55.9; H, 3.4%).

The methyl ester, prepared by treatment with ethereal diazomethane, crystallised from benzene-hexane in colourless needles, m.p. 148-149° (Dimroth[21] 149°; Rios[62] 149-151°) (Found: C, 57.8; H, 4.2. $C_{12}H_{10}O_6$ requires: C, 57.6; H, 4.0%).

Action of borontrifluoride-acetic acid complex on 1,3-dihydroxyanthraquinone (xanthopurpurin)

A mixture of 1,3-dihydroxyanthraquinone (0.4 g) and boron trifluoride-acetic acid complex (5 ml) was refluxed for 4 hr. It was poured into a saturated solution of sodium acetate (100 ml). The separated solid was filtered and dried (0.3 g). It was identified as the starting material from the mixed melting point and chromatographic behaviour.

2-Acetylxanthopurpurin (28)

To a melt prepared from anhydrous aluminium chloride (15 g) and dry sodium chloride (3 g) at 150-160°, a mixture of phthalic anhydride (1.5 g) and 2-acetyl-resorcinol monomethyl ether (1 g) was added and the mixture stirred at 180° for 15 min. It was cooled and poured in 2% hydrochloric acid (25 ml), and the separated

brown product filtered, washed free of acid and dried). It was taken in benzene and passed through a short silica gel column. The benzene extract on concentration gave yellow needles (0.5 g), m.p.200-202° (Found: C, 68.5; H, 3.6. $C_{16}H_{10}O_5$ requires: C, 68.1; H, 3.5%). It gives violet colour in sodium hydroxide solution, orange in conc. sulphuric acid, and deep orange with alcoholic ferric chloride.

Methylation of 2-acetylxanthopurpurin

A mixture of 2-acetylxanthopurpurin (0.08 g), acetone (15 ml), anhydrous potassium carbonate (0.5 g) and dimethyl sulphate (0.2 ml) was refluxed on water bath for 24 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which crystallised from methanol in yellow needles (0.05 g), m.p. 152.3° (Found: C, 70.1; H, 4.7. $C_{18}H_{14}O_5$ requires: C, 69.7; H, 4.5%).

4,6-Dichloro-2-acetylresorcinol (29)

A mixture of 2-acetylresorcinol (0.7 g), and sulphuryl chloride (0.45 ml) in dry ether (15 ml) was allowed to stand at room temperature for 24 hr. Removal of the solvent and crystallisation of the solid from ethanol gave yellow needles (0.25 g), m.p. 176-178° (Found: C, 43.6; H, 2.8; Cl, 31.7 $C_8H_6Cl_2O_3$ requires: C, 43.4; H, 2.7; Cl, 32.1%).

31·7 43·4 32·1

4 or 6-Chloro-2-acetylresorcinol-1-methylether (30)

A mixture of 2-acetylresorcinol monomethyl ether (5 g) and sulphuryl chloride (2.8 ml) in dry ether (50 ml) was allowed to stand at room temperature for 24 hr. Ether was distilled off and the yellow oil obtained was taken in ether, washed with sodium bicarbonate solution and water. Ethereal solution was dried over anhydrous sodium sulphate. Concentration of ether gave a yellow oil (4 g), which was distilled under reduced pressure, b.p.150°/5 mm. (Found: C, 53.8; H, 4.4; Cl, 18.0. $C_9H_9ClO_3$ requires: C, 53.9; H, 4.5; Cl, 17.8%). It gave a positive halogen test.

Condensation of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (27) with 4- or 6-chloro-2-acetylresorcinol-1-methyl ether (30).

To a melt prepared from anhydrous aluminium chloride (8) and dry sodium chloride (1.5 g) at 150-155°, a mixture of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.5 g) and mono-chloro-2-acetylresorcinol-1-methyl ether (0.4 g) was added and the mixture stirred at 180° for 15 min. The melt was cooled and poured in 2% hydrochloric

acid. The dark black product was filtered, washed with water and dried (0.3 g). It did not show any test for hydroxyanthraquinones.

Condensation of the anhydride (27) with 3,6-dihydroxy-2-methoxyacetophenone (32) using phosphorous oxychloride and phosphorous pentoxide.

A mixture of the anhydride (0.3 g), 3,6-dihydroxy-2-methoxyacetophenone (0.25 g), phosphorous oxychloride (5 ml) and phosphorous pentoxide (1 g) was boiled for 12 hr. It was cooled and poured on ice, a dark brown resinous product was obtained, which was filtered and dried (0.1 g). It did not show any test for anthraquinones.

Condensation of 3,6-dihydroxy-2-methoxyacetophenone (32) with 3-methyl-4-carboxy-5-methoxyphthalic anhydride (27): (34).

To a melt prepared from anhydrous aluminium chloride (20 g) and dry sodium chloride (4 g) at 150-160°, a mixture of 3,6-dihydroxy-2-methoxyacetophenone (1.1 g) and 3-methyl-4-carboxy-5-methoxyphthalic anhydride (1 g) was added and the mixture stirred at 180° for 15 min. The melt was cooled and poured in 2% hydrochloric acid. The brick-red

precipitate thus obtained was filtered, washed with water and dried (0.5 g). The product was purified by chromatography on oxalated silica gel plates [benzene-alcohol (4:1)]. It was collected, washed with water to remove oxalic acid and dried. The silica gel was extracted with acetone, which on concentration gave a red crystalline compound (0.08 g). It was crystallised from acetic acid in red needles, m.p. dec. above 260°, (Found: C, 57.8; H, 3.1. $C_{18}H_{12}O_9$ requires: C, 58.1; H, 3.2%). The Rf values of this product and the sample of kermesic acid, obtained from Professor K. Dimroth, on TLC over oxalated silica gel using benzene-alcohol (4:1), were found to be different. Rf: synthetic product 0.47; kermesic acid 0.33.

Condensation of the anhydride (27) with 2,3,6-trimethoxyacetophenone (33)

To a melt prepared from anhydrous aluminium chloride (10 g) and dry sodium chloride (2 g) at 150-155°, a mixture of 2,3,6-trimethoxyacetophenone (0.5 g) and the anhydride (0.6 g) was added and the mixture stirred at 180° for 15 min. The melt was cooled and poured in 2% hydrochloric acid. The brick red precipitate thus obtained was filtered, washed with water and dried (0.3 g). Chromatography over oxalated silica gel and benzene

alcohol (9:1) gave a red product (0.04 g). It crystallised from acetic acid in red needles, m.p. dec. above 260°. It was found to be identical with the product (34) by chromatographic behaviour on oxalated TLC plate.

Kermesic acid

A sample of the natural product was obtained from Professor K. Dimroth. The slight impurity which was observed on oxalated silica gel plate was removed by crystallisation. It crystallised from acetic acid in red needles, m.p. sinters at 284-285° and dec. 290-291°.

Methylation of kermesic acid

A mixture of kermesic acid (0.15 g), anhydrous potassium carbonate (3 g), dimethyl sulphate (1 ml), and acetone (50 ml) was refluxed on a water bath for 72 hr. The reaction mixture was tested for ferric chloride colouration occasionally. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained. It was taken in chloroform. The chloroform solution was chromatographed on silica gel plates (benzene:acetone 4:1). The major fraction was collected

extracted with acetone and crystallised from methanol in yellow needles, m.p. 196° (0.11 g) (Found: C, 62.8; H, 4.9. $C_{21}H_{20}O_8$ requires: C, 63.0; H, 5.0%). The IR spectrum of this compound in KBr shows bands at 1680 (quinone C=O), 1735 cm^{-1} (ester C=O). The minor fraction which gave a colour with ferric chloride was crystallised from methanol (0.02 g), m.p. 244°.

Trimethyl ether-ester of kermesic acid (37)

Method A: A mixture of kermesic and pentamethyl ether-ester (0.5 g) in dry methylene chloride (3 ml) and hydrobromic acid in acetic acid (30%; 4 ml) was left at room temperature for 24 hr. and then poured over ice cold water. The orange-red compound was filtered, washed with water and dried (0.4 g). It crystallised from acetic acid in long orange needles, m.p. 316° (Dimroth[21] 310°) (Found: C, 61.4; H, 4.5. $C_{19}H_{16}O_8$ requires: C, 61.2; H, 4.3%).

Method B: A mixture of kermesic acid (0.5 g) and a solution of diazomethane in dry ether was allowed to stand at 0-5° for 24 hr. The excess of diazomethane was destroyed by addition of a few drops of acetic acid.

and the ether was distilled off. The orange compound (0.4 g) was bicarbonate insoluble. It was crystallised from acetic acid, m.p. 316°.

1,4-Dihydroxy-6-methoxy-7-carbomethoxy-8-methyl-anthraquinone

A mixture of kermesic acid trimethyl ether-ester (0.5 g), sodium hydroxide solution (2%; 50 ml) and sodium hydrosulphite (0.8 g) was stirred on water bath for 2 hr. The original violet red colour changed to brown. It was cooled and air-oxidised for 1 hr. Acidification with hydrochloric acid gave an orange precipitate which was filtered, washed with water and dried (0.3 g). It crystallised from methanol in shining plates; m.p. 232° (Found: C, 63.4; H, 4.2. $C_{18}H_{14}O_7$ requires: C, 63.2; H, 4.1%). The compound showed all characteristic properties of quinizarin. The IR spectrum of this compound in chloroform shows bands at 1740 (ester C=O) and 1630 cm^{-1} (bonded C=O).

1,4,6-Trimethoxy-7-carbomethoxy-8-methylanthraquinone

A mixture of the above compound (0.25 g), dimethyl sulphate (2 ml), anhydrous potassium carbonate (2 g) and dry acetone (50 ml) was refluxed on water bath

for 24 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which crystallised from methanol as yellow needles (0.2 g), m.p. 250-252° (Found: C, 64.6; H, 4.6. $C_{20}H_{18}O_7$ requires: C, 64.8; H, 4.8%).

Xanthokermesic acid (43)

Kermesic acid (1 g), in aqueous sodium hydroxide solution (2%; 50 ml), was hydrogenated in presence of Raney-nickel. After absorption of two moles of hydrogen, the colour of the solution turned from violet to yellow. It was then air oxidised for 1 hr. Acidification with conc. hydrochloric acid gave a yellow precipitate, which was filtered, washed with water and dried (0.8 g). It was crystallised from methanol, m.p.$>$300°. The Rf value was identical with that of laccaic acid D on TLC plate (oxalated silica gel, benzene:alcohol 8:2).

Methylation of xanthokermesic acid (44)

A mixture of the above product (0.8 g), dimethyl sulphate (2 ml), potassium carbonate (5 g), and acetone (50 ml), was refluxed on a water bath for 6 hr. After removal of the solvent and dilution of the

mixture with water, a yellow product was obtained, which crystallised from methanol (0.7 g), m.p. 226°, undepressed when mixed with the methylated laccaic acid D[31] and their IR spectra were superposable.

Decarboxylation of xanthokermesic acid (45)

A mixture of xanthokermesic acid (0.5 g) and freshly distilled diethylaniline (2 ml) was refluxed for 2 hr. The solution was cooled and diluted with hydrochloric acid. The mixture was extracted with ether (3x20 ml) and shaken thrice with dilute hydrochloric acid and then with water. Removal of ether gave a solid (0.3 g); its chromatographic behaviour was identical with the desoxyerythrolaccin[31] on oxalated silica gel plate (benzene:alcohol 4:1).

Methylation of the above product (47)

A mixture of the above product (0.25 g), dimethyl sulphate (1 ml), anhydrous potassium carbonate (3 g) and acetone (50 ml) was refluxed for 12 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which crystallised from methanol in yellow needles, m.p. 204°; undepressed with a sample of desoxyerythrolaccin trimethyl ether.[31]

Condensation of 3-methyl-4-carboxy-5-methoxyphthalic anhydride with 1,2,4-triacetoxy benzene.

To a melt prepared from anhydrous aluminium chloride (10 g) and dry sodium chloride (3 g) at 150-155°, a mixture of 1,2,4-triacetoxybenzene (0.5 g) and 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.5 g) was added and the mixture stirred at 180° for 15 min. It was cooled and poured over 2% hydrochloric acid. The semi-solid separated was filtered and dried (0.2 g).

Methylation of the above product

A mixture of the above product (0.1 g), dimethyl-sulphate (0.5 ml), potassium carbonate (2 g), and acetone (25 ml) was refluxed on water bath for 12 hr. After removal of the solvent and dilution with water, a dark brown gummy product separated which was taken in chloro-form. Concentration of chloroform gave a brown resinous product, which failed to crystallise. Its chromatographic behaviour on TLC silica gel plates (benzene:acetone 4:1) was found to be different from the methyl tetramethyl kermesate.

Condensation of phthalic anhydride and hydroquinone using boron trifluoride etherate.

A mixture of phthalic anhydride (0.2 g), hydroquinone (0.15 g) and boron trifluoride etherate (2 ml)

was heated on water-bath for 4 hr. The colour of the mixture turned red at the end of the reaction. It was cooled and poured on water. The solid obtained was filtered, washed with bicarbonate, water and dried (0.14 g). It crystallised from acetic acid in red plates, m.p. 198-199° (lit.[34] 199-200°). It was identified as quinizarin from m.p. and chromatographic behaviour.

Condensation of phthalic anhydride and 2-methoxy-hydroquinone using boron trifluoride etherate

A mixture of phthalic anhydride (0.2 g), 2-methoxyhydroquinone (0.18 g) and boron trifluoride etherate (2 ml) was heated on water bath for 4 hr. The colour of the mixture turned red at the end of the reaction. It was cooled and poured on ice cold water. The solid obtained was filtered, washed with bicarbonate, water and dried (0.19 g). It crystallised from acetic acid, m.p. 237° (lit.[63] 238°). It was identified as purpurin-2-methylether from melting point and chromatographic behaviour.

Condensation of 3-chlorophthalic anhydride and hydroquinone using boron trifluoride etherate

A mixture of 3-chlorophthalic anhydride (0.2 g), hydroquinone (0.12 g) and boron trifluoride etherate (2 ml) was heated on water bath for 4 hr. It was cooled and poured on ice cold water. The semi solid product was extracted with chloroform and passed through short column of silica gel and benzene. Concentration of the solvent gave a red product (0.03 g), which crystallised from methanol in red needles, m.p. 242° (lit.[64] 243°).

Condensation of phthalic anhydride and 4-methylcatechol monomethylether using boron trifluoride etherate.

A mixture of phthalic anhydride (0.3 g), 4-methylcatechol monomethyl ether (0.28 g) and boron trifluoride etherate (3 ml) was heated on water bath for 4 hr. It was cooled and poured on ice cold water. The semisolid product was taken in chloroform and passed through a short column of silica gel, a yellow band was eluted with benzene. The benzene fraction on concentration gave a product (0.06 g), which crystallised from methanol as yellow needles, m.p. 202-204°.

Its chromatographic behaviour on TLC silica gel plate (benzene) was identical with 1-hydroxy-2-methoxy-4-methylanthraquinone.

Condensation of 2-methoxyhydroquinone (51) with the anhydride (27) using boron trifluoride etherate

A mixture of 2-methoxyhydroquinone (0.1 g), 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.18 g), and boron trifluoride etherate (5 ml) was heated on water bath for 4 hr. The brown-red solution was cooled and poured on water. The solid separated was taken in ethanol and warmed for 10-15 minutes on water bath. Removal of the solvent gave a red compound (0.04 g). Chromatography over oxalated silica gel TLC plate, using benzene-alcohol mixture (9:1) showed two spots; one major and a minor fraction.

Methylation of the above product (54)

A mixture of the above product (0.04 g), dimethyl sulphate (0.5 ml), potassium carbonate (1 g) and acetone (25 ml) was refluxed on a water bath for 24 hr. After removal of the solvent and dilution of the mixture with water gave a product, which in its

chromatographic behaviour on TLC silica gel plates, using benzene-acetone mixture (4:1) showed two spots (Rf 0.32 and 0.38). The major fraction (Rf 0.32) was separated by PLC over silica gel plates, using the same solvent system. It crystallised from methanol as yellow needles, m.p. 211° (0.03 g). (Found: C, 62.6; H, 5.1. $C_{21}H_{20}O_8$ requires: C, 63.0; H, 5.0%). The IR spectrum in chloroform shows band at 1740 (ester C=O), 1680 (quinone C=O) and 1490 cm^{-1} (C=C). The minor fraction (Rf = 0.38) (negligible amount) in its chromatographic behaviour on TLC silica gel plate, using benzene:acetone mixture (4:1) was found to be identical with pentamethyl ether-ester of kermesic acid.

Condensation of 2-methoxyhydroquinone with 3-methyl-4-carboxy-5-methoxyphthalic anhydride

To a melt prepared from anhydrous aluminium chloride (5 g) and dry sodium chloride (0.5 g) at 150-155°, a mixture of 2-methoxyhydroquinone (0.2 g) and 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.25 g) was added and the mixture stirred at 180° for 20 min. It was cooled and poured over 2% hydrochloric acid. The product obtained was filtered, washed with water and dried (0.1 g).

Methylation of the above product

A mixture of the above product (0.1 g), dimethyl sulphate (0.5 ml), potassium carbonate (2 g) and acetone (25 ml) was refluxed on water bath for 12 hr. After removal of the solvent, and dilution of the mixture with water, a yellow product was obtained. The product showed two spots on its chromatographic behaviour over TLC using benzene:acetone (4:1). The major fraction (Rf 0.32), m.p. 210-211°, was found to be identical with the isokermesic acid pentamethyl ether-ester from its m.p. and chromatographic behaviour over TLC plates. The minor fraction (Rf 0.38) was found to be identical with the pentamethyl ether-ester of the kermesic acid by its chromatographic behaviour on TLC silica gel using benzene:acetone (4:1) mixture as a solvent.

1,4,6-Trihydroxy-8-methylanthraquinone-7-carboxylic acid (57)

To a melt prepared from anhydrous aluminium chloride (6 g) and dry sodium chloride (2 g) at 150-155°, a mixture of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.5 g) and hydroquinone dimethyl ether (0.3 g) was added

and the mixture stirred at 180° for 15 min. The melt was poured in 2% hydrochloric acid. The brick-red precipitate was filtered, washed with water and dried (0.3 g). It crystallised from acetic acid in red needles, m.p. darkens at 292° and does not melt $<$300° (Found: C, 61.4; H, 3.3. $C_{16}H_{10}O_7$ requires: C, 61.2; H, 3.2%).

1,4-Dihydroxy-6-methoxy-7-carbomethoxy-8-methylanthraquinone (58)

A mixture of the above product (0.5 g) in ethereal diazomethane solution (excess) was left overnight at 0-5°. Excess of diazomethane was destroyed by the addition of few drops of acetic acid. Removal of ether gave an orange product, which was dissolved in chloroform and passed through a short column of silica gel and eluted with benzene. The benzene eluate on concentration gave a compound which crystallised from chloroform-methanol in orange needles, m.p. 232°. Mixed m.p. with 1,4-dihydroxy-6-methoxy-7-carbomethoxy-8-methylanthraquinone, obtained from the trimethyl ether-ester of kermesic acid, remained undepressed.

1,4,6-Trimethoxy-7-carbomethoxy-8-methylanthraquinone (59)

A mixture of 1,4,6-trihydroxy-8-methylanthraquinone-7-carboxylic acid (0.1 g), dimethyl sulphate (0.5 ml), potassium carbonate (1 g) and acetone (25 ml) was refluxed on water bath for 24 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which crystallised from methanol in yellow needles, m.p. 250-252° (Found: C, 64.5; H, 4.7. $C_{20}H_{18}O_7$ requires: C, 64.8; H, 4.8%).

6-Methoxy-7-carbomethoxy-8-methyl-1,4,9,10-anthradiquinone (60)

A mixture of 1,4-dihydroxy-6-methoxy-7-carbomethoxy-8-methyl anthraquinone (0.8 g), lead tetraacetate (1.6 g) and glacial acetic acid (3 ml) was triturated in a pestal and mortar for 5 min. The colour slowly changed from red to brown. It was diluted with water and the yellow product filtered and dried (0.65 g). It crystallised from benzene:hexane mixture in brown needles, m.p. 184-185° (Found: C, 63.2; H, 3.7. $C_{18}H_{12}O_7$ requires: C, 63.5; H, 3.5%).

Acetylation of the diquinone (61A & 61B)

A mixture of the above product (0.5 g), acetic anhydride (5 ml) and perchloric acid (0.1 ml) was left overnight at room temperature. It was poured on water, the yellow precipitate was filtered and dried (0.3 g). The product without any purification was used for further reaction.

Hydrolysis of the acetate (62 and 63)

A mixture of the above acetate (0.4 g), ethanol (25 ml) and conc. hydrochloric acid (2.5 ml) was refluxed on water bath for 3 hr. After removal of the solvent and dilution of the mixture with water, a dark red product was obtained. Chromatographic behaviour on oxalated silica gel plate using benzene-alcohol mixture (9:1), the product showed two spots. Separation of the two fractions was achieved by PLC over oxalated silica gel using the same solvent system.

Fraction I (0.07 g) crystallised from methanol as red needles, m.p. 240-241° (Found: C, 60.7; H, 4.1. $C_{18}H_{14}O_8$ requires: C, 60.4; H, 3.9%). The IR spectrum in nujol shows bands at 1735 (C=O ester) and 1625 cm-1 (bonded carbonyl).

Fraction II (0.05 g) crystallised from methanol in red needles, m.p. 255-257°. The IR spectrum in nujol shows bands at 1730 (C=O ester) and 1625 (bonded carbonyl). Both the fractions I and II were characterised as their methyl ether-esters.

Methylation of fraction I

A mixture of the above fraction I (0.07 g), dimethyl sulphate (0.5 ml), potassium carbonate (1 g) and acetone (25 ml) was refluxed on water bath for 24 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which crystallised from methanol as yellow needles (0.05 g), m.p.196°. Mixed m.p. with the pentamethyl ether-ester of kermesic acid shows no depression. The IR spectra of this product and the pentamethyl ether-ester of kermesic acid are superposable.

Methylation of the Fraction II

A mixture of the fraction II (0.04 g), dimethyl sulphate (0.5 ml), potassium carbonate (1 g) and acetone (25 ml) was refluxed on water bath for 24 hr. After removal of the solvent and dilution of the mixture with

water, a yellow product was obtained, which crystallised from methanol in yellow needles (0.03 g), m.p. 211°. Mixed melting point with the pentamethyl ether-ester of isokermesic acid shows no depression.

1,3,4,6-Tetrahydroxy-8-methylanthraquinone-7-carboxylic acid

To a melt, prepared from anhydrous aluminium chloride (0.25 g) and dry sodium chloride (0.05 g), 1,3,4,6-tetramethoxy-7-carbomethoxy-8-methylanthraquinone (0.025 g) was added and the mixture stirred at 150° for 5 min. The melt was poured in 2% hydrochloric acid. The red product was filtered, washed with water and dried (0.01 g). It crystallised from acetic acid in red needles, m.p. sinters at 284-285° and dec. 290-291°. Mixed m.p. with the kermesic acid showed no depression in melting point. Chromatographic behaviour of the two products on oxalated silica gel plates using benzene-alcohol (9:1) mixture was identical.

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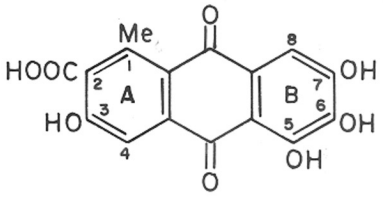
P A R T III

CEROALBOLINIC ACID

***

Introduction

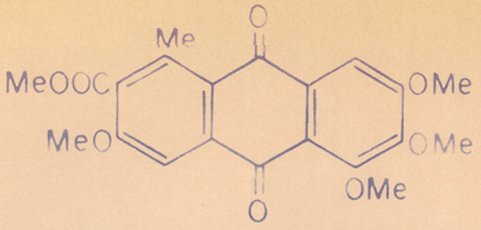
Ceroalbolinic acid, an anthraquinone pigment related to carminic acid, was isolated by Rios[1] from the insects Ceroplastes albolineatus. Ceroplastes albolineatus is a Coccidae, the females of which infest during the rainy season the shrub popularly known as "Palo loco" (Senecio praecox, Cav.). The females were extracted with chloroform to remove the waxes and from the ethanolic extract of wax-free insects a red substance was isolated, which was named as ceroalbolinic acid and assigned the structure (1).



(1)

The acid, $C_{16}H_{10}O_8$, is optically inactive and begins to decompose at 290° and carbonizes without melting. It gave a positive ferric colour and showed in its IR spectrum bands at 3500 (hydroxyl), 1725 (carboxyl), 1643 (bonded C=O) and 1590 cm^{-1} (C=C bond). Acetylation with perchloric acid and acetic anhydride gave a bicarbonate soluble tetraacetate. Methylation with dimethyl sulphate,

potassium carbonate and acetone yielded pentamethyl ether-ester (2), m.p. 201-204°. Treatment of the acid with diazomethane gave a tetramethyl ether-ester (3), m.p. 245-248°. The reductive acetylation product obtained by treating the acetate of the tetramethyl ether-ester with zinc and sodium acetate in acetic anhydride, showed λmax at 277, 349, 368 and 407 mμ, which are characteristic values for substituted anthracene derivatives. This was supported by the additional evidence that the tetramethyl ether-ester of the acid (1) gave 1-methylanthracene when distilled over zinc. Decarboxylation of ceroalbolinic acid with copper chromite in quinoline gave 3,5,6,7-tetrahydroxy-1-methylanthraquinone, which was characterised by its tetraacetate and trimethyl ether (4), m.p. 221-223°. Oxidation of (3) with potassium permanganate yielded a tricarboxylic acid monomethyl ester monomethyl ether (5), the trimethyl ester of which was found to be identical with that of cochenillic acid methyl ether trimethyl ester. Isolation of cochenillic acid derivative fixed the positions of the methyl at C-1, a hydroxyl at C-3 and a carboxyl group at C-2 in the A ring of the anthraquinone nucleus. The three hydroxyl groups have to be located on the B ring of the anthraquinone, either at 5,6,7 or 6,7,8-positions; the other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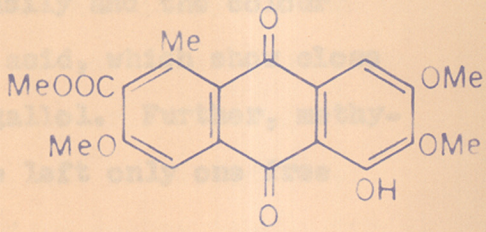
MeO
OMe
OMe
OMe
O
O

(2)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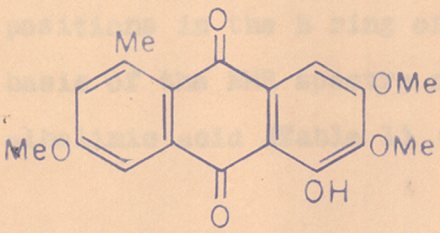
MeO
OMe
OMe
OH
O
O

(3)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OMe
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OH
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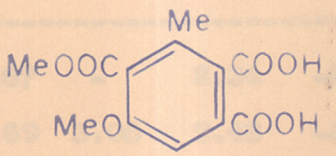
O

(4)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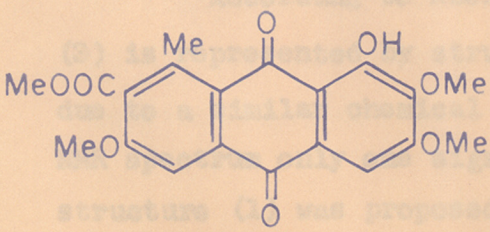
MeO
COOH
COOH

(5)


Me
OH
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OMe
OMe
O
O

(6)

sequences 5,6,8 or 5,7,8 are excluded on the basis of the IR spectra of ceroalbolinic acid derivatives, which show only one bonded quinone easily and the colour reactions of the ceroalbolinic acid, which show close resemblance to those of anthragallol. Further, methylation of (1) with diazomethane left only one free hydroxyl group intact.

The location of hydroxyl groups, at 5,6 and 7 positions in the B ring of (1), was established on the basis of the NMR spectra of some derivatives of ceroalbolinic acid (Table 1) (chemical shifts on τ scale).

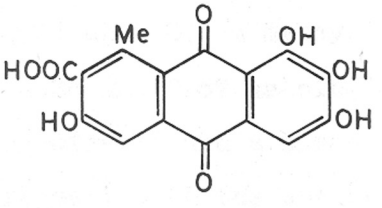
Table 1

Compound in $CDCl_3$	$-CH_3$ (1)	OMe (3)	-COOMe (2)	H(4)	H(2)	H(8)	OH
(3)	7.31	6.05	5.99	2.67	-	2.34	-2.09
(4)	7.25	6.08 6.00	-	2.69	3.05	2.43	-3.05

According to Rios[1] "if the trimethyl ether ester (3) is represented by structure (6), the C-4 and C-5 protons due to a similar chemical environment should exhibit in the NMR spectrum only one signal for the two protons". Therefore structure (1) was proposed for ceroalbolinic acid.

PRESENT WORK

The reason given by Rios in favour of structure (1) in preference to (7) for ceroalbolinic acid on the basis of the NMR spectral data is not valid, because the two aromatic protons in structures (1) and (7) are in different environments, and are expected to have different chemical shifts in either case. Biogenesis by the acetate route (Part II) will in fact favour (7). The synthesis of ceroalbolinic acid was undertaken with the object of removing this ambiguity regarding its structure.



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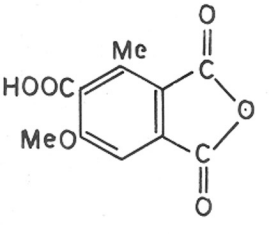


(8)

Condensation of 3-methyl-4-carboxy-5-methoxy-phthalic anhydride (8) with pyrogallol trimethyl ether in aluminium chloride-sodium chloride melt at 180° for 20 minutes gave a product which in its chromatographic behaviour on oxalated silica gel plates using benzene

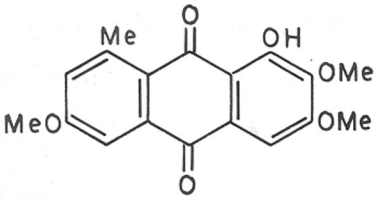
alcohol (9:1) showed a single product. It darkened at 290-295° and did not melt below 320°. Structure (1) was assigned to this compound on the analogy of the reaction of the anhydride (8) with 2-methoxyhydroquinone, when it gave under similar conditions isokermesic acid and not kermesic acid (Part II).

Methylation of the above product with ethereal diazomethane gave a compound, m.p. 220-22°, which in its IR spectrum showed bands at 1729 (ester C=O), 1664 (C=O) and 1630 cm^{-1} (bonded C=O). The NMR spectrum of this tetramethyl ether-ester in $CDCl_3$ shows four methoxyl groups at 6.00, a methyl at 7.37, two aromatic protons at 2.38 and 2.67 as singlets, assignable to 4 and 8 protons respectively and a bonded hydroxyl at -2.6, and is in agreement with the structure (3).

Methylation of the synthetic product with dimethyl sulphate, potassium carbonate in boiling acetone yielded (2), which in its chromatographic behaviour on TLC showed a major fraction (R_f 0.65) and a minor fraction (Rf 0.7). The major fraction crystallised from methanol in yellow needles, m.p. 228-230°. The other fraction which was insignificant was not examined.

The NMR spectrum of the pentamethyl ether-ester in $CDCl_3$ (Fig. 1) shows five methoxyls (between 5.95 and 6.05), a methyl (7.30) and two aromatic proton signals at 2.26 and 2.37 assignable to 4 and 8 protons respectively. In the NMR spectrum of anthragallol trimethyl ether the 4-proton appears at 2.32.

Unambiguous evidence in support of structure (1) for the condensation product was obtained by subjecting it to decarboxylation by refluxing the product in diethylaniline for 4 hr. Methylation of the decarboxylated compound with ethereal diazomethane gave an orange compound (4), m.p. 193-194°. The non-identity of this compound (4) with the trimethyl ether (9) of isoerythrolaccin,[2] the colouring matter of seed lac, was showed by depression in the mixed melting point and also by chromatography on silica gel using benzene: acetone (9.5:0.5). (Rf isoerythrolaccin trimethyl ether, 0.56 and the synthetic product, 0.47). The structure (10) for isoerythrolaccin, was confirmed by its unambiguous synthesis.[2]



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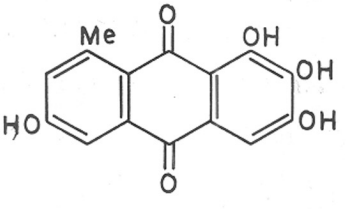

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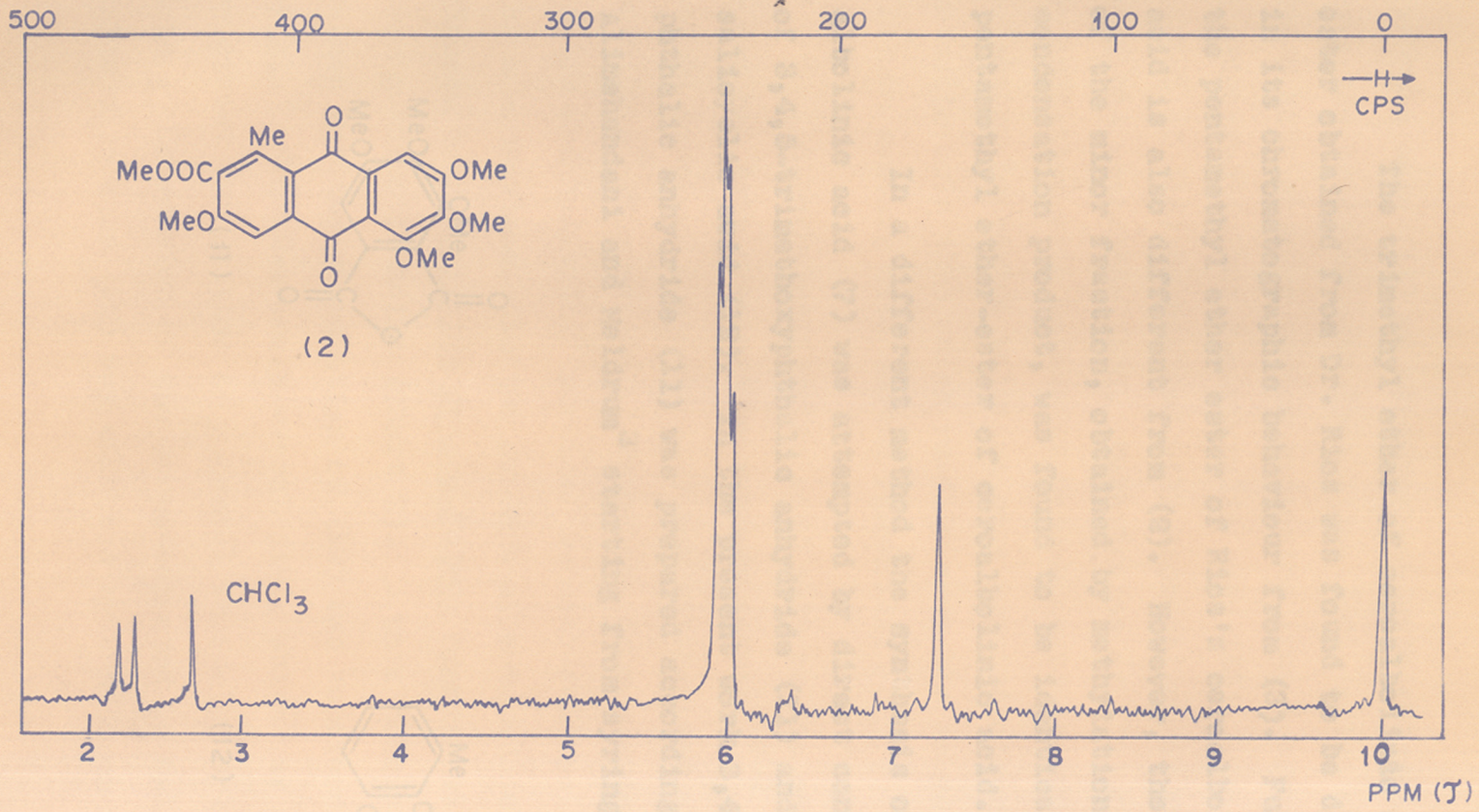
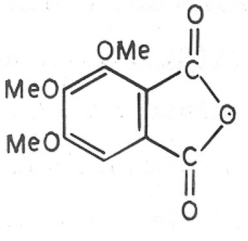

500
400
300
200
100
0
H
CPS
Me
MeOOC
MeO
OMe
OMe
OMe
O
O
O
(2)
CHCl3
2
3
4
5
6
7
8
9
10
PPM (δ)

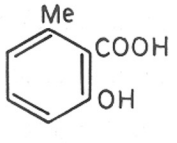
Fig. 1

The trimethyl ether of ceroalbolinic acid methyl ester obtained from Dr. Rios was found to be different in its chromatographic behaviour from (3). Further, the pentamethyl ether ester of Rios's ceroalbolinic acid is also different from (2). However, the Rf value of the minor fraction, obtained by methylation of the condensation product, was found to be identical with the pentamethyl ether-ester of ceroalbolinic acid.

In a different method the synthesis of ceroalbolinic acid (7) was attempted by direct condensation of 3,4,5-trimethoxyphthalic anhydride (11) and 6-methylsalicyclic acid (12). In the present work 3,4,5-trimethoxyphthalic anhydride (11) was prepared according to Alimehandani and Meldrum[3] starting from syringic acid(13).



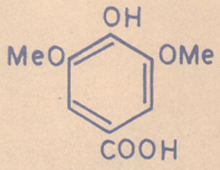
(11)



(12)

Syringic acid (13) on condensation with chloral hydrate gave the trichloromethylphthalide (14), which on hydrolysis with aqueous sodium hydroxide gave 4-hydroxy-3,5-dimethoxyphthalide-2-carboxylic acid (15). Decarboxylation of (15) by heating with naphthalene at 200-210° gave the phthalide (16). Methylation of (16) followed by oxidation with potassium permanganate in aqueous potassium hydroxide solution gave the trimethoxy phthalic acid (18) which readily gave the anhydride (11) on heating with acetic anhydride.

6-Methylsalicyclic acid (12) or its methyl-ether has been prepared by various methods.[4-8] The methyl ether of the acid (12) was synthesised earlier by Gibson[4] by a series of reactions from m-cresol (Chart 1). Anslow and Raistrick[5] prepared 6-methylsalicyclic acid, for a comparison with the product of metabolism of glucose by Penicillium gresco-fulvum Dierckx starting from o-nitrotoluidine (Chart 2). A more convenient method for the preparation of (12) was reported by Eliel et al.[6] from 2-bromo-m-cresol (19), which was obtained by direct bromination of m-cresol in fuming sulphuric acid . Conversion of the bromo-compound to 6-methyl-salicyclic acid (12) was effected by a halogen metal



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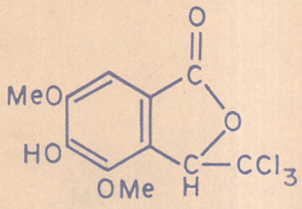

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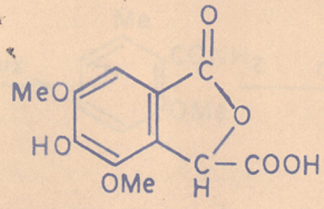

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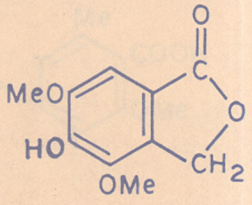

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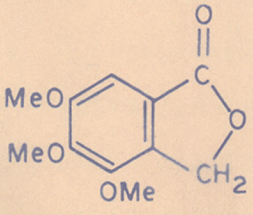

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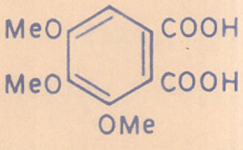


(18)

CHART-1

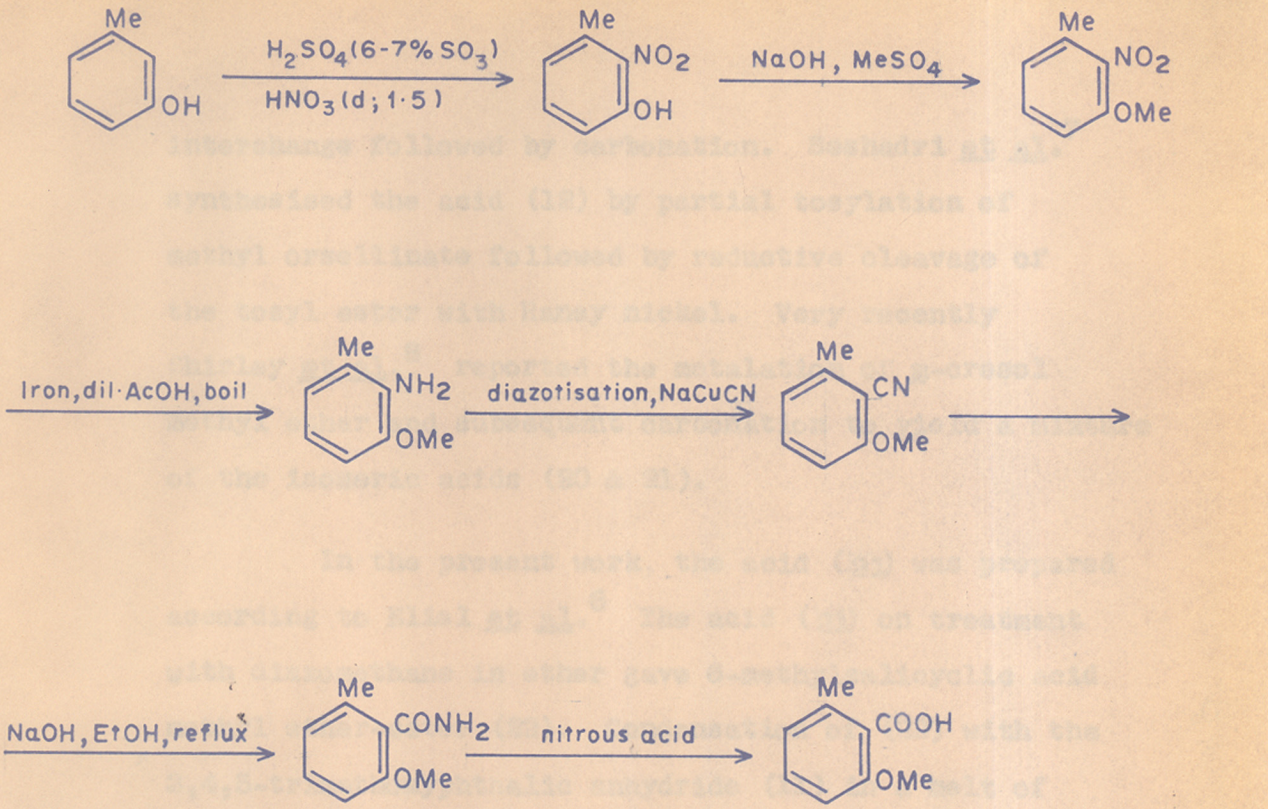
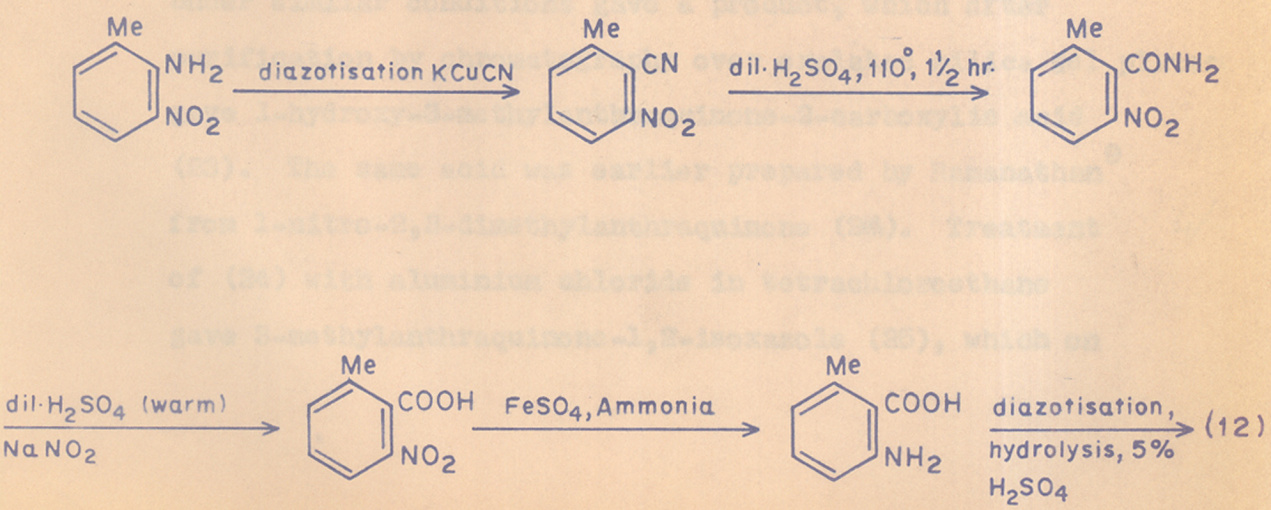
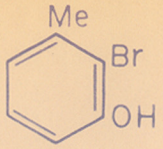


CHART-2



interchange followed by carbonation. Seshadri et al.[7] synthesised the acid (12) by partial tosylation of methyl orsellinate followed by reductive cleavage of the tosyl ester with Raney nickel. Very recently Shirley et al.[8] reported the metalation of m-cresol methyl ether and subsequent carbonation to yield a mixture of the isomeric acids (20 & 21).

In the present work, the acid (21) was prepared according to Eliel et al.[6] The acid (21) on treatment with diazomethane in ether gave 6-methylsalicyclic acid methyl ether-ester (22). Condensation of (22) with the 3,4,5-trimethoxyphthalic anhydride (11) in a melt of aluminium chloride-sodium chloride mixture at 180° for 20 minutes gave a dark product, which did not give the characteristic colour reactions for hydroxyanthraquinones. However, phthalic anhydride on condensation with (22) under similar conditions gave a product, which after purification by chromatography over oxalated silica gel plates gave 1-hydroxy-3-methylanthraquinone-2-carboxylic acid (23). The same acid was earlier prepared by Ramanathan[9] from 1-nitro-2,3-dimethylanthraquinone (24). Treatment of (24) with aluminium chloride in tetrachloroethane gave 3-methylanthraquinone-1,2-isoxazole (25), which on



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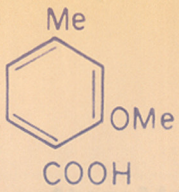

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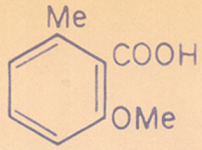

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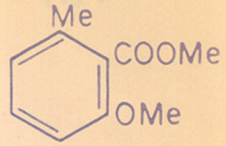

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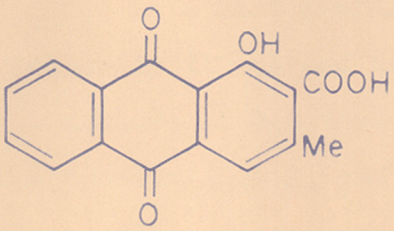

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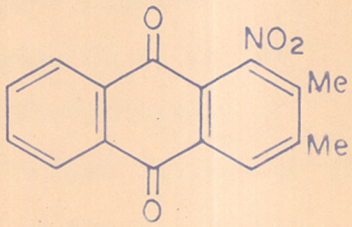

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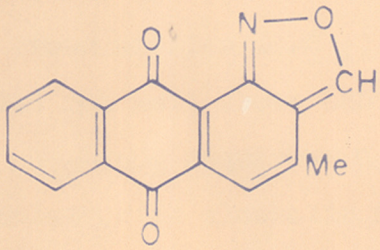

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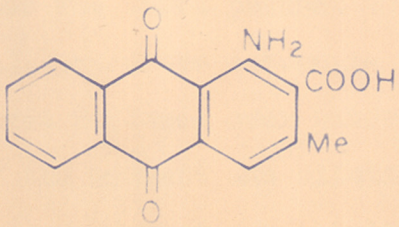

(26)

hydrolysis with methanolic baryta gave the amino carboxylic acid (26), which on diazotization and hydrolysis gave (23).

EXPERIMENTAL

Condensation of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (8) and pyrogallol trimethyl ether

To a melt prepared from anhydrous aluminium chloride (10 g) and dry sodium chloride (2 g) at 150°, a mixture of 3-methyl-4-carboxy-5-methoxyphthalic anhydride (0.6 g) and pyrogallol trimethyl ether (0.3 g) was added and stirred at 180° for 20 minutes. It was cooled and poured over 2% hydrochloric acid. The product was filtered, washed with water and dried. It was taken in acetone and on concentration of the solvent, it gave brown needles (0.2 g). It darkens at 290-295° and did not melt above 320° (Found: C, 58.5; H, 3.3. $C_{16}H_{10}O_8$ requires: C, 58.2; H, 3.0%). It gave a brown colour with alcoholic ferric chloride solution and green in aqueous sodium hydroxide solution.

Methylation of the above product

A mixture of the above product (0.1 g), dimethyl sulphate (0.5 ml), potassium carbonate (2 g), and acetone (25 ml) was refluxed over water bath for 24 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained. The product

showed two spots (one major and a minor) on its chromatographic behaviour, on TLC silica gel plate using benzene-acetone mixture (4:1). Separation of the two fractions was achieved over PLC silica gel plates using the same solvent system.

The major fraction crystallised from methanol in yellow needles (0.07 g), m.p. 228-230° (Found: C, 63.4; H, 4.9. $C_{21}H_{20}O_8$ requires: C, 63.0; H, 5.0%).

The minor fraction on its chromatographic behaviour over TLC silica gel plate was found to be identical with the pentamethyl ether-ester of cercalbolinic acid.

Diazomethane methylation of the condensation product

A mixture of the condensation product (0.1 g) and ethereal diazomethane solution was left overnight at 0-5°. The excess of diazomethane was destroyed by addition of a few drops of acetic acid and ether was distilled off. The compound crystallised from benzene-hexane mixture in yellow needles (0.05 g), m.p. 220-22° (Found: C, 62.5; H, 4.9. $C_{20}H_{18}O_8$ requires: C, 62.2; H, 4.7%). It gave a brown colour with alcoholic ferric chloride solution.

Decarboxylation of the condensation product

A mixture of the condensation product (0.1 g) and freshly distilled diethylaniline (2 ml), was refluxed for 4 hr. The mixture was cooled and diluted with 2% hydrochloric acid. The brown solid obtained was filtered, washed twice with bicarbonate and then with water and dried. It crystallised from dilute alcohol in brown needles (0.04 g), m.p. above 300°. It was characterised as its trimethyl ether.

Diazomethane methylation of the above compound

A mixture of the above compound (0.04 g), and ethereal diazomethane solution was left overnight at 0-5°. Excess of diazomethane was destroyed by addition of few drops of acetic acid, and ether was distilled off. The chloroform solution of this compound was passed through a short column of silica gel and eluted with benzene-acetone mixture (4:1). Concentration of the solvent gave orange crystals (0.015 g), m.p. 193-94° (Found: C, 66.4; H, 5.1. $C_{18}H_{16}O_6$ requires: C, 65.9; H, 4.9%). Mixed m.p. depressed by 20° with the sample of isoerythrolaccin trimethyl ether. Rf values for this compound and trimethyl ether of isoerythrolaccin

were found to be different on TLC silica gel plate using benzene-acetone mixture (9.5:0.5).

3,4,5-Trimethoxyphthalic anhydride

It was prepared according to the method of Alimchandani and Meldrum.[3]

6-Methylsalicyclic acid methyl ether

It was prepared according to the method of Eliel et al,[6] from 2-bromo-m-cresol methyl ether.

Condensation of 3,4,5-trimethoxyphthalic anhydride and 6-methylsalicyclic acid dimethyl ether ester.

To a melt prepared from anhydrous aluminium chloride (5 g) and dry sodium chloride (0.5 g) at 150° a mixture of 3,4,5-trimethoxyphthalic anhydride (0.2 g) and 6-methylsalicylic acid methyl ether methyl ester (0.2 g) was added and stirred at 180° for 20 minutes. It was cooled and poured over 2% hydrochloric acid. The dark gray product was filtered, washed with water and dried (0.1 g). The product did not show any reactions characteristic of hydroxyanthraquinones.

Condensation of phthalic anhydride and 6-methylsalicylic acid dimethyl ether ester

To a melt prepared from anhydrous aluminium chloride (5 g) and dry sodium chloride (0.5 g) at 150°, a mixture of phthalic anhydride (0.2 g), and 6-methylsalicyclic acid methyl ether methyl ester (0.2 g) was added and stirred at 180° for 20 minutes. It was cooled and poured over 2% hydrochloric acid. The yellow product was filtered, washed with water and dried (0.1 g). It was crystallised from benzene in yellow needles, m.p. 275-276° (lit.[9] 276°). It gave brown colour with alcoholic ferric chloride solution.

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PART IV

SYNTHESIS OF 1,3,6-TRIHYDROXYANTHRAQUINONE

and

3-HYDROXYANTHRAQUINONE-1,2-DICARBOXYLIC ACID

***

Synthesis of 1,3,6-trihydroxyanthraquinone

Introduction:

Of the fourteen possible trihydroxyanthraquinones thirteen are known. Synthesis of 1,3,6-trihydroxyanthraquinone has not so far been reported. Synthesis of 1,3,5; 1,3,7- and 2,3,6-trihydroxyanthraquinones and a new improved synthesis of 1,3,8-trihydroxyanthraquinone has been reported from this laboratory.[1-3]

1,3,5-Trihydroxyanthraquinone has been synthesised by the following route. Chlorination of 1-amino-5-benzamidoanthraquinone, followed by deamination, yielded 1-benzamido-6,8-dichloroanthraquinone. Further chlorination of this compound, followed by hydrolysis and deamination yielded 1,3,5-trichloroanthraquinone, which was converted into the trimethoxy derivative. The latter on demethylation with aluminium bromide in benzene gave 1,3,5-trihydroxyanthraquinone.[1]

1,3,7-Trihydroxyanthraquinone was synthesised from 1-amino-6,8-dichloroanthraquinone. Bromination of 1-amino-6,8-dichloroanthraquinone, followed by deamination, yielded 7-bromo-1,3-dichloroanthraquinone, which was converted into the corresponding trimethoxyanthraquinone.

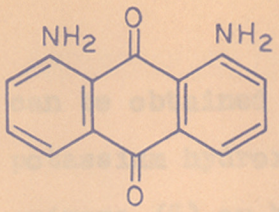
Demethylation with aluminium bromide in boiling benzene yielded 1,3,7-trihydroxyanthraquinone.[2] 1,3-Dichloro-8-hydroxyanthraquinone, prepared from 1-amino-6,8-dichloroanthraquinone was converted to the corresponding trimethoxyanthraquinone. Demethylation by the above method yielded 1,3,8-trihydroxyanthraquinone.[2]

Condensation of 4-bromophthalic anhydride with pyrocatechol gave 2,3-dihydroxy-6-bromoanthraquinone, which was converted to the corresponding trimethoxyanthraquinone. Demethylation with aluminium chloride-sodium chloride gave 2,3,6-trihydroxyanthraquinone.[3] The preparative methods of the other known trihydroxyanthraquinones are described by Coffey and van Alphan[4] and in the Elsevier's Encyclopaedia of Organic Chemistry.[5]

Jayaraman[6] attempted to synthesise 1,3,6-trihydroxyanthraquinone by brominating 1,8-diaminoanthraquinone (1) in acetic acid at 90-100°, which gave a dibromo derivative (2); on deamination, it gave a dibromoanthraquinone; identified as the 2,7-dibromoanthraquinone(3). The bromine atoms in (2) were assigned to the ortho positions with reference to the amino groups because of the ortho directing influence of the amino groups. Hence

(2) was constituted as 1,8-diamino-2,7-dibromoanthraquinone. On further bromination of (2), with calculated quantities of bromine in nitrobenzene at 150-160°, it gave a diaminotribromoanthraquinone (4), the third bromine atom entering one of the two para positions. Hence (4) was constituted as 1,8-diamino-2,4,7-tribromoanthraquinone. Deamination of (4) gave 1,3,6-tribromoanthraquinone (5). Conversion of 1,3,6-tribromoanthraquinone to the corresponding trimethoxy derivative proved difficult. Heating (5) with sodium and methanol under pressure at 120° for 4 hr. gave a dibromo-dimethoxyanthraquinone,[6] (m.p. 220-221°).

The present work was undertaken to find out a suitable method for the substitution of bromine atoms by methoxyl or phenoxyl groups and subsequent conversion to the hydroxy derivative. Treatment of (5) with sodium methoxide in dry methanol and silver nitrate (as a catalyst) gave a product not free from bromine. Failure in obtaining the trimethoxy derivative can be attributed to the poor solubility of (5) even in boiling methanol. It has been reported that phenoxyanthraquinones can be prepared by heating halogeno derivatives with phenol and potassium carbonate,[7] and the corresponding methoxy derivatives



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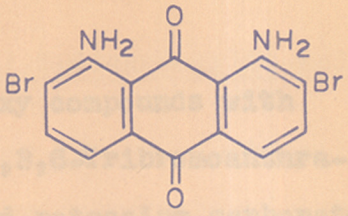

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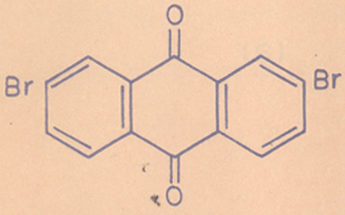

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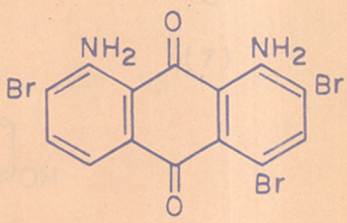

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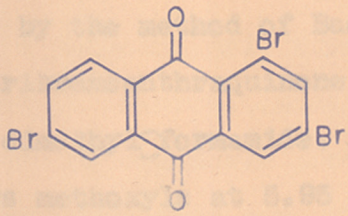
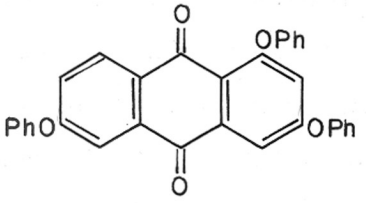


(5)

can be obtained by treating the phenoxy compounds with potassium hydroxide and methanol.[8] 1,3,6-Tribromoanthraquinone (5) on heating with phenol and potassium carbonate



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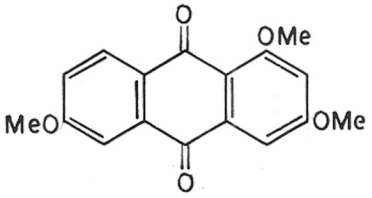

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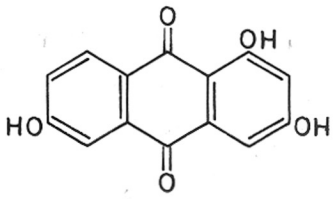
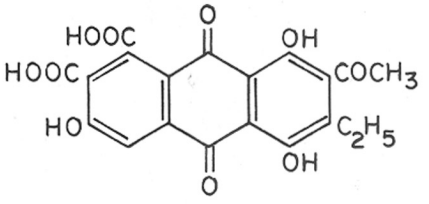


(8)

gave 1,3,6-triphenoxyanthraquinone (6), which on prolonged refluxing with potassium hydroxide and pyridine in methanol gave 1,3,6-trimethoxyanthraquinone (7). The same compound could be obtained by the method of Bacon and Hill,[9] by refluxing 1,3,6-tribromoanthraquinone with sodium methoxide, copper oxide and dimethylformamide. The NMR spectrum of (7) in $CDCl_3$ shows methoxyls at 5.95 and 5.99, and five aromatic protons [3.22 (2-H, d; J=3 cps); 2.74 (7-H, q), 2.51 (4-H, d; J=3 cps), 2.26 (5-H, d; J=3 cps) and 1.8 (8-H, d; J=9 cps)]. Demethylation of (7) with aluminium chloride-sodium chloride yielded 1,3,6-trihydroxyanthraquinone (8).

Synthesis of 3-hydroxyanthraquinone-1,2-dicarboxylic acid

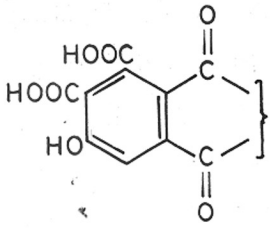
"Laccaic acid", the colouring matter of stick lac,[10] was found to be a mixture of hydroxyanthraquinone derivatives. Further, colour reactions showed that the major constituents of laccaic acid were purpurin derivatives[11] and there was no evidence for a C-acetyl or C-ethyl group as in the structure (9) suggested by Mayer[12] based on the results of Dimroth and Goldschmidt.[13] Laccaic acid A[10] on oxidation with alkaline hydrogen



(9)

peroxide yielded a phenolic acid which on methylation proved to be the tetramethyl ester of anisole-2,3,4,5-tetracarboxylic acid, the structure of which was proved by synthesis. The above evidence confirmed that one of the benzene rings of the anthraquinone is substituted by two carboxylic acid groups and a hydroxyl group. This will lead to the three possible

orientations (10, 11, 12) of the two carboxylic groups and a hydroxyl group on an anthraquinone ring of laccaic acid A. The structure (12) was ruled out on the basis of the anhydride obtained from the dibasic acid, obtained from the alkaline hydrolysis of MXLA III (methylation product of xantholaccaic acid A).[10] To differentiate



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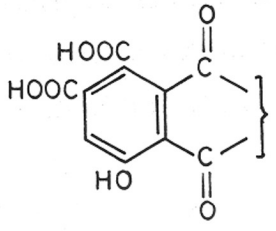

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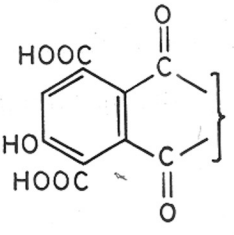
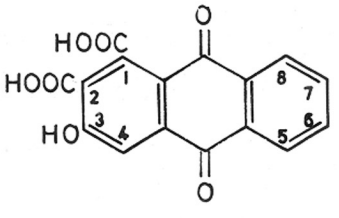


(12)

between the other two alternate structures (10 and 11), it was considered desirable at an early stage of this work on lac pigments in this laboratory, to synthesise 3-hydroxyanthraquinone-1,2-dicarboxylic acid (13) and to obtain exact chemical shifts of the proton at 4 and to compare them with the laccaic acid A derivatives.



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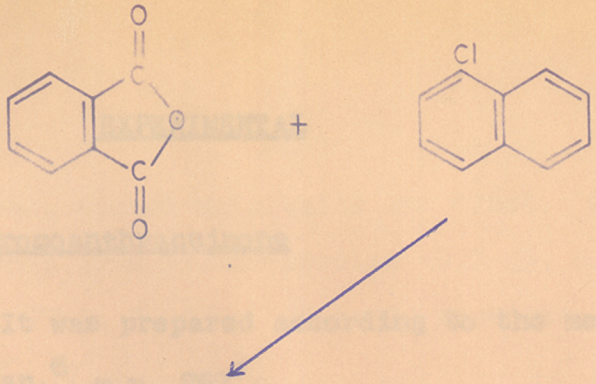
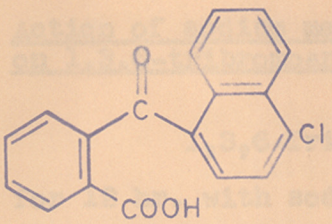
Anthraquinone-1,2-dicarboxylic acid was prepared in 75% yield by oxidising 1,2-benzanthraquinone with potassium permanganate in dilute sulphuric acid and in a small yield (22%) with nitric acid (d, 1.15) at 190°.[14] It was also obtained in 90% yield by oxidising 2-methyl-anthraquinone-1-carboxylic acid with nitric acid (d, 1.1) at 190°.[15] In the present work 3-hydroxyanthraquinone-1,2-dicarboxylic acid was prepared from 3-chloro-1,2-benzanthraquinone.[16] Condensation of phthalic anhydride and 1-chloronaphthalene in tetrachloroethane and aluminium chloride gave the corresponding keto acid (14). Cyclisation of (14) with conc. sulphuric acid at 60-70° gave 3-chloro-1,2-benzanthraquinone (15). Oxidation of (15) with potassium permanganate in dilute sulphuric acid at water-bath temperature for 6 hr. gave 3-chloroanthraquinone 1,2-dicarboxylic acid (16) in 41% yield. Acid (16), when refluxed for 6 hr. with sodium methoxide in absolute methanol gave 3-methoxy-anthraquinone-1,2-dicarboxylic acid (17). The NMR spectrum of the dimethyl ester of (17) in $CDCl_3$ shows three methoxyl groups at 5.94, 6.00 and 6.09; a singlet at 2.17 (4-H) and two multiplets at 2.24 (6 and 7-H) and 1.8 (5 and 8-H). The corresponding proton in methylated laccaic acids[10] appears around 2.2. The dimethyl ester of (17) on heating with aluminium chloride and sodium chloride gave 3-hydroxyanthraquinone-1,2-dicarboxylic acid (13).





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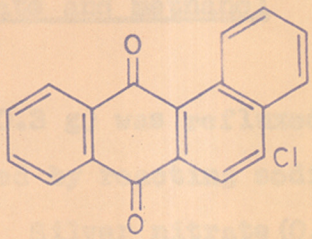

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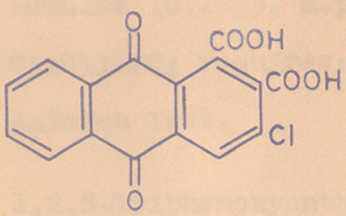

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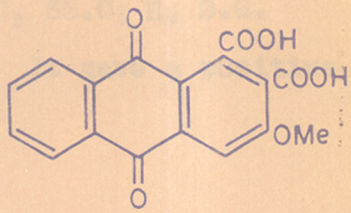

(17)

EXPERIMENTAL

1,3,6-Tribromoanthraquinone

It was prepared according to the method described by Jayaraman,[6] m.p. 252°.

Action of sodium methoxide, silver nitrate and methanol on 1,3,6-tribromoanthraquinone

1,3,6-Tribromoanthraquinone (0.3 g) was refluxed for 12 hr. with sodium methoxide prepared by reacting sodium (0.3 g) with absolute methanol (50 ml). Silver nitrate (0.1 g) was added in small lots at an interval of one hr. On removal of the solvent and dilution of the mixture with water, a yellow product was obtained. It was filtered, washed with water and dried. It crystallised from benzene in yellow needles (0.1 g), m.p. 220-221° (Found: C, 55.0; H, 3.6. $C_{16}H_{11}BrO_4$ requires: C, 55.3; H, 3.2%). It gave a positive halogen test.

1,3,6-Triphenoxyanthraquinone (6)

A mixture of 1,3,6-tribromoanthraquinone (0.5 g), phenol (5 ml) and anhydrous potassium carbonate (0.5 g) was refluxed for 6 hr. It was diluted with ethanol, and then poured over 2% sodium hydroxide. The yellow precipitate

was filtered, washed with water and dried. Crystallisation from methanol gave yellow needles (0.3 g), m.p. 174-175° (Found: C, 79.0; H, 4.3. $C_{32}H_{20}O_5$ requires: C, 79.3; H, 4.1%).

1,3,6-Trimethoxyanthraquinone (7)

Method A:

A mixture of 1,3,6-triphenoxyanthraquinone (0.4 g), absolute methanol (40 ml), potassium hydroxide (2 g) and pyridine (2 ml) was refluxed on a water bath for 72 hr. After removal of the solvent and dilution of the mixture with water, a yellow product was obtained, which was filtered, washed with water and dried. It was dissolved in benzene and passed through a short column of alumina. Benzene extract on concentration gave yellow needles (0.1 g), m.p. 224-226° (Found: C, 68.4; H, 4.9. $C_{17}H_{14}O_5$ requires: C, 68.5; H, 4.8%).

Method B

A mixture of 1,3,6-tribromoanthraquinone (0.4 g), sodium methoxide (prepared by dissolving sodium (1.5 g) in absolute methanol (25 ml) and distilling the excess solvent), and copper oxide (1 g) in dry dimethylformamide (40 ml) was refluxed for 48 hr. The mixture was filtered and

washed with hot dimethyl formamide. The solvent was completely removed and diluted with water. The precipitate was filtered, washed with water and dried. It crystallised from benzene in yellow needles (0.1 g), m.p. 224-226°. It was identical with the above compound by mixed m.p. and chromatographic behaviour on TLC silica gel plate, using benzene-acetone (9:1).

1,3,6-Trihydroxyanthraquinone (8)

To a melt prepared from anhydrous aluminium chloride (0.5 g) and dry sodium chloride (0.1 g), 1,3,6-trimethoxyanthraquinone (0.05 g) was added, and the mixture stirred at 150° for 10 min. It was cooled and poured on ice cold 2% hydrochloric acid. The precipitate was filtered, washed with water and dried (0.01 g). It crystallised from o-dichlorobenzene in brown needles, m.p. 316-317° (Found: C, 65.4; H, 3.4. $C_{14}H_8O_5$ requires: C, 65.6; H, 3.1%).

The IR spectrum in (Nujol) shows bands at 1667 (C=O) and 1634 cm^{-1} (bonded carbonyl). It gave brown colour with alcoholic ferric chloride.

3-Chloro-1,2-benzanthraquinone (15)

It was prepared according to the method described by Heller.[16]

3-Chloroanthraquinone-1,2-dicarboxylic acid (16)

A solution of 3-chloro-1,2-benzanthraquinone (5 g) in conc. sulphuric acid (50 ml), was added slowly to water (400 ml). Finely powdered potassium permanganate (20 g) was added in small lots for a period of 1 hr. and the mixture heated on a steam bath for 6 hr. under stirring. It was then decolourised by the addition of a hot solution of oxalic acid. The precipitate was filtered and extracted with hot dilute ammonia solution. The insoluble residue from this treatment was unchanged 3-chloro-1,2-benzanthraquinone (1.2 g). The ammonical solution was acidified with hydrochloric acid and the precipitate filtered off, washed with water and dried. It crystallised from methanol in yellow needles (1.8 g), m.p. 320-321° (Found: C, 57.9; H, 2.3. $C_{16}H_7ClO_6$ requires: C, 58.2; H, 2.1%). The methyl ester, prepared by treatment with diazomethane, crystallised from ethanol in yellow plates, m.p. 180-182° (Found: C, 60.6; H, 3.3. $C_{18}H_{11}ClO_6$ requires: C, 60.2; H, 3.0%).

3-Methoxyanthraquinone-1,2-dicarboxylic acid (17)

A mixture of 3-chloroanthraquinone-1,2-dicarboxylic acid (1 g) and sodium (0.4 g) in absolute methanol (25 ml) was refluxed for 12 hr. Finely powdered silver nitrate was added in small lots at an interval of one hr. Methanol was distilled off and the reaction mixture diluted with water. On acidification, it gave a precipitate which was filtered, washed with water and dried. It crystallised from ethanol in lemon yellow needles (0.6 g), m.p. 280° (Found: C, 62.6; H, 3.3. $C_{17}H_{10}O_7$ requires: C, 62.5; H, 3.1%).

The methyl ester prepared by treatment with ethereal diazomethane crystallised from methanol in yellow plates, m.p. 190-191° (Found: C, 64.1; H, 3.7. $C_{19}H_{14}O_7$ requires: C, 64.4; H, 3.9%).

3-Hydroxyanthraquinone-1,2-dicarboxylic acid (13)

To a melt prepared from anhydrous aluminium chloride (1 g) and dry sodium chloride (0.2 g), 3-methoxyanthraquinone-1,2-dicarboxylic acid dimethyl ester (0.1 g) was added and the mixture stirred at 150° for 10 minutes.

It was cooled and poured on ice cold 2% hydrochloric acid. The yellow precipitate was filtered, washed with water and dried (0.05 g). It crystallised from acetic acid in yellow needles, m.p. 332-334° (Found: C, 61.1; H, 2.2. $C_{16}H_8O_7$ requires: C, 61.5; H, 2.5%). It gave an orange colour in sodium hydroxide, yellow in conc. sulphuric acid and red with alcoholic ferric chloride solution.

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S U M M A R Y

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PART I: Naturally occurring anthraquinone carboxylic acids

A brief review is made of the hydroxyanthraquinone carboxylic acids occurring in nature with special reference to their physiological properties, occurrence, isolation, colour reactions, constitution and classification. Since the present work deals mainly with anthraquinone carboxylic acids, a separate table of the naturally occurring hydroxyanthraquinone carboxylic acids is included, followed by a brief account of the facts on which their structures are based and the routes adopted for their syntheses wherever reported.

PART II: Kermesic acid

Synthesis of phenol-2,3,4,5-tetracarboxylic acid

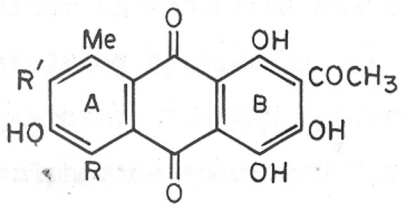
In the course of a programme of work on the anthraquinonoid insect pigments, a phenol tetracarboxylic acid was obtained by the oxidation of laccaic acid with alkaline hydrogen peroxide. It was found necessary to synthesise the ether-ester of the above phenolic acid for direct comparison with the methylated product of

the acid obtained by oxidation of laccaic acid. This was synthesised starting from 5-methoxytoluene-2,3,6-tricarboxylic acid, which was reported earlier, by Bhatia and Venkataraman, by a series of reactions starting from 3,5-dimethylanisole. Oxidation of 5-methoxytoluene-2,3,6-tricarboxylic acid gave anisole-2,3,4,5-tetracarboxylic acid, the tetramethyl ester on treatment with aluminium chloride-sodium chloride melt gave phenol-2,3,4,5-tetracarboxylic acid. The trimethyl ester of 5-methoxytoluene-2,3,6-tricarboxylic acid was shown to be identical, by Bhatia and Venkataraman, with the trimethyl ester of cochenillic acid methyl ether, obtained by oxidation of carminic acid, by alkaline hydrogen peroxide, followed by methylation with ethereal diazomethane.

Structure and synthesis of kermesic acid

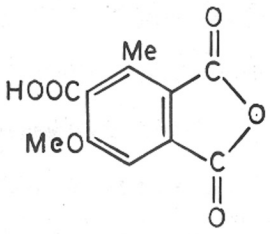
Extensive work on kermesic acid, the colouring matter of kermes was carried out by Dimroth, who assigned to it the structure (1a). It was shown by Overeem and van der Kerk and by Bhatia and Venkataraman independantly that in carminic acid, the colouring matter of cochineal,

the carboxyl group in the A-ring, is in the β-position. Since both carminic acid and kermesic acid yield cochenillic acid by oxidation, Overeem and van der Kerk concluded that kermesic acid is constituted as (1b). An attempt was made, in the present work, to synthesise (1b) by condensing 3-methyl-4-carboxy-5-methoxy-phthalic anhydride (2) with 3,6-dihydroxy-2-methoxy-



(1a ; R = COOH ; R′=H)

(1b ; R = H ; R′= COOH)

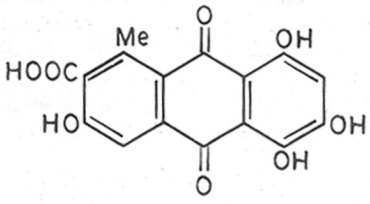


(2)

acetophenone in an aluminium chloride-sodium chloride melt at 180°. After chromatography on oxalated silica gel plates and benzene-alcohol (4:1) as the solvent, a product was isolated in 5 per cent yield, which ultimately proved to be the isomer of (1b) with the hydroxyl and acetyl groups in the B-ring interchanged. It crystallised in red needles (dec. above 260°) from acetic acid and the colour reactions were similar to those described by Dimroth. The NMR spectrum in NaOD-D_2O (three singlets

of intensity 1:3:3, the 3-proton singlets appearing at 4.46 and 4.65 ppm higher than the aromatic proton signal) confirmed the structure (1b) or its isomer. Since we had difficulty in preparing the tetraacetate and trimethyl ether described by Dimroth, it was clear that a direct comparison with natural kermesic acid was necessary before any conclusion could be drawn. After an extensive search, a sample of kermesic acid, isolated by Dimroth was obtained from Professor Karl Dimroth. The NMR spectrum of kermesic acid in dimethyl sulphoxide shows two single-proton signals at 2.34 and 3.40. The former corresponds to the α-proton in the A-ring of (1b), but the signal at 3.4 can only be assigned to a β-proton in anthraquinone flanked by hydroxyl groups as in purpurin. A signal at -3.62 and a broad signal at -2.90 exchange with D_2O, and can be assigned to chelated hydroxyl groups. The NMR spectrum in pyridine shows only one signal at 6.75, assignable to an α-methyl group in anthraquinone adjacent to a carboxyl group as in (1b). The absence of the C-acetyl group thus indicated was confirmed by the NMR spectrum of the ether-ester (yellow needles

from methanol, m.p. 196°) of kermesic acid prepared by the usual dimethyl sulphate-potassium carbonate method. The mass spectral molecular weight of the pentamethyl ether-ester of kermesic acid is 400, showing conclusively that kermesic acid is constituted as (3).



(3)

A careful study of Dimroth's papers shows that no positive evidence for the C-acetyl group in the structure (1a) was obtained. Its presence was surmised from elemental analysis of kermesic acid and some of its derivatives.

Treatment of kermesic acid with aqueous sodium hydroxide and dithionite (or absorption of two moles of hydrogen in presence of palladium on carbon) gave xantho-kermesic acid, which proved to be identical with laccaic acid D. Methylation by the usual method gave the tetra-methyl ether-ester, m.p. 226°, identical with the ether-

ester of laccaic acid D (m.p., mixed m.p. and superimposable IR spectra). Decarboxylation of xanthokermesic acid by refluxing with diethylaniline for 2 hr., followed by methylation with dimethyl sulphate, potassium carbonate and acetone, gave a product, identical with desoxyerythrolaccin trimethyl ether. The structure of desoxyerythrolaccin, the colouring matter of seed lac, was shown unambiguously as 1,3,6-trihydroxy-8-methylanthraquinone.

Condensation of the anhydride (2) with 2-methoxyhydroquinone in presence of boron trifluoride-etherate at 100° for 4 hr. gave a product, which on methylation yielded a pentamethyl ether-ester, m.p. 211°, different from methyl tetramethyl kermesate, m.p. 196°.

The synthesis of kermesic acid was ultimately achieved by the following route. Condensation of hydroquinone dimethyl ether with the anhydride (2), in aluminium chloride-sodium chloride melt at 180° gave 1,4,6-trihydroxy-8-methylanthraquinone-7-carboxylic acid, which on treatment with ethereal diazomethane gave 1,4-dihydroxy-6-methoxy-7-carbomethoxy-8-methylanthraquinone,

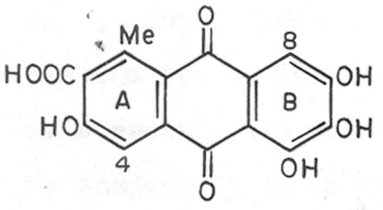
which was converted into 6-methoxy-7-carbomethoxy-8-methyl-1,4,9,10-anthradiquinone, by treatment with lead tetraacetate in acetic acid. Thiele acetylation of the diquinone gave a mixture of two compounds, which on acid hydrolysis followed by chromatographic separation gave two products (a major and a minor fraction). Methylation of the major fraction gave a product identical in all respects with the pentamethyl ether-ester of kermesic acid. While the product of methylation of the minor fraction was identified as the pentamethyl ether-ester of isokermesic acid. Demethylation of the former with aluminium chloride-sodium chloride melt at 180° gave kermesic acid identical in all respects with the natural product.

The biogenesis of kermesic acid and related insect pigments is discussed.

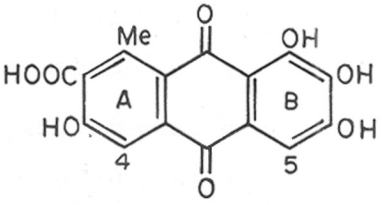
PART III: Ceroalbolinic acid

Structure (4) was assigned by Rios to ceroalbolinic acid, a pigment produced by the insect Ceroplastes albolineatus. The isomeric structure (5)

was considered less probable for a reason which does not appear to be valid: the two aromatic protons of the tetramethyl ether-ester showed up in the NMR spectrum as two separate signals at 2.34 and 2.67, and they should have appeared as a single signal for the structure (5). The two aromatic protons in (4) or (5) are in different environments and may be expected to have different chemical shifts in either case. Biogenesis by the acetate route will in fact favour (5).



(4)



(5)

Condensation of pyrogallol trimethyl ether with the 3-methyl-4-carboxy-5-methoxyphthalic anhydride (2) in an aluminium chloride-sodium chloride melt at 180° for 20 minutes gave (4) which darkened at 290-295°, and did not melt below 320°. The tetramethyl ether-ester, m.p. 220-222°, obtained by treatment with ethereal diazomethane, and the pentamethyl ether-ester,

m.p. 228-230°, obtained by the dimethyl sulphate-potassium carbonate method, differed in their m.ps from the corresponding derivatives of ceroalbolinic acid (245-248° and 201-204° respectively). The Rf values (TLC on silica gel; benzene-acetone) of the two sets of ether-esters were also different. The NMR spectrum of the penta-methyl ether-ester of (4) in $CDCl_3$ shows five methoxyls (between 5.95 and 6.05), a methyl (7.30) and two singlet aromatic proton signals (2.26 and 2.37), assignable to the 4- and 8- protons. Ceroalbolinic acid consequently has the structure (5). Unambiguous evidence in support of structure (4) for the condensation product was obtained by subjecting it to decarboxylation by refluxing in diethylaniline for 4 hr. Methylation of the decarboxylated compound with ethereal diazomethane gave an orange compound, different from the trimethyl ether of isoerythrolaccin, the colouring matter of seed lac, as shown by depression in mixed melting point and their different chromatographic behaviour (silica gel benzene-acetone, 9.5:0.5). The structure of isoerythrolaccin was confirmed by its unambiguous synthesis in this Laboratory. An attempt to synthesise ceroalbolinic acid by condensing

3,4,5-trimethoxyphthalic anhydride with 6-methylsalicylic acid dimethyl ether-ester, was unsuccessful.

PART IV: Synthesis of 1,3,6-trihydroxyanthraquinone and 3-hydroxyanthraquinone-1,2-dicarboxylic acid

Synthesis of 1,3,6-trihydroxyanthraquinone

Of the fourteen possible trihydroxyanthraquinones only one, i.e. 1,3,6-trihydroxyanthraquinone is unknown. 1,8-Diaminoanthraquinone on bromination in two steps gave 1,8-diamino-2,4,7-tribromoanthraquinone which on deamination gave 1,3,6-tribromoanthraquinone. 1,3,6-Tribromoanthraquinone was converted to 1,3,6-trimethoxyanthraquinone. Demethylation with aluminium chloride and sodium chloride gave 1,3,6-trihydroxyanthraquinone.

Synthesis of 3-hydroxyanthraquinone-1,2-dicarboxylic acid

In connection with the work on lac pigments in this laboratory, it was necessary to prepare 3-hydroxyanthraquinone-1,2-dicarboxylic acid. Condensation of phthalic anhydride and 1-chloronaphthalene gave the corresponding keto acid, which was cyclised to 3-chloro-

1,2-benzanthraquinone. Oxidation of 3-chloro-1,2-benzanthraquinone gave 3-chloroanthraquinone-1,2-dicarboxylic acid. Replacement of chlorine by methoxyl group and subsequent methylation gave dimethyl ester of 3-methoxyanthraquinone -1,2-dicarboxylic acid, which on demethylation gave 3-hydroxyanthraquinone-1,2-dicarboxylic acid.

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D. D. Gadgil

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