NATURALLY OCCURRING QUINONE COLOURING MATTERS, STRUCTURE OF THE MAIN CONSTITUENT OF LAC DYE.

A

THESIS

SUBMITTED BY

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To

THE UNIVERSITY OF POONA

For

THE DEGREE OF Ph.D.

NATIONAL CHEMICAL LABORATORY

POONA

1968.

**

C O N T E N T S

PART I

CONSTITUTION OF LACCAIC ACID A

***

INTRODUCTION

Lac is a sticky resinous product secreted by the tiny lac insect (*Laccifer lacca*, Kerr[1]), a species of scale insect (family *Coccidae*) thriving on certain Indian trees, commonly known as lac hosts. The most important among them are Palas (Butea monosperma, Lamk., Taub.), Ber (*Zizyphus jujuba*, Lamk.), Kusum (*Schleichera trijuga*, Willd.), Ghont (*Zizyphus xylopyra*, Willd.), Fig (*Ficus religiosa*). It is harvested mainly for the production of shellac and lac dye. Shellac and other forms of lac are the only commercial resins of animal origin. India, Siam and Burma are the main cultivators of lac and 90% of the world's production is obtained from the Indian cultivation.

The crude lac, collected by scraping the lac-bearing twigs is called stick lac. Lac-bearing twigs which are collected few days before the larval emergence, are called as brood lac, and are mainly used for artificial infection of the host. From two strains, Kusumi and Rangeeni, four crops of lac are obtained. They are known as 'baisaki' and 'jethwi' (summer crops), 'katki' and 'aghani' (winter crops).[1a]

Stick lac is a complex mixture of several intermediate esters of various polyhydroxy carboxylic acids,[1b,c] water-

insoluble yellow colouring matter, and water soluble red dye (lac dye), sugars, salts, albuminous matter etc. The resinous matter, which remains behind, after removal of water soluble matter, is called as seed-lac from which shellac of commerce is obtained by a process of hot filtration or by solvent extraction.[1c]

Lac dye was considerably used for dyeing wool, silk and leather and in the nineteenth century it was one of the major items of export trade. After the advent of synthetic dyes, however, its use declined slowly. At present it is obtained only as an unimportant by-product of shellac industry.

The earlier methods[1c,2,3,4] of isolation of lac dye mainly involved the precipitation of the dye as lead[2] or calcium salt[3] from an acidic or aqueous extract of stick lac and the decomposition of the salt with sulphuric or hydrochloric acid. During the early stage of the present work, the calcium salt of lac dye supplied by M/s Angelo Bros. Ltd., Calcutta, was used for getting laccaic acid. The finely powdered calcium salt was purified by solvent extraction and then boiled with 2N hydrochloric acid and filtered. The filtrate on standing for 8 days gave a red crystalline dye.

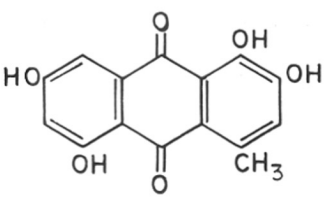
The previous methods of isolation involved a drastic treatment such as heating with sulphuric acid. Such a drastic

treatment might cause changes in the constitution of the dye molecule.

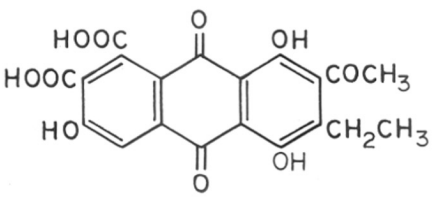
In this laboratory, a much more satisfactory method for the isolation of lac dye was developed,[5] which involves (a) the extraction of the powdered stick lac with distilled water, (b) passing of the aqueous extract through a column of cation exchange resin (H-form) from cashew-nut shell liquid, and (c) the concentration of the eluate under reduced pressure in a flash evaporator, and at 40-55°. On keeping at 5-10°, the dye was obtained in crystalline form. Paper chromatography of the dye, using n-butanol-formic acid-water (4:1:5) revealed two spots with R_f values 0.09 and 0.45 (major fraction) respectively. After a second passage through CNSL resin or Dowex-50 the major component (R_f value 0.45) was separated. On repeated crystallisation from water, a crystalline laccaic acid containing about 1% nitrogen was obtained.

The residue left after removal of laccaic acid from stick lac is called seed lac from which a pigment known as erythrolaccin was isolated.[2,6] The structure (I) was assigned to erythrolaccin and was confirmed by its synthesis.[7,8]

Schmidt[9] in 1887, assigned to laccaic acid the molecular formula $C_{16}H_{12}O_8$, revised by Dimroth and Goldschmidt[10] to $C_{20}H_{14}O_{10}$. On the basis of a series of oxidations which ultimately yielded phenol-2,3,4,5-tetracarboxylic acid, and by comparison with the reactions of carminic acid, Dimroth and Goldschmidt[10] concluded that "a considerable fragment of the molecule has been structurally established with certainty;" but they did not propose a complete structure. After summarising their results, Mayer and Cook[11] suggested structure (II) for the laccaic acid.



I



II

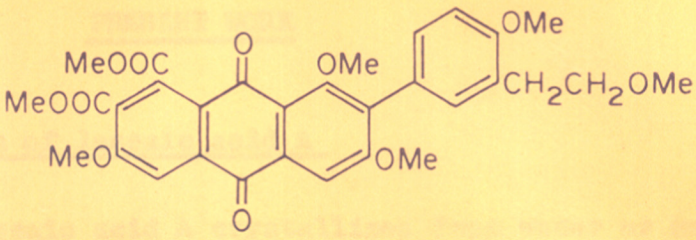
Evidence for the presence of the anthraquinone nucleus was further indicated by the zinc dust distillation studies by Tschirch and Ludy.[2] The presence of nitrogen in laccaic acid was first noted by Venkataraman,[10a] Kulkarni,[10b] and by Kamath and Potnis[3] by the analysis of crude calcium salt of laccaic acid.

Earlier work in this laboratory showed[12] that Dimroth's laccaic acid is a complex mixture containing about 2% nitrogen. Colour reactions and a study of the UV-visible absorption spectra of the vats obtained from laccaic acid, hydroxyanthraquinones and hydroxynaphthaquinones showed that the major constituents of laccaic acid are purpurin derivatives. Potentiometric titrations indicated that laccaic acid is a dibasic acid. There was no evidence for a C-acetyl or C-ethyl group as in (II). Methylation of laccaic acid with dimethyl sulphate and potassium carbonate in boiling acetone yielded a product which on chromatography on silica gel yielded five crystalline ether-esters designated as MLA I, MLA II, MLA III, MLA IV, and MLA V. Only MLA III contains nitrogen. On treatment with aqueous dithionite laccaic acid underwent the purpurin $\longrightarrow$ xanthopurpurin reduction[13] and gave "xantholaccaic acid". On methylation with methyl iodide and silver oxide in dimethylformamide xantholaccaic acid gave a product which on chromatography gave three crystalline ether-esters designated as MXLA I, MXLA II and MXLA III; only the last contains nitrogen. The molecular weight of MXLA I is 578 (mass spectrum), corresponding to $C_{31}H_{30}O_{11}$. MLA V has molecular weight 608 (652) and molecular formula, $C_{33}H_{32}O_{14}$. On hydrolysis with methanolic potassium hydroxide MXLA I gave a crystalline dibasic acid, which on decarboxylation yielded a compound with the molecular formula

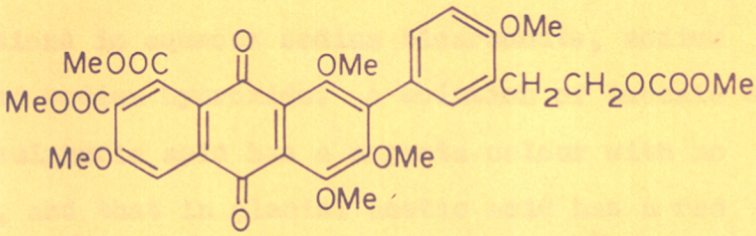
$C_{27}H_{26}O_7$ (mol.wt. by mass spectrum 462). Based mainly on NMR evidence, structures (III), (IV) and (V) were assigned[12] to MXLA I, MLA V and the decarboxylation product obtained from the dibasic acid which in its turn was obtained from MXLA I. This work showed that MXLA I and MLA V are probably originated from the same constituent of laccaic acid.

Isolation of laccaic acid A

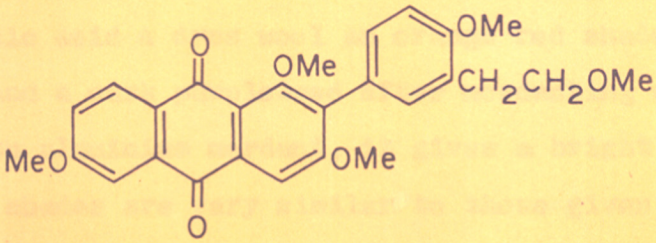
The isolation of laccaic acid A from crude laccaic acid was achieved by the method developed by I.N. Shaikh[14] in this laboratory. Crude laccaic acid (2.2 g) was dissolved in n-butanol saturated with 0.3N hydrochloric acid, the mixture filtered and the clear bright red solution in the organic phase was chromatographed on a column of poly-caprolactam powder (300 g., 100 x 5 cm^2) using the same solvent system for development and elution. A minor fast-moving fraction was followed successively by two main fractions. The eluate (1 lit.) which contained the first major fraction was extracted with a saturated solution of sodium acetate or sodium bicarbonate; the extracts were acidified (Congo Red) with conc. hydrochloric acid and kept overnight in the refrigerator, when laccaic acid A separated in bright red needles (1.0 g).


MeOOC
MeOOC
MeO
O
OMe
OMe
OMe
CH2CH2OMe
O

III


MeOOC
MeOOC
MeO
O
OMe
OMe
OMe
OMe
CH2CH2OCOOMe
O

IV


MeO
O
OMe
OMe
OMe
CH2CH2OMe
O

V

PRESENT WORK

Constitution of laccaic acid A

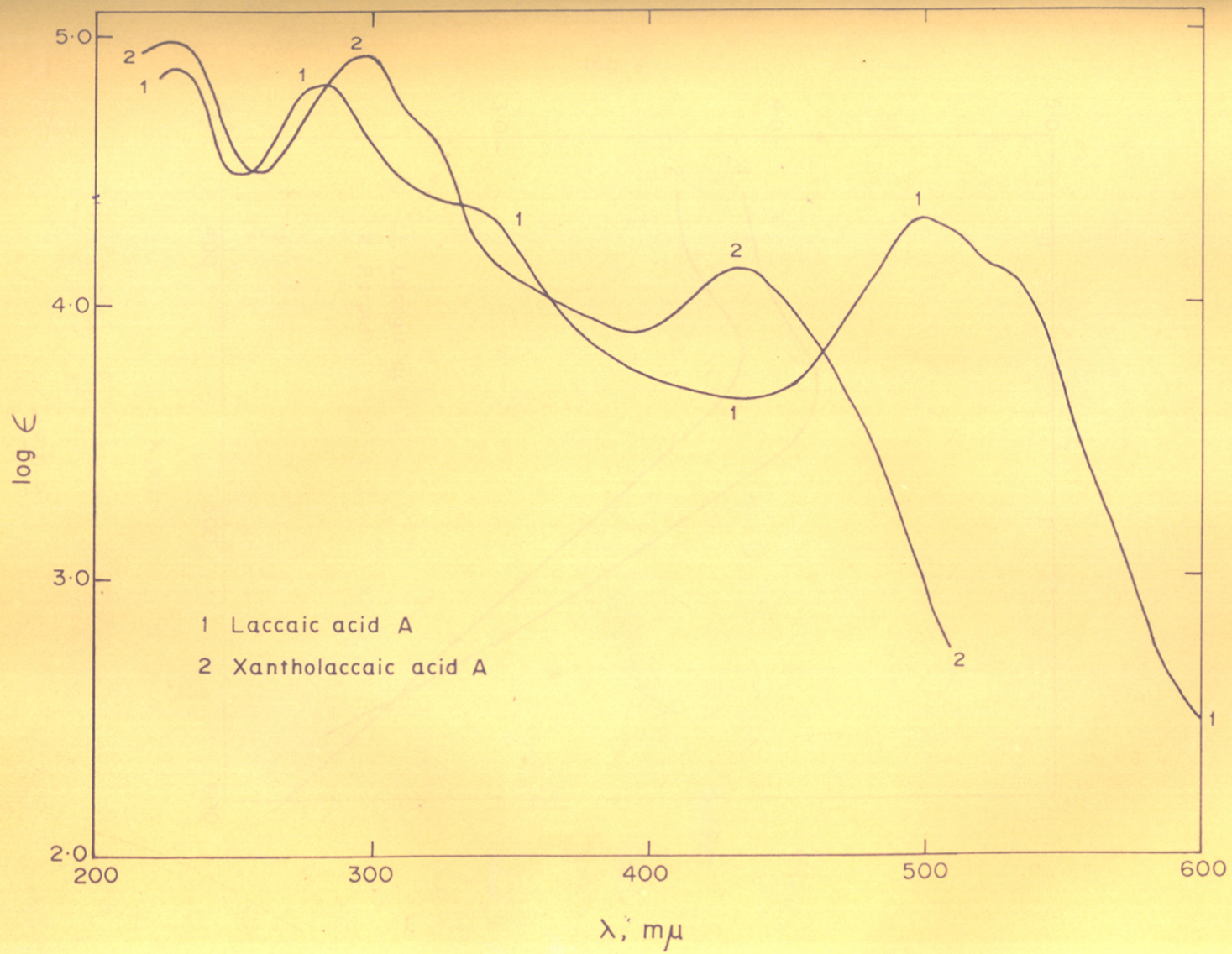
Laccaic acid A crystallizes from water or methanol in bright red needles which sintered at 230° and charred at higher temperature. It is readily soluble in water, methanol, ethanol, pyridine, acetic acid, and dimethylformamide, difficultly soluble in acetone and n-butanol and insoluble in benzene, chloroform and ether. It forms purple solutions in aqueous sodium bicarbonate, sodium carbonate and sodium hydroxide. A solution of laccaic acid A in conc. sulphuric acid has a magenta colour with no fluorscence, and that in glacial acetic acid has a red colour with faint fluorscence in ultra violet light. It gives a bluish gray colouration with ethanolic ferric chloride and a purple with methanolic magnesium acetate. Its purple solution in aqueous sodium hydroxide turns red on treatment with sodium hydrosulphite, and remains red after air oxidation.

Laccaic acid A dyes wool an orange red shade from an acid bath and a dark purple-red after mordanting with chromium. With aluminium mordant, it gives a bright red colour. These shades are very similar to those given by purpurin.

The elemental analysis of laccaic acid A gave C, 57.7; H, 3.8 and nitrogen 2.3%, which corresponds to the molecular formula $C_{26}H_{19}NO_{12}$. The molecular formula was deduced from the methylated derivatives of laccaic acid A, which will be discussed afterwards. Kuhn Roth estimation indicated the presence of a C-methyl group.

The UV-visible absorption spectra of the laccaic acid A (Fig. 1) has a close resemblance with that of crude laccaic acid in ethanol, thereby showing that the main chromophore of its constituents is likely to be the same. The electronic spectra of the laccaic acid in ethanol and in alkaline dithionite were reported in the earlier publications,[12] and from the results it is certain that the laccaic acid behaves as an anthraquinone and in particular like purpurin. Laccaic acid A in ethanol, shows a peak at 500 mμ in the visible region (purpurin in ethanol, λ_{max} 480 mμ). The spectrum of laccaic acid A as the vat in alkaline dithionite (Fig. 2) has a strong resemblance to that of purpurin (laccaic acid λ_{max} 452 mμ; purpurin λ_{max} 446 mμ).

The infrared spectrum of laccaic acid A in nujol (Fig. 3) showed peaks at 1715, 1692, 1667 and 1626 cm^{-1}. The 1692 and 1715 cm^{-1} peaks can be assigned to carboxylic groups, the peak at 1626 cm^{-1} to two chelated quinone carbonyl groups as in quinizarin and purpurin and the 1667 cm^{-1} peak to an additional carboxyl group.


5·0
4·0
3·0
2·0
log ∈
200
300
400
500
600
λ, mμ
1 Laccaic acid A
2 Xantholaccaic acid A

FIG·1

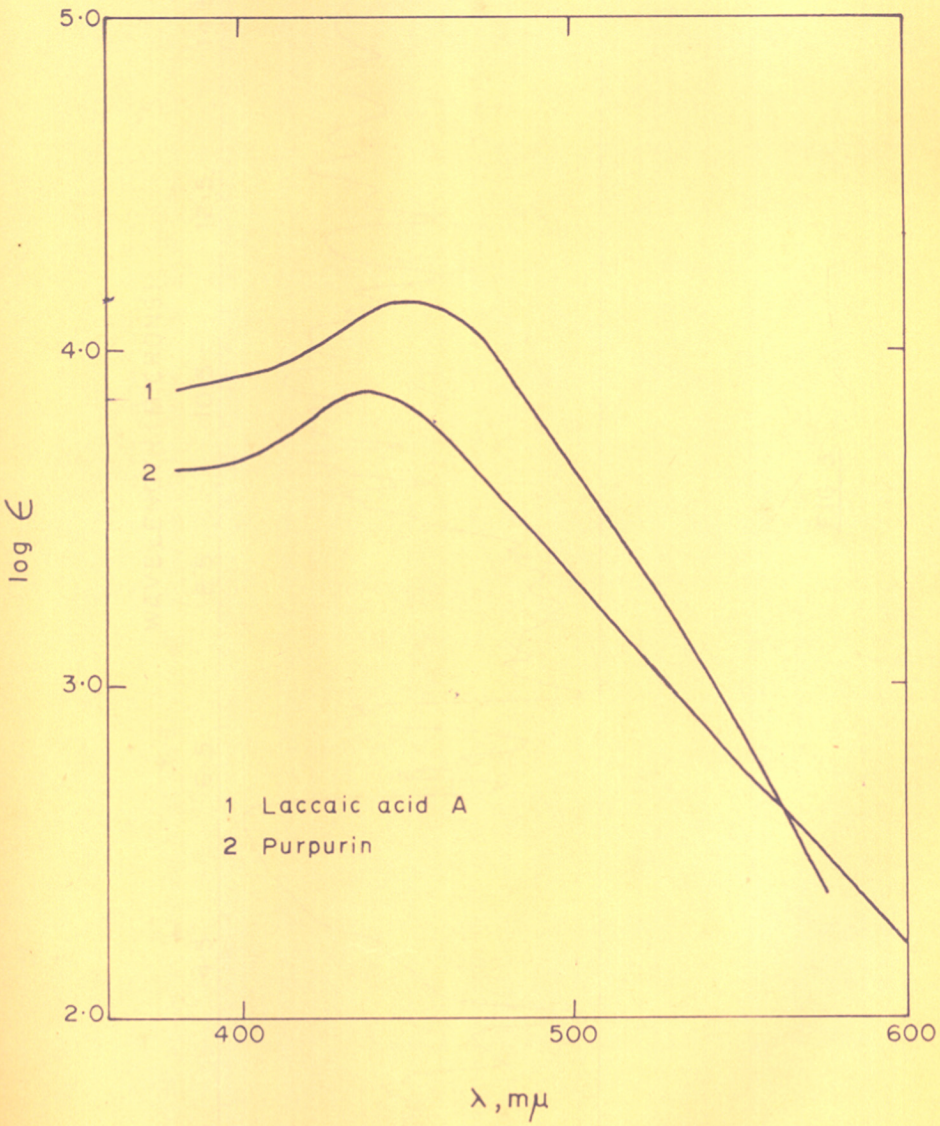


FIG. 2

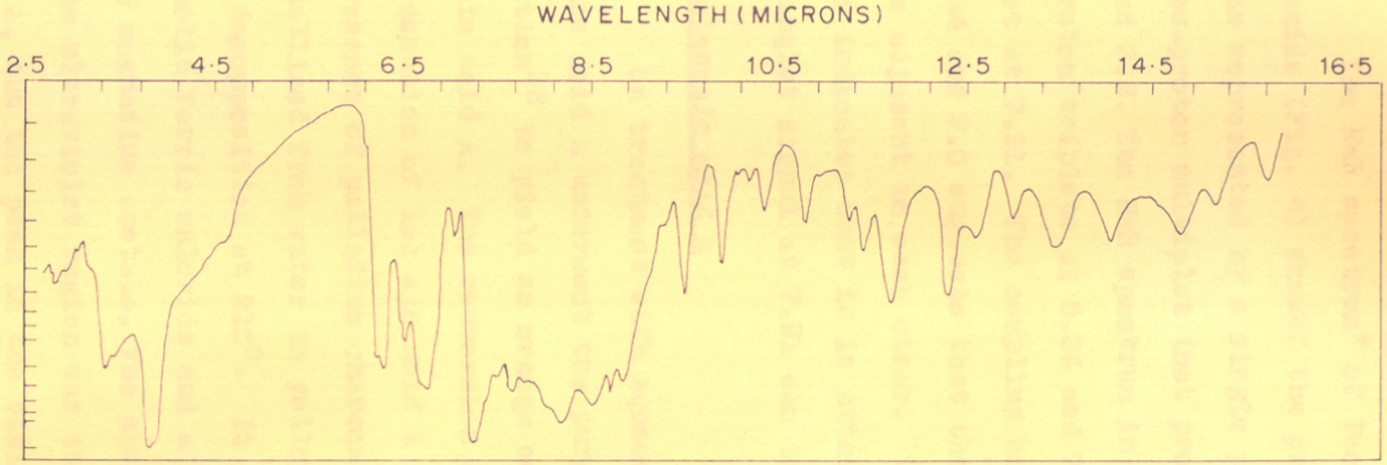


FIG. 3

The NMR spectrum* of laccaic acid A in dimethylsulphoxide (Fig. 4) showed the presence of four aromatic protons represented by a single proton singlet at 2.22 and a three-proton multiplet (not properly resolved) between 2.7 and 3.3. The NMR spectrum in pyridine showed two broad two-proton triplets at 6.34 and 7.0 and a three-proton singlet at 7.91. The coupling constant of the two signals at 6.34 and 7.0 suggests that they are due to two methylene groups adjacent to each other. The chemical shift of the latter indicates that it is attached to an aromatic ring. The singlet signal at 7.91 can be assigned to a methyl group.

Xantholaccaic acid A

On treatment with aqueous alkaline sodium dithionite, laccaic acid A underwent the purpurin→ xanthopurpurin reduction[13] to yield an orange compound designated as xantholaccaic acid A. The conversion could also be achieved by hydrogenation of laccaic acid A in aqueous sodium hydroxide in presence of palladium charcoal. Xantholaccaic acid A crystallized from water in yellowish orange needles melting with decomposition at 210°. It gives brown colour with ethanolic ferric chloride and a brownish orange with methanolic magnesium acetate. The absorption spectrum (Fig. 1) in the ultraviolet region was the same as that of laccaic acid A, but the peak in the visible region showed a marked

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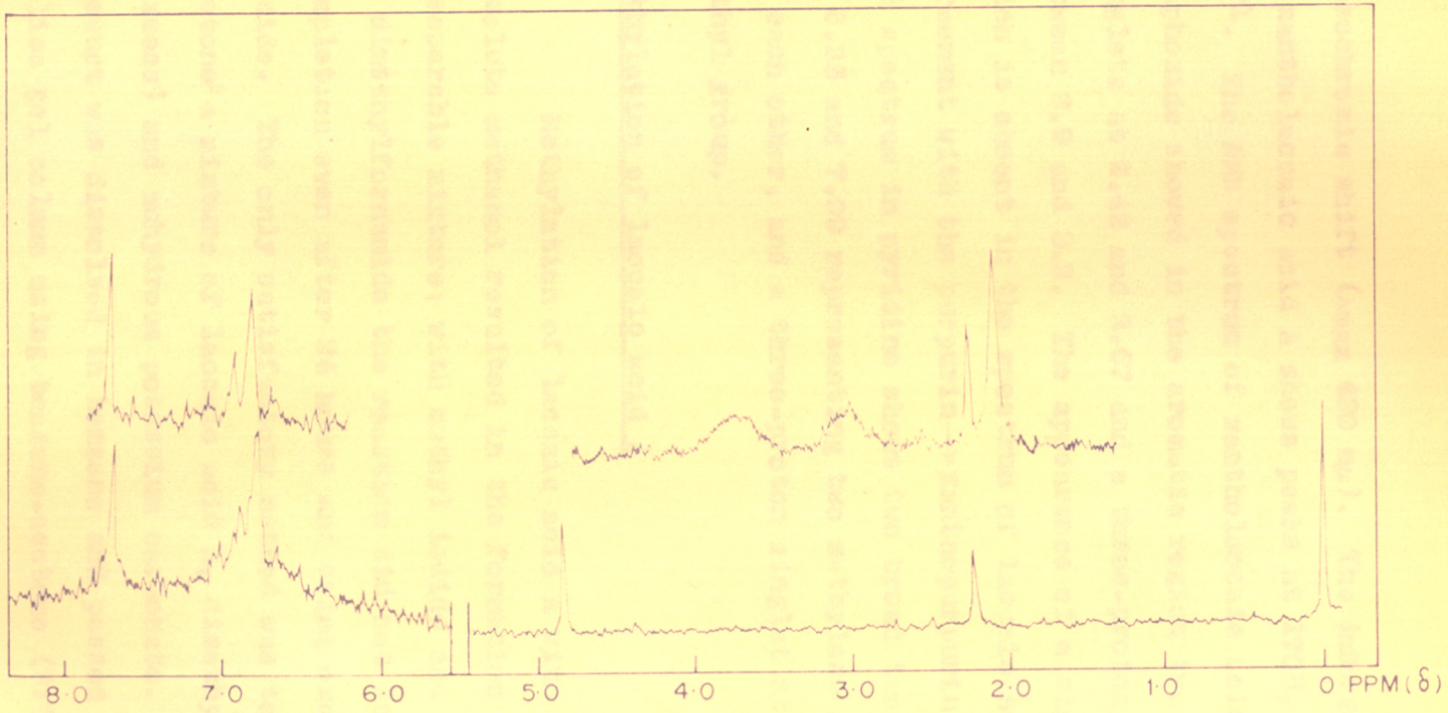
* Chemical shifts are cited on the τ scale.



FIG·4

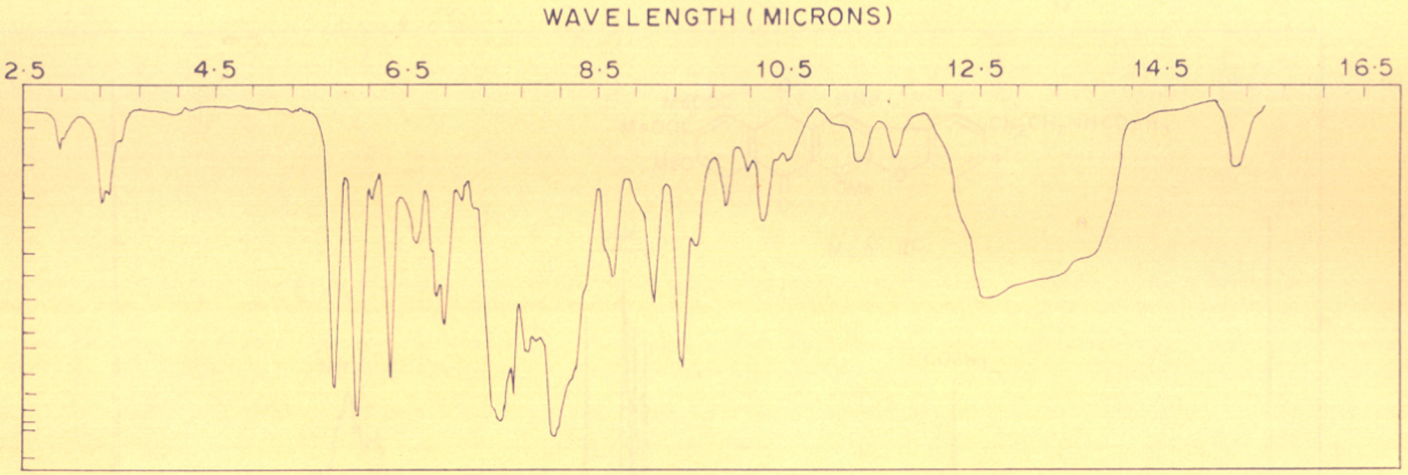
hypsochromic shift (λ_{max} 430 mμ). The infrared spectrum of xantholaccaic acid A shows peaks at 1708, 1675 and 1623 cm^{-1}. The NMR spectrum of xantholaccaic acid A in dimethyl sulphoxide showed in the aromatic region two single-proton singlets at 2.42 and 2.67 and a three-proton multiplet between 2.9 and 3.2. The appearance of a signal at 2.67, which is absent in the spectrum of laccaic acid A, is in agreement with the purpurin→xanthopurpurin conversion. NMR spectrum in pyridine shows two broad two-proton triplets at 6.35 and 7.09 representing two methylene groups adjacent to each other, and a three-proton singlet at 7.99 for a methyl group.

Methylation of laccaic acid A

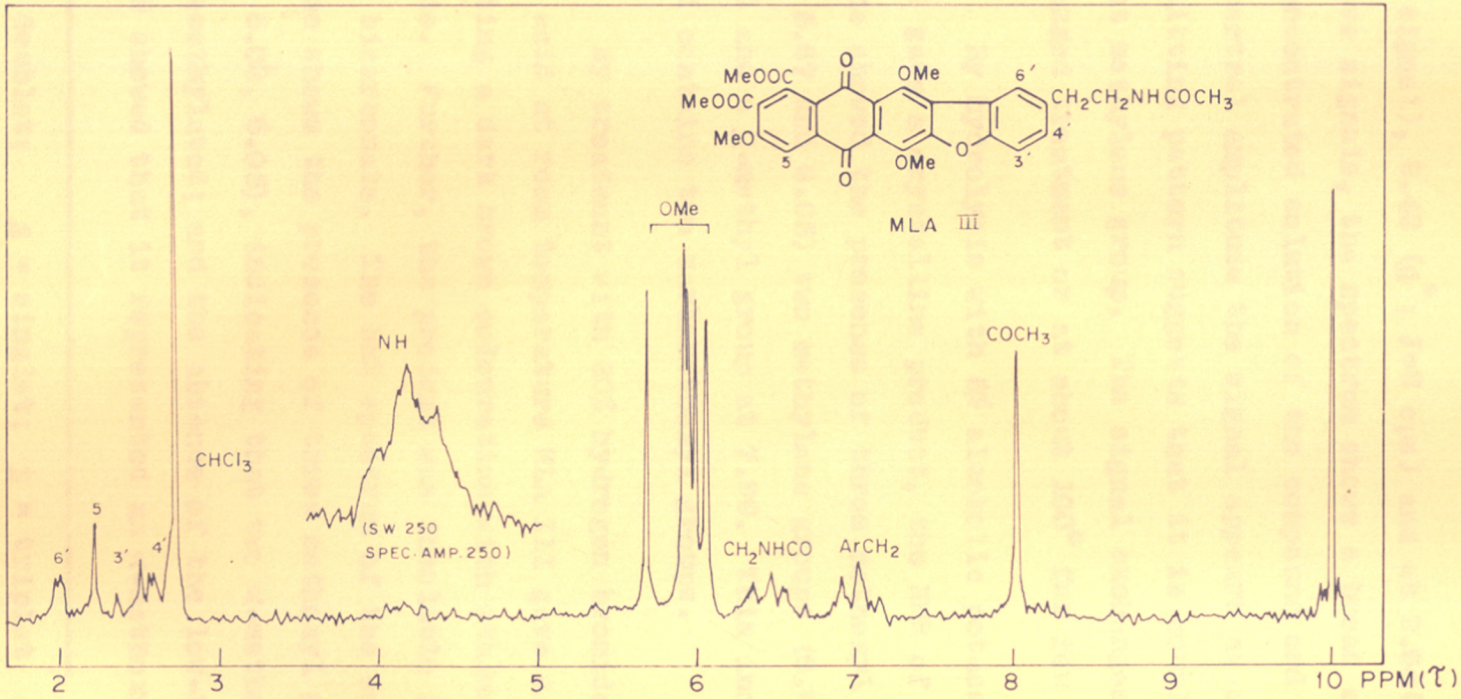
Methylation of laccaic acid A with diazomethane in absolute methanol resulted in the formation of a complex inseparable mixture; with methyl iodide and silver oxide in dimethylformamide the reaction did not proceed to completion even after 24 hours and using excess of methyl iodide. The only satisfactory method was to reflux in acetone a mixture of laccaic acid A, dimethyl sulphate (excess) and anhydrous potassium carbonate. The crude product was dissolved in benzene and passed through a silica gel column using benzene-acetone (8:2) mixture as eluant. The residue obtained by the concentration of the eluate crystallized from methanol in yellow microscopic

needles, melting at 243°. It was found to be identical with MLA III which was obtained earlier[12] along with MLA I, MLA II, MLA IV and MLA V, during the silica gel chromatography of the methylation product of crude lac dye.

From the elemental analysis of MLA III (C, 63.2; H, 4.9; N, 2.5%) and the molecular weight 589 (mass spectrum), the molecular formula was assigned as $C_{31}H_{27}NO_{11}$. The molecular formula was in agreement with the proton count in the NMR spectrum. The infrared spectrum in $CHCl_3$ showed (Fig. 5) bands at 1742 and 1672 cm^{-1}; attributed to ester carbonyl and quinone carbonyl groups reppectively, and a band at 3448 probably due to -NH- vibration. The NMR spectrum in $CDCl_3$ (Fig. 6) gives a total proton count of 27 and shows the presence of five methoxyl groups in the range of 5.68 to 6.07, although the signal at 5.68 is unusually low for a methoxyl group on an anthraquinone. The sharp singlet at 8.02 can be assigned to a C-methyl group. Further it shows two two-proton triplets at 7.02 and 6.47 indicating the presence of two methylene groups adjacent to each other; the chemical shifts correspond to a benzylic methylene and a methylene attached to oxygen or to an electron-withdrawing group. The aromatic region shows signals for four protons. The singlet at 2.22 can be assigned to an α-proton in anthraquinone. The other three protons, constituting an ABC pattern appear at 1.98



FIG·5


MeOOC
MeOOC
MeO
OMe
OMe
O
O
O
6′
4′
3′
5
$CH_2CH_2NHCOCH_3$
MLA III
OMe
NH
(SW 250
SPEC. AMP. 250)
$CHCl_3$
$COCH_3$
CH_2NHCO
$ArCH_2$
6′
5
3′
4′
2
3
4
5
6
7
8
9
10 PPM(τ)

FIG·6

(broad signal), 2.43 (d*; J=8 cps) and at 2.64(q). Besides the above signals, the spectrum shows a broad signal at 4.2. In a concentrated solution of the compound and at an increased spectral amplitude the signal appears as a broad triplet. The splitting pattern suggests that it is coupled with an adjacent methylene group. The signal exchanges with D_2O by a prolonged treatment or at about 100° for few minutes.

By hydrolysis with 5% alcoholic potassium hydroxide MLA III gave a crystalline product, the NMR of which in pyridine showed the presence of three methoxyl groups (5.57, 5.67 and 6.05) two methylene groups (6.22 t and 6.83 t) and a C-methyl group at 7.89. This indicated that MLA III contains two carbomethoxyl groups.

By treatment with 30% hydrogen bromide in glacial acetic acid at room temperature MLA III gave a product exhibiting a dark brown colouration with ethanolic ferric chloride. Further, the product was insoluble in aqueous sodium bicarbonate. The NMR spectrum of the compound in pyridine shows the presence of three methoxyl groups (5.84, 6.02, 6.05), indicating that two ∝-methoxyl groups were demethylated; and the absence of the low-field signal at 5.68 showed that it represented an ∝-methoxyl group.

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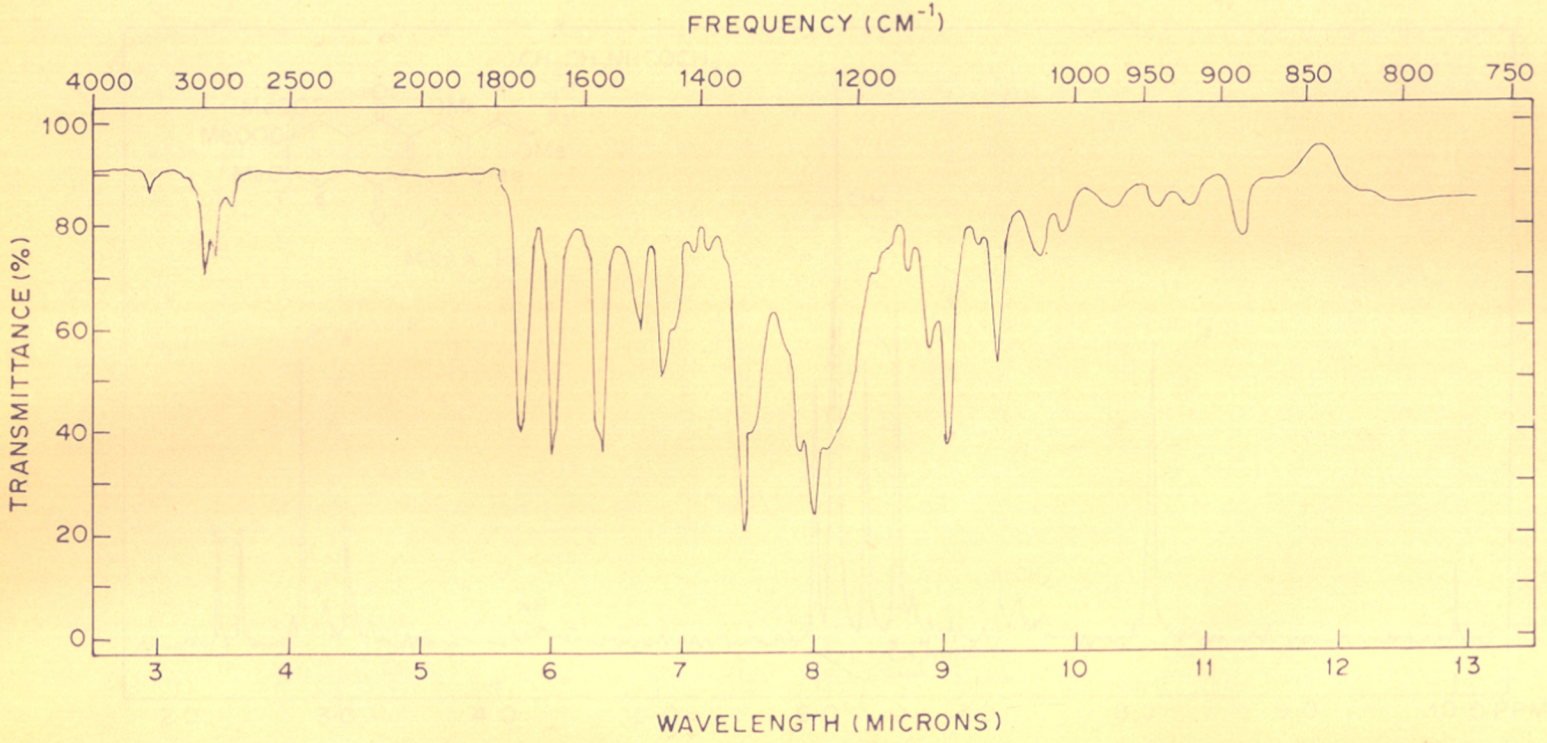
* d = doublet; s = singlet; t = triplet
q = quartet; m = multiplet.

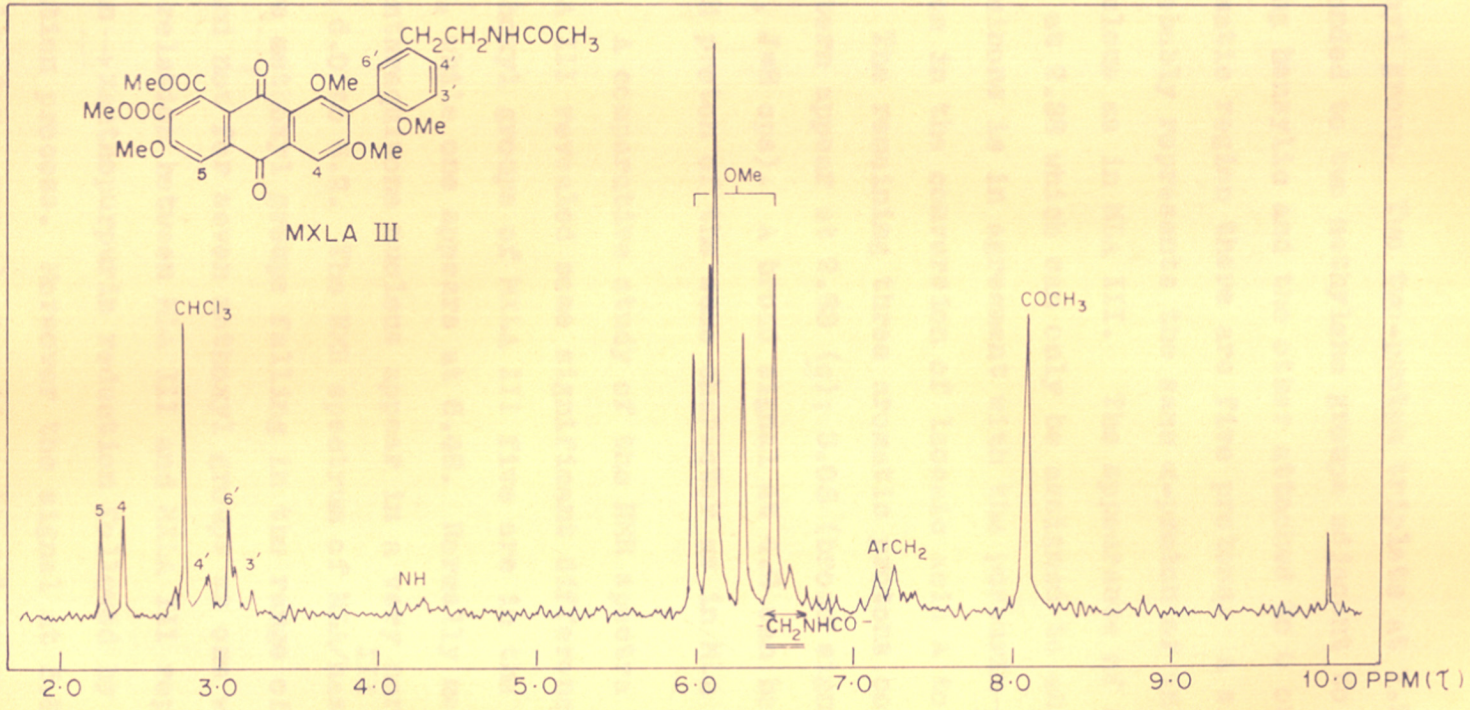
Comparing the NMR spectrum of this compound with that of the product obtained by the alkaline hydrolysis of MLA III, it is clear that only one β-methoxyl group at 6.05 remains intact under both hydrolytic experiments.

Methylation of xantholaccaic acid A

Xantholaccaic acid A was methylated with methyl iodide in presence of silver oxide in dimethyl formamide at room temperature during 24 hours. The crude product gave on crystallization from methanol yellow plates melting at 169°. The compound was found to be identical with MXLA III which was obtained previously[5,12] along with MXLA I and MXLA II, during the chromatography of crude product of methylation of xantholaccaic acid. The molecular weight by mass spectrum was 605, corresponding to the molecular formula $C_{32}H_{31}NO_{11}$, confirmed by the proton count in the NMR spectrum. Kuhn-Roth estimation showed the presence of a C-methyl group. The infrared spectrum (in $CHCl_3$; Fig. 7) shows bands at 1734 and 1670 cm^{-1}, assigned to ester carbonyl and quinone carbonyl respectively, and a band at 3495 cm^{-1} assigned to NH vibration. The NMR spectrum in $CDCl_3$ (Fig. 8) gives a total proton count of 31, and shows the presence of six methoxyl groups as singlets at 5.98, 6.08, 6.1, 6.28 and 6.48, integrating for 3,3,6,3 and 3 protons respectively. A sharp singlet at 8.08 could be assigned to


FREQUENCY (CM^{-1})
4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13
WAVELENGTH (MICRONS)

FIG·7


CH2CH2NHCOCH3
MeOOC
MeOOC
MeO
OMe
OMe
OMe
MXLA III
CHCl3
OMe
COCH3
ArCH2
NH
CH2NHCO
5
4
6'
4'
3'
2·0
3·0
4·0
5·0
6·0
7·0
8·0
9·0
10·0 PPM(τ)

FIG·8

a C-methyl group. Two two-proton triplets at 7.25 and 6.57 corresponded to two methylene groups adjacent to each other, one being benzylic and the other attached to O or N-CO-. In the aromatic region there are five protons. A singlet at 2.25 probably represents the same α-proton of an anthraquinone nucleus as in MLA III. The appearance of an additional singlet at 2.38 which can only be assigned to an α-proton in anthraquinone is in agreement with the purpurin→xanthopurpurin reduction in the conversion of laccaic acid A to xantholaccaic acid A. The remaining three aromatic protons constituting ABC pattern appear at 2.83 (q); 3.05 (broad signal) and 3.14 (d, J=8 cps). A broad signal at 4.2 can be assigned to an NH proton of the same character as in MLA III.

A comparative study of the NMR spectra of MLA III and MXLA III revealed some significant differences. Out of six methoxyl groups of MXLA III five are in the range of 5.98 to 6.08, while one appears at 6.48. Normally methoxyl groups on an anthraquinone nucleus appear in a very narrow region between 6.0 to 6.2. The NMR spectrum of MLA III has signals for five methoxyl groups falling in the range of 5.68 to 6.07, and not for seven methoxyl groups as one would expect if the relation between MLA III and MXLA III represented a purpurin → xanthopurpurin reduction followed by a normal methylation process. Moreover the signal at 5.68 in MLA III is too low for a normal aromatic methoxyl group.

MXLA III on hydrolysis with 5% methanolic potassium hydroxide gave a crystalline product, the NMR spectrum of which in pyridine shows the presence of four methoxyl groups, two methylene groups and a C-methyl group. The compound did not give a colouration with ethanolic ferric chloride, and on treatment with diazomethane in dry ether was reconverted to MXLA III. This indicated that MXLA III contains two carbomethoxyl groups.

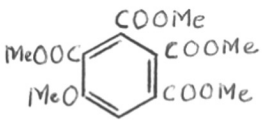
On treatment with boron trifluoride etherate in acetic anhydride at room temperature for 24 hours, MXLA III yielded a compound which gave a brown colouration with ethanolic ferric chloride, showing the presence of a chelated hydroxyl group. The compound was not soluble in aqueous sodium carbonate indicating the absence of a β-hydroxyl and free carboxyl groups. The NMR spectrum shows the presence of five methoxyl groups. The disappearance of the high-field signal at 6.48 indicated that an α-methoxyl group of an anthraquinone nucleus has undergone demethylation. The absence of an aliphatic methoxyl group in MXLA III in contrast with MXLA I,[12] is shown by the fact that the NMR spectrum does not indicate the replacement of a methoxyl by an acetoxyl group. The same product was obtained by treatment of MXLA III with 30% hydrogen bromide in glacial acetic acid at room temperature for 18 hours.

Attempts to decarboxylate the dibasic acid obtained from MXLA III were not successful.

Comparison of the values 8.02 and 8.08 for C-methyl signals in the NMR spectra of MLA III and MXLA III with those of various methylanthraquinones (Table 1) shows the absence of C-methylanthraquinone in laccaic acid A. The presence of a C-acetyl group was excluded by the fact that Clemmenson or borohydride reduction of MXLA III gave an amorphous product in which a signal at 8.02 was still present.

Nitric acid oxidation of MLA III

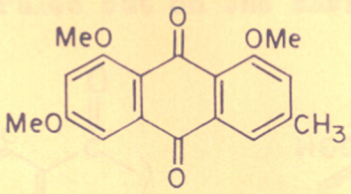
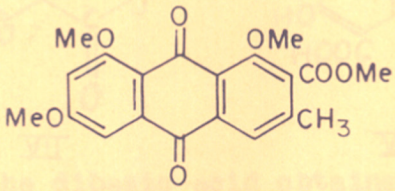
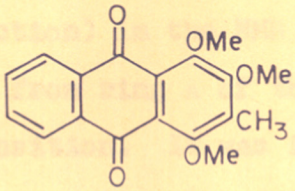
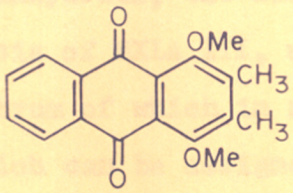
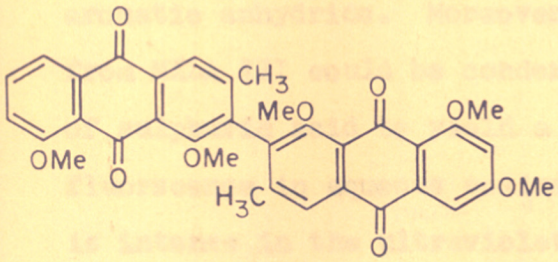
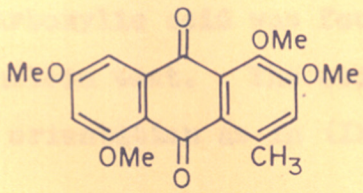
MLA III on oxidation with 35% nitric acid, followed by a methylation with diazomethane, yielded tetramethyl anisole-2,3,4,5-tetracarboxylate (VI), m.p. 130°. It was found



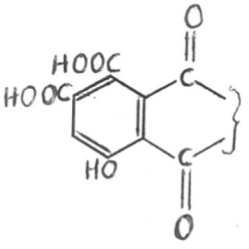
VI

to be identical with a synthetic sample[15] (mixed melting point remained undepressed). Formation of the compound (VI) showed that one of the rings of the anthraquinone nucleus of laccaic acid A is substituted with a hydroxyl group and two carboxyl groups, the other two carboxyl groups being formed due to the cleavage of quinone carbonyls. The oxidative degradation product leads to the three possible orientations (VII), (VIII) and (IX) of the two carboxyl groups and a hydroxyl group on an

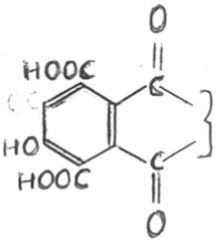
TABLE-1

COMPOUND	SOLVENT	$-C-CH_3$ SIGNAL (τ)
	$CDCl_3$	7·58
	$CDCl_3$	7·6
	$CDCl_3$	7·7
	$CDCl_3$	7·7
	$CDCl_3$	7·85
	$CDCl_3$	7·25

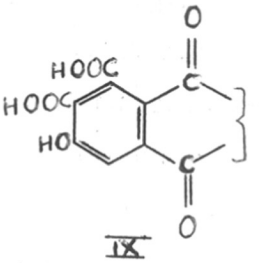
anthraquinone ring of laccaic acid A. The structure (VII) was ruled out on the basis of the decarboxylation product



VII



VIII

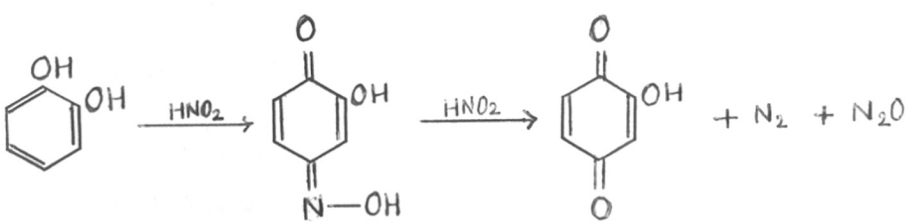


IX

of the dibasic acid obtained from MXLA I (mentioned in the introduction) in the NMR spectrum of which three protons arising from ring A of the anthraquinone are substituted at 1,2,4-position. It was found that on treatment with boiling acetic anhydride, the dibasic acid obtained from the alkaline hydrolysis of MXLA III, was converted into its anhydride, the IR spectrum of which in nujol showed peaks at 1775 and 1830 cm^{-1} which can be assigned to the carbonyl groups of the aromatic anhydride. Moreover, the dibasic acid obtained from MXLA III could be condensed with resorcinol in presence of sulphuric acid to yield a phthalein which gives a weak fluorscence in aqueous sodium hydroxide, the fluorscence is intense in the ultraviolet light. Anthraquinone 1,2-dicarboxylic acid was found to behave similarly during phthalein test. The evidence described above, supports the orientation as in (IX).

Nature of nitrogen in laccaic acid A

Although the presence of nitrogen in crude laccaic acid was detected much earlier, its exact nature was unknown for a long time, mainly because the nitrogen containing laccaic acid was not obtained as a pure crystalline substance giving elemental analysis for C,H and N in agreement with a specific molecular formula. After getting a constant analytical value of 2.3-2.5% for nitrogen by the Kjeldahl and Dumas methods, laccaic acid A was subjected to van Slyke estimation, which gave a value of 1.8%, but the conclusion that an ∝-amino acid group was present proved to be erroneous, because the nitrogen free fractions of lac dye also gave van Slyke N of 1.2 to 1.8%. It has been shown by Kainz and Huber[16] that such anomalous van Slyke values are given by phenols due to C-nitrosation at ortho and para positions to phenolic hydroxyl groups. Thus in the case of catechol, the anomalous reaction might proceed as follows. On treatment with excess nitrous acid, degradation of the benzoquinone took place to



yield oxalic acid with evolution of carbon dioxide and a mixture of nitrogen and nitrous oxide.

The IR spectrum of MLA III in $CHCl_3$ (Fig. 5) shows a peak at 3460 cm^{-1} and that of MXLA III in $CHCl_3$ (Fig. 7) at 3495 cm^{-1}. These peaks can be attributed to NH stretching.

A broad single-proton triplet observed in the NMR spectra of MXLA III and MLA III and which underwent deuterium exchange can be assigned to an NH proton. The multiplicity of this signal indicates that the NH group is attached to a methylene group. Further, the methylene attached to the NH group is subjected to a paramagnetic shift, compared to one which is attached to an aliphatic amine. The two methylene groups and the methyl are in the right position if the NH is further linked to an acetyl group. In the NMR spectrum of N-acetyl-β-phenylethylamine[17] in $CDCl_3$, the two methylene groups are at 7.2 ($ArCH_2$) and 6.52 ($NH-CH_2$) and the NH proton appears at 3.5. N-acetyl determination of MXLA III under the usual conditions gave 0.6 mole of acetic acid, but more drastic treatment with potassium hydroxide in boiling ethylene glycol gave the correct acetyl value. All these evidence leads to show the presence of a $ArCH_2CH_2NHCOCH_3$ group in laccaic acid A and its derivatives MLA III and MXLA III.

It was observed during the course of this study that the NH proton of N-acetyl β-phenylethylamine and its derivatives show a characteristic solvent shift when its spectrum is recorded in carbon tetrachloride and deutero-

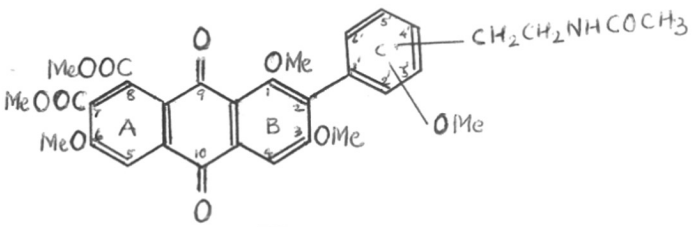
chloroform. Table 2 shows that in CCl_4, the NH proton appears around 2.5 and suffers a diamagnetic shift of more than 1 ppm in $CDCl_3$. This shift is probably due to the solvent solute interaction. The large shift may be due to the specific interaction of the carbonyl group of an amide with the proton of $CHCl_3$. The solvent shift is consistent and is helpful in distinguishing the NH proton of an amide from other signals which may absorb in the same region.

Table 2

No.	Compound	NH signal in CCl_4	NH signal in $CDCl_3$
1.	N-acetyl benzylamine	2.1	3.4
2.	N-acetyl-β-phenylethylamine	2.2	3.5
3.	N-acetyl-p-nitro-β-phenyl-ethylamine	(insoluble)	4.25
4.	N-acetyl-3,4-dimethoxy-β-phenylethylamine	2.2	4.95

Structure of xantholaccaic acid A hexamethyl ether-ester

Having obtained the proof that one of the rings of anthraquinone is substituted with two carboxyl groups and a hydroxyl group and further knowing that the laccaic acid A undergoes purpurin → xanthopurpurin transformation it is now possible to represent MXLA III tentatively as (X).



X

The substitution of the phenyl group at 2-position as in (X) is substantiated by the absence in its NMR spectrum (Fig. 8) of any signal beyond 3.0 which can be assigned to a β-proton, and the diamagnetic shift suffered by the two methoxyl groups at positions 1 and 3. The signal at 6.48 is assigned to α-methoxyl group at position 1, as this has disappeared in the boron trifluoride etherate-acetic anhydride reaction product and is replaced by a chelated -OH group.

The ABC pattern of the three aromatic protons suggests 1,2,4 substitution on the phenyl ring C. One of the substituents has been already shown to be an ethylacetamido group. Further it is clear that the sixth methoxyl group has to be

placed on the phenyl ring. The orientation of the two substituents (-OMe and $-CH_2CH_2NHAc$) will be discussed later.

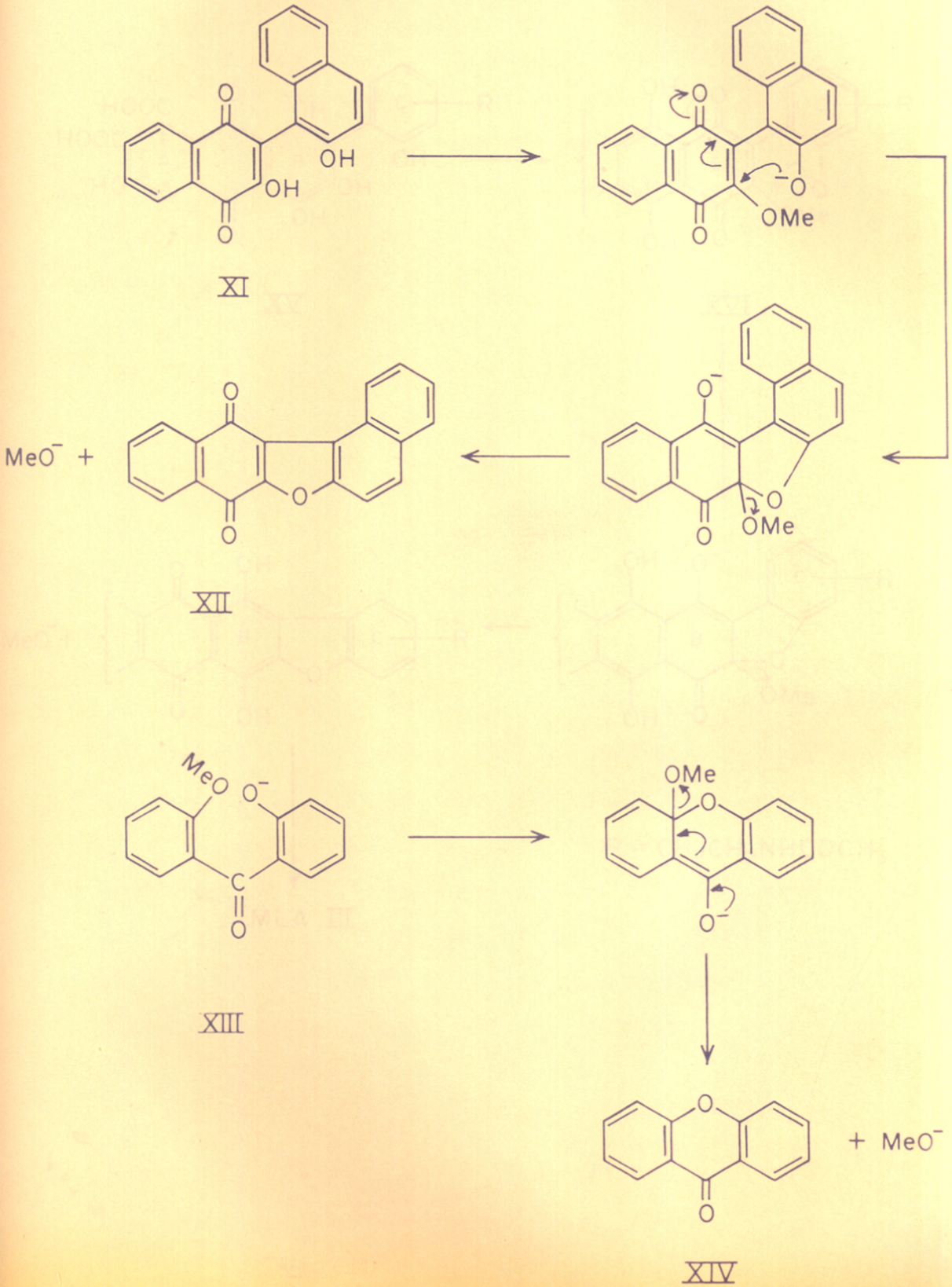
Structure of MLA III

Although MLA III and MXLA III are derived from the same laccaic acid A which has been shown to be a purpurin derivative, MXLA III contains one methoxyl group more than MLA III and not one less as one would expect from a normal purpurin $\longrightarrow$ xanthopurpurin change. Out of the five methoxyl signals which appeared in the NMR spectrum of MLA III (Fig.6), two were assigned to two α-methoxyl groups situated on one ring of the anthraquinone nucleus, the remaining three to two carbomethoxyls and a β-methoxyl on the other ring of anthraquinone. The absence of any additional aromatic signals in the NMR spectrum of MLA III and the similarity of the splitting pattern of the three aromatic protons from the β-phenyl ring in MLA III and MXLA III, clearly indicates that MLA III and MXLA III are similarly substituted. However, these three aromatic protons in MLA III, are shifted downfield, compared to these in MXLA III. Thus a broad signal at 3.05 showing a meta coupling in MXLA III, appears at 1.98 in MLA III. This show that some structural changes might have taken place during the methylation of laccaic acid A. Probably these changes involve a cyclization between two phenolic hydroxyl groups resulting into a dibenzofuran system. This is possible only if a β-phenyl ring carries a hydroxyl group, ortho to

the point of attachment to the anthraquinone nucleus. However, it is not clearly understood how the elimination of water takes place between two phenolic hydroxyl groups under the conditions of methylation. Probably its formation involves the displacement of a 3-methoxyl group in 1,4-dihydroxy-anthraquinone by the phenolate ion from the adjacent phenyl ring. This view is in accordance with the observation that the dihydric phenol (XI) produced by the alkaline fission of the 1,2-benzobrazanquinone (XII) undergoes recyclization to the parent quinone (XII) by treatment for about an hour with excess of dimethyl sulphate and potassium carbonate in boiling acetone. The more acidic hydroxyl of the naphtha-quinone may undergo methylation first and the conversion of (XI) to (XII) may proceed as shown by the mechanism in Chart 1.

A similar mechanism for the formation of xanthones under mild basic conditions from the corresponding 2-hydroxy-2'-methoxybenzophenones (XIII) to (XIV) was suggested by Barton and Scott,[18] (Chart 1).

From these observations it seems probable that during the conversion of laccaic acid A to MLA III, laccaic acid A (XV) attains a tautomeric form (XVI) in which the 1,4-hydroxyls and 9,10-quinone functions are interchanged. Hence the mechanism for this conversion can be written as shown in Chart 2.



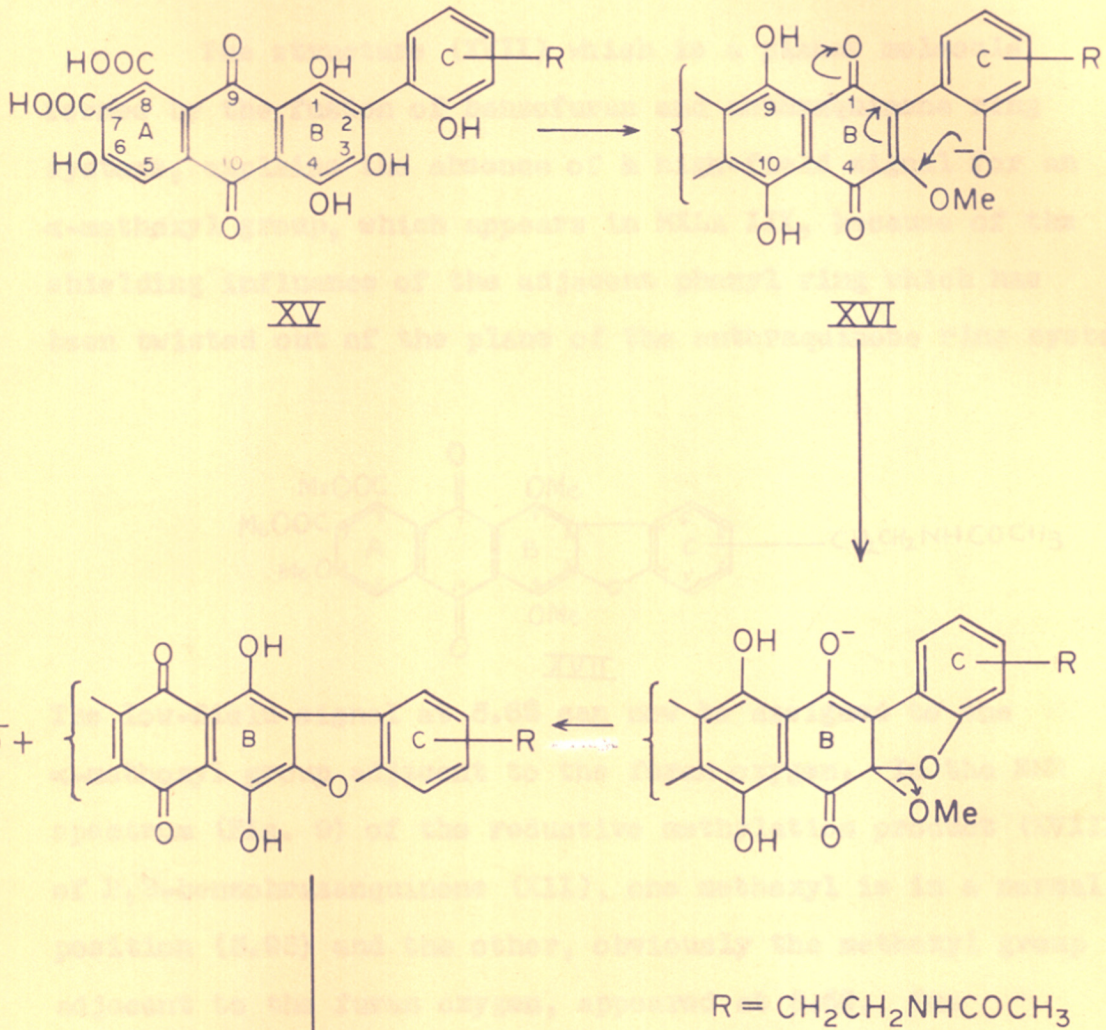
XI

XII

XIII

XIV

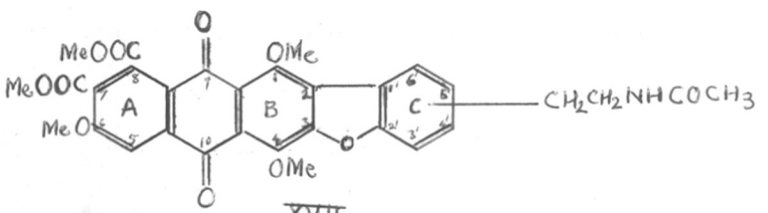
CHART-2



XV XVI

A partial structure (XVII) for MLA III can now be assigned.

The structure (XVII) which is a planar molecule formed by the fusion of benzofuran and anthraquinone ring systems, explains the absence of a high-field signal for an α-methoxyl group, which appears in MXLA III, because of the shielding influence of the adjacent phenyl ring which has been twisted out of the plane of the anthraquinone ring system.



XVII

The low-field signal at 5.68 can now be assigned to the α-methoxyl group adjacent to the furan oxygen. In the NMR spectrum (Fig. 9) of the reductive methylation product (XVIII) of 1,2-benzobrazanquinone (XII), one methoxyl is in a normal position (5.95) and the other, obviously the methoxyl group adjacent to the furan oxygen, appeared at 5.62. The reductive methylation product (XVIIIA) of 3-hydroxybrazanquinone shows in its NMR spectrum in $CDCl_3$, signals for two methoxyl groups at 5.89 and 6.11 and a signal at 5.67, which is due to a methoxyl group adjacent to furan oxygen.

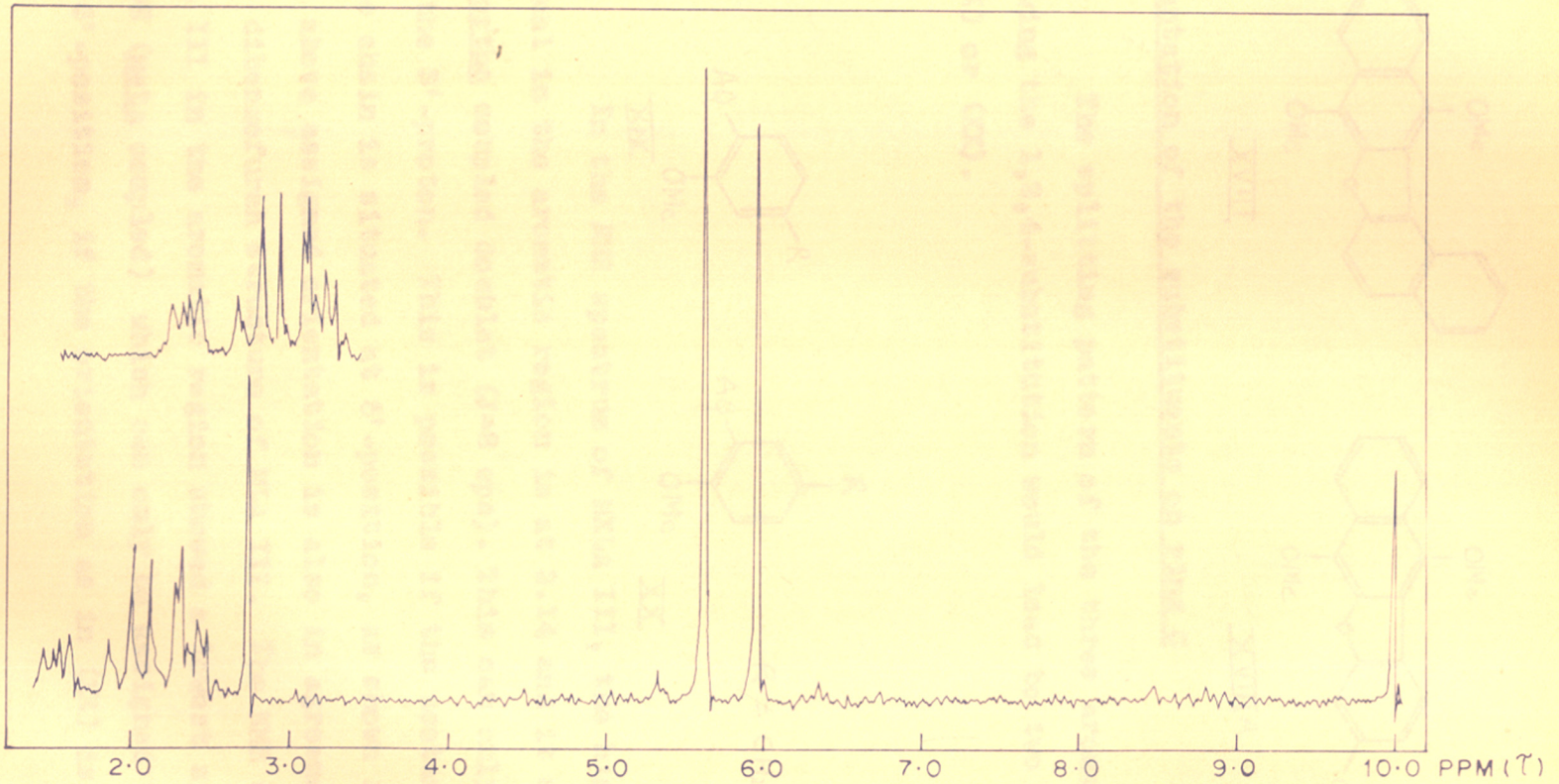
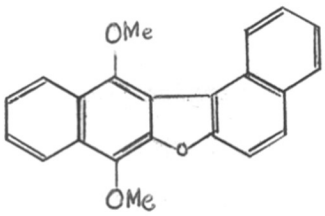
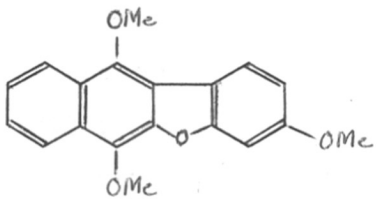


FIG. 9



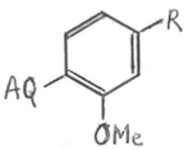
XVIII



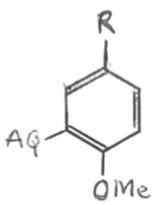
XVIIIA

Orientation of the substituents on ring C

The splitting pattern of the three aromatic protons showing the 1,2,4-substitution would lead to two possibilities (XIX) or (XX).



XIX

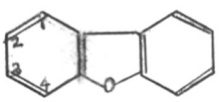


XX

$R = CH_2CH_2NHCOCH_3$

In the NMR spectrum of MXLA III, the highest signal in the aromatic region is at 3.14 and it appears as an ortho coupled doublet (J=8 cps). This can only be assigned to the 3'-proton. This is possible if the acetamidoethyl side chain is situated at 5'-position, as shown in (XX). The above assigned orientation is also in agreement with the dibenzofuran structure of MLA III. The NMR spectrum of MLA III in the aromatic region showed a lowest signal at 1.98 (meta coupled) which can only be assigned to a proton at 6'-position, if the orientation as in (XX) is present.

The above conclusion is based on the comparison with the NMR spectrum of dibenzofuran (XXI) in CCl_4, which shows a lowest signal at 2.55 for the proton at 1-position.[19]

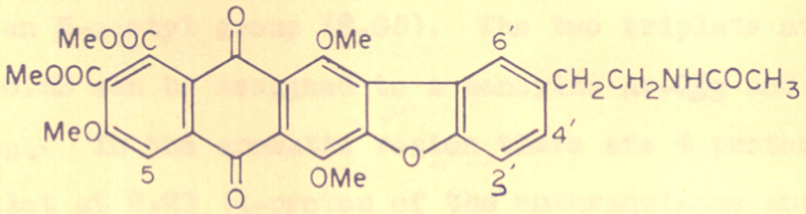


XXI

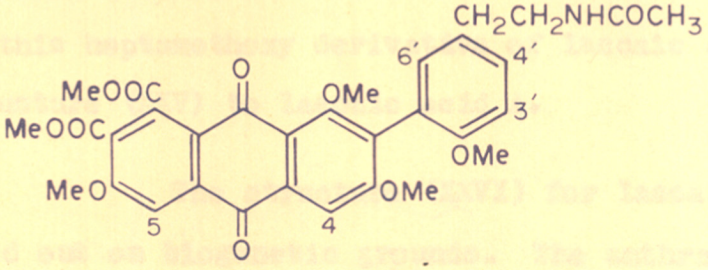
Based on the above evidence, the structures (XXII) and (XXIII) can be assigned to MLA III and MXLA III respectively.

Structure of laccaic acid A

The signals for three aromatic protons exhibiting ABC pattern in the NMR spectrum of laccaic acid A, have a close resemblance with those present in the NMR spectra of xantholaccaic acid A and MXLA III. This clearly indicates the absence of a furan structure and the presence of a free phenyl group at the 2-position of the anthraquinone unit in laccaic acid A. In order to ascertain whether MLA III was the sole product obtained by the methylation of laccaic acid A or the corresponding heptamethyl ether-ester was also formed as indicated by the above evidence, the crude product of methylation of laccaic acid was chromatographed carefully on silica gel plates using benzene-acetone mixture (8:2). After repeated developments, a yellow band with R_f value (0.3) separated from that of MLA III (R_f value 0.27). After working up, this band yielded a crystalline


MeOOC
MeOOC
MeO
OMe
OMe
O
O
O
5
6′
4′
3′
$CH_2CH_2NHCOCH_3$

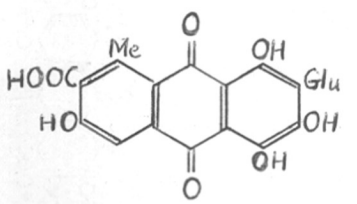
XXII


MeOOC
MeOOC
MeO
OMe
OMe
OMe
O
O
O
5
4
6′
4′
3′
$CH_2CH_2NHCOCH_3$

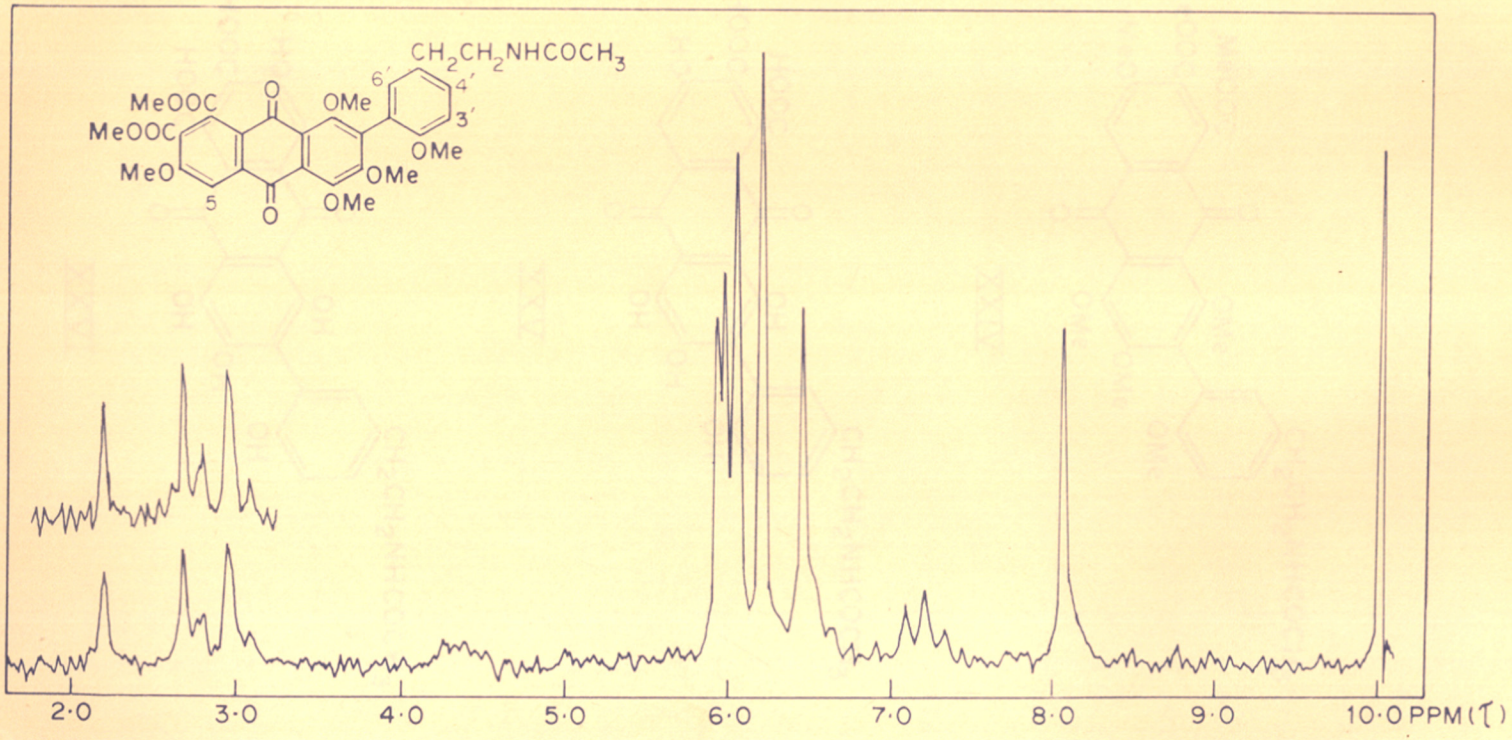
XXIII

compound melting at 115-116°. The NMR spectrum of this compound in $CDCl_3$ (Fig. 10) showed the presence of 7-methoxyl groups (5.95, 5.98, 6.05(2), 6.2(2) and 6.45) and an N-acetyl group (8.05). The two triplets at 7.22 and 6.53 can be assigned to a benzylic Ar-CH_2 and an N-CH_2 group. In the aromatic region there are 4 protons, a singlet at 2.22 (α-proton of the anthraquinone and three protons (from ring C) at 2.72 (q), 2.95 (d, J=2.5 cps) and 3.02 (d, one side of the signal overlapping with the doublet at 2.95). The broad signal at about 4.4 can be assigned to the NH proton. Based on the above evidence the structure (XXIV) is assigned to this heptamethoxy derivative of laccaic acid A, and the structure (XXV) to laccaic acid A.

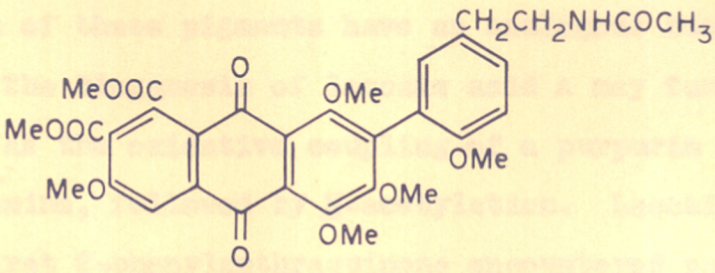
The structure (XXVI) for laccaic acid A is ruled out on biogenetic grounds. The anthraquinone residue of laccaic acid A, as shown in (XXV) has a strong resemblance



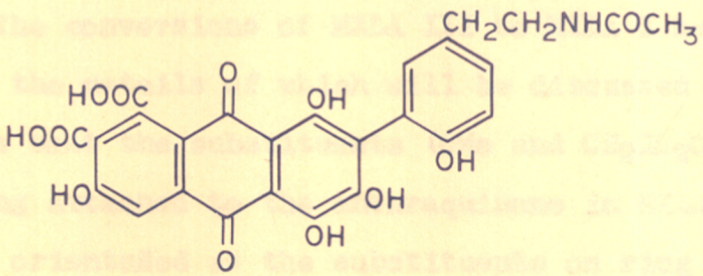
XXVII


CH2CH2NHCOCH3
6'
4'
3'
MeOOC
MeOOC
MeO
OMe
OMe
OMe
OMe
5
O
O
2·0
3·0
4·0
5·0
6·0
7·0
8·0
9·0
10·0 PPM(τ)

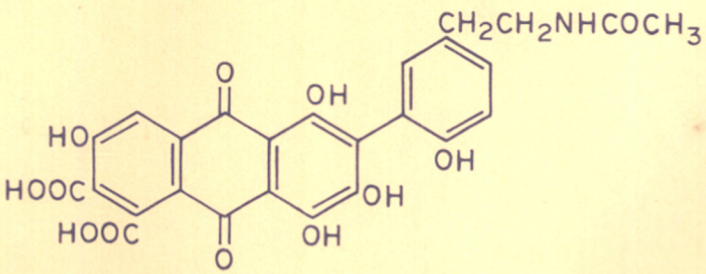
FIG·10



XXIV



XXV



XXVI

to that of carminic acid[19a] (XXVII) which is a pigment derived from the insect Coccus cacti (family Coccidae to which the lac insect belongs). It is therefore possible that both of these pigments have an analogous biogenetic origin. The biogenesis of laccaic acid A may further be pictured as the oxidative coupling of a purpurin derivative with tyramine, followed by N-acetylation. Laccaic acid A is the first 2-phenylanthraquinone encountered as a natural product except that of cassiamin,[19b] a 2,2'-bianthraquinonyl, may also be regarded as a derivative of 2-phenylanthraquinone.

The conversions of MXLA III to MXLA I has been effected; the details of which will be discussed elsewhere. This shows that the substituents (OMe and CH_2CH_2OMe) on the phenyl ring attached to the anthraquinone in MXLA I are similarly orientated as the substituents on ring C in laccaic acid A.

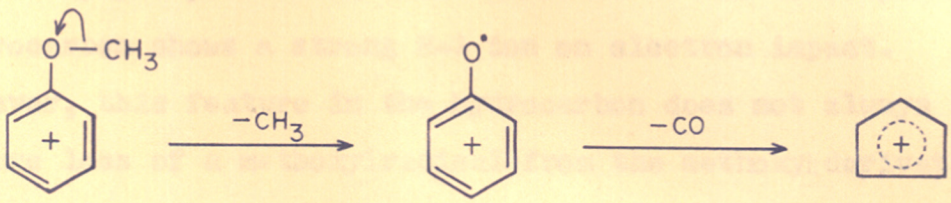
Mass spectra of MLA III and MXLA III

Before proceeding to a discussion of the mass spectra of the ether esters of laccaic acid A and xantholaccaic acid A, it is useful to review briefly the fragmentation under electron impact of aromatic ethers and esters, naphthaquinones, anthraquinones and benzofurans, since these are structural units present in the laccaic acid derivatives.

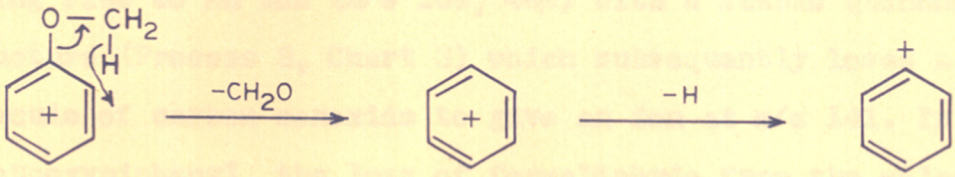
Mass spectra of simple aryl methyl ethers

A mass spectral study of substituted anisoles[20-24] has shown the presence of four major pathways. The first of these arises by the loss of a methyl radical from the molecular ion, which is followed by a loss of a carbon monoxide molecule to give a sequence M→M-15→M-43 (Process 1, Chart 3). The second pathway is due to aryl-oxygen fission with a migration of one hydrogen atom (loss of formaldehyde), which sometimes is followed by the expulsion of a hydrogen atom to give the even-electron M-31 ion (Process 2, Chart 3). Besides the above two processes, a direct loss of 43 mass units (CH_3CO group) has been observed in the mass spectrum of 9-methoxyphenanthrene.[24] A direct loss of a methoxyl radical has been suggested[24] in the fragmentation of some methyl anisoles. However, the presence of metastable ions indicative of such an aryl-oxygen fission, has been reported[24] in case of monomethoxystilbenes and 3,4-bis(4'-methoxyphenyl)

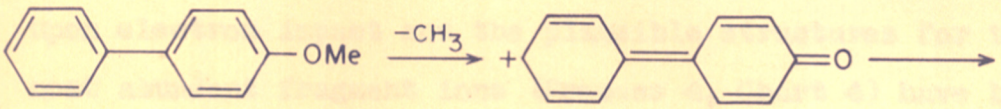
CHART-3



---(1)



---(2)



---(3)

m/e 184

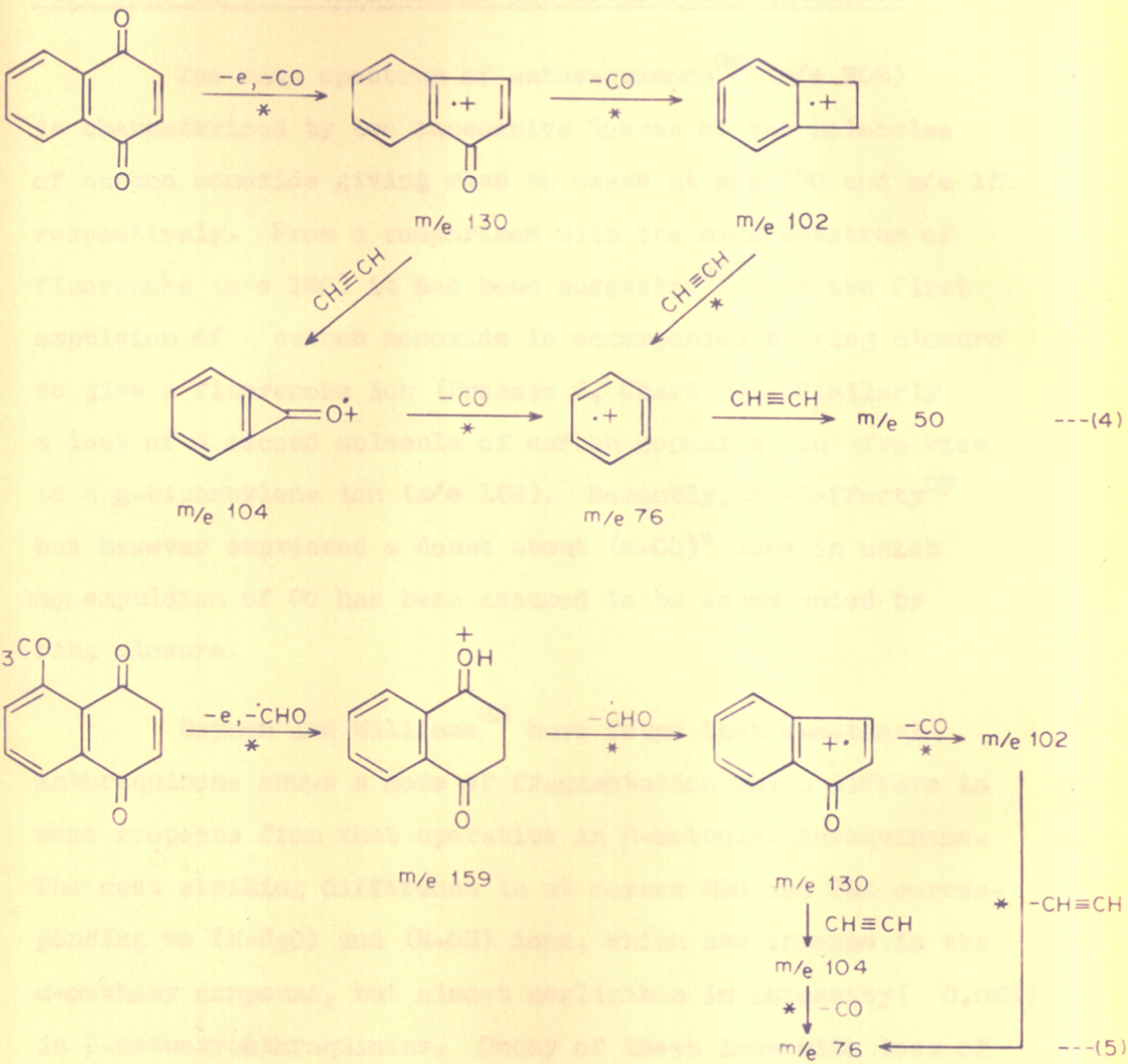
m/e 169

pyridine. These authors[24] believe that the direct loss of a methoxyl group occur in those cases where the corresponding hydrocarbon shows a strong M-1 ion on electron impact. However, this feature in the hydrocarbon does not always ensure loss of a methoxyl radical from the methoxyl derivative, because the possibility of forming a stable quinonoid ion may make loss of a methyl radical the preferred course. Thus 4-methoxybiphenyl (m/e 184), undergoes an alkyl-oxygen fission giving rise to an ion (m/e 169, 49%) with a stable quinonoid structure (Process 3, Chart 3) which subsequently loses a molecule of carbon monoxide to give an ion at m/e 141. In 3-methoxybiphenyl, the loss of formaldehyde from the molecular ion, was prominent.[24]

Mass spectra of naphthaquinones and methoxynaphthaquinones

Naphthaquinone breaks down in a well defined manner upon electron impact and the plausible structures for the most abundant fragment ions (Process 4, Chart 4) have been suggested by Bowie et al.[25] It was shown[25] that 5-methoxy-naphthaquinone on electron impact loses two formyl radicals successively according to the process 5 shown in chart 4. The isomeric 2-methoxynaphthaquinone behaves differently and shows intense $M-CH_3$ and $M-CH_2O$ ions.

CHART-4

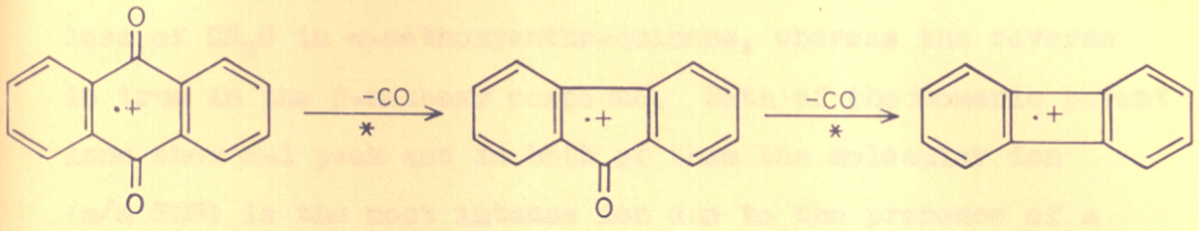

-e,-CO
*
m/e 130
-CO
*
m/e 102
CH≡CH
CH≡CH
*
-CO
*
CH≡CH
m/e 50
m/e 104
m/e 76
---(4)
H_3CO
-e,-·CHO
*
+
OH
m/e 159
-·CHO
*
-CO
*
m/e 102
m/e 130
CH≡CH
m/e 104
*
-CO
m/e 76
*
-CH≡CH
---(5)

Mass spectra of anthraquinone and methoxyanthraquinones

The mass spectrum of anthraquinone[26] (m/e 208) is characterized by two successive losses of the molecules of carbon monoxide giving rise to peaks at m/e 180 and m/e 152 respectively. From a comparison with the mass spectrum of fluorenone (m/e 180) it has been suggested[26] that the first expulsion of a carbon monoxide is accompanied by ring closure to give a fluorenone ion (Process 6, Chart 5). Similarly a loss of a second molecule of carbon monoxide can give rise to a ɑ-biphenylene ion (m/e 152). Recently, Mc-Lafferty[27] has however expressed a doubt about $(M-CO)^+$ ions in which the expulsion of CO has been assumed to be accompanied by ring closure.

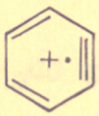
Beynon and Williams[26] have found that ∝-methoxyanthraquinone shows a mode of fragmentation which differs in some respects from that operative in β-methoxyanthraquinone. The most striking difference is at masses 220 and 221 corresponding to $(M-H_2O)$ and (M-OH) ions, which are intense in the ∝-methoxy compound, but almost negligible in intensity(0.05%) in β-methoxyanthraquinone. Decay of these ions with loss of 28 mass units to give ions at 192 and 193, and further loss of 28 to give ions at 164 and 165, find no parallel in the case of 2-methoxyanthraquinones. Both of the methoxyanthraquinones show peaks due to the loss of 31, 30 and 29 mass units due to the cleavage of $-OCH_3$, $-CH_2O$ and -CHO groups from the molecular

CHART-5



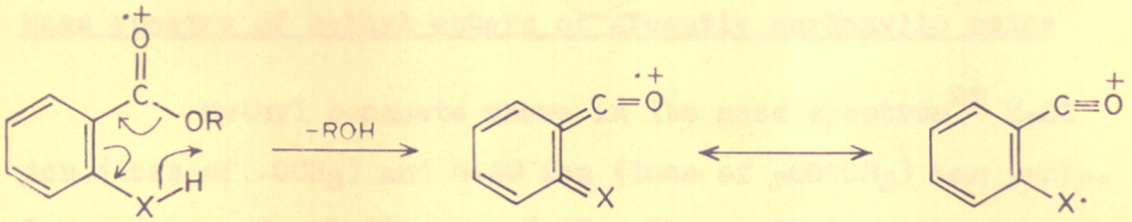
m/e 180

m/e 152



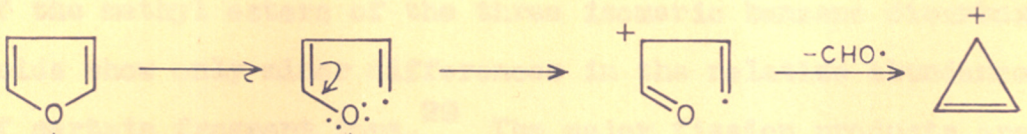
m/e 76

---(6)



(X≡CH_2, O, NH)

---(7)



m/e 68

a

b, m/e 39

---(8)

ion. A loss of CHO is more prominent than that due to the loss of CH_2O in α-methoxyanthraquinone, whereas the reverse is true in the β-methoxy compound. Both of the isomeric parent ions show M-1 peak and in both of them the molecular ion (m/e 238) is the most intense ion due to the presence of a quinonoid structure.

A presence of M-43 ion formed by the direct loss of CH_3CO group from the molecular ion has been reported[28] in the mass spectrum of 1,8-dihydroxy-6-methoxy-3-methyl-7-chloro-anthraquinone.

Mass spectra of methyl esters of aromatic carboxylic acids

Methyl benzoate shows in its mass spectrum[29] M-31 ion (loss of $-OCH_3$) and M-59 ion (loss of $-COOCH_3$) ion peaks. In presence of substituents (-OH, $-NH_2$ or CH_3), ortho to carbomethoxyl group, a fragmentation mode, as shown in process 7 (Chart 5) has been observed.[29-31] The mass spectra of the methyl esters of the three isomeric benzene dicarboxylic acids show only minor differences in the relative abundance of certain fragment ions.[29] The major fission products are due to the loss of the methoxyl (M-31), carbomethoxyl (M-59) and two carbomethoxyl (M-118) ions.

Mass spectra of furans, benzofurans and dibenzofurans

Because of the presence of six electrons furan has a stability greater than the corresponding noncyclic divinyl

ether and this is reflected by the fact that the molecular ion (m/e 68) in the spectrum of furan[31] accounts for 25% of the total ion current. The most favoured rupture on electron impact probably takes place as shown in process 8 (Chart 5). As we go from furan to benzofuran[33] and dibenzafuran,[34] the stability of the molecular ion increases more and more. Thus the mass spectrum of dibenzofuran[34] is largely dominated by the molecular ion, since the fragmentation modes leading to ions a and b from furan (Process 8), cannot result in the fragmentation in this case.

Mass spectrum of MLA III

The peaks in the mass spectrum of a complicated molecule like MLA III, could be assigned with certainty only after labelling of various functional groups. In the following discussion only the probabilities are mentioned. Some of the peaks are assigned by the comparison of the mass spectra of model compounds.

In the mass spectrum of MLA III the molecular ion is the most intense ion, the peaks ranging from m/e 589 to m/e 383, are more intense than those falling in the same range in the mass spectrum of MXLA III. This higher intensity of the peaks is probably due to the presence of a dibenzofuran system in MLA III. An intense peak (relative intensity 65%), at m/e 530 which is formed by the loss of 59 mass units from the

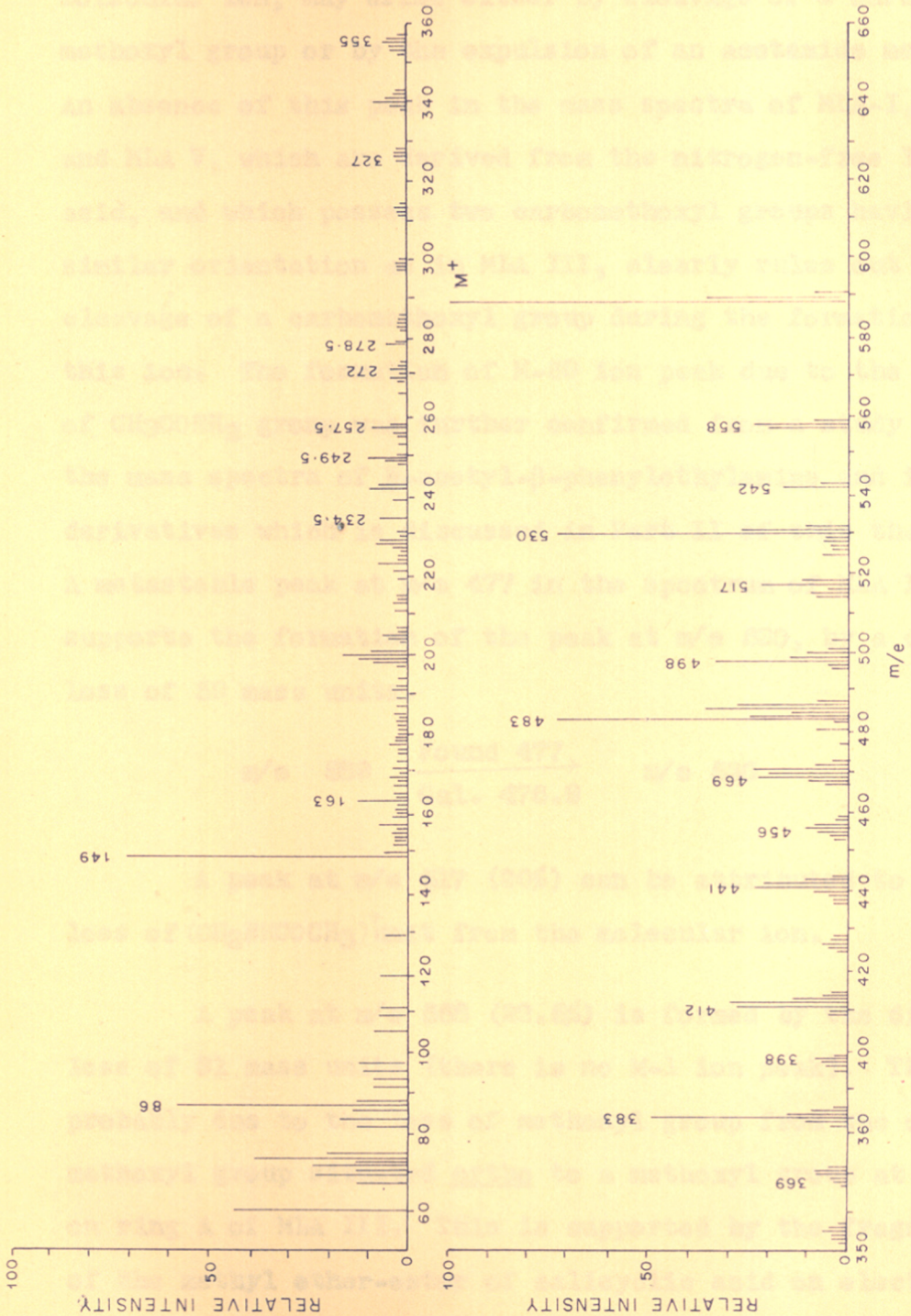

RELATIVE INTENSITY
100
50
0
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
86
149
163
234·5
249·5
257·5
272
278·5
327
355
M+
RELATIVE INTENSITY
100
50
0
350
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
660
369
383
398
412
441
456
469
483
498
517
530
542
558
m/e

FIG.11

molecular ion, may arise either by cleavage of a carbomethoxyl group or by the expulsion of an acetamide molecule. An absence of this peak in the mass spectra of MLA-I, MXLA-I and MLA V, which are derived from the nitrogen-free laccaic acid, and which possess two carbomethoxyl groups having a similar orientation as in MLA III, clearly rules out the cleavage of a carbomethoxyl group during the formation of this ion. The formation of M-59 ion peak due to the loss of CH_3CONH_2 group was further confirmed from a study of the mass spectra of N-acetyl-β-phenylethylamine and its derivatives which is discussed in Part II of this thesis. A metastable peak at m/e 477 in the spectrum of MLA III supports the formation of the peak at m/e 530, by a direct loss of 59 mass units.

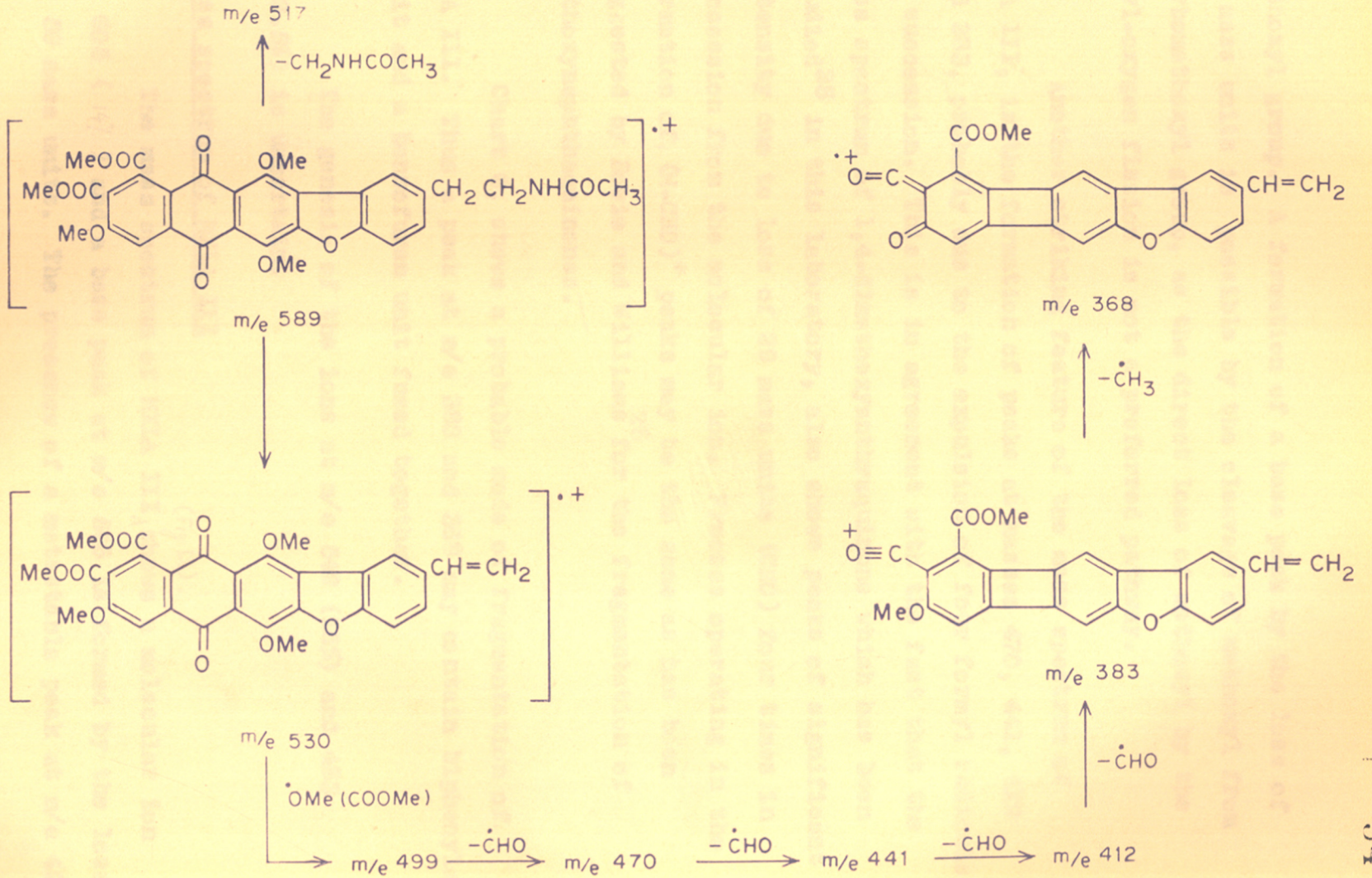
$$\text{m/e } 589 \xrightarrow[\text{Cal. } 476.9]{\text{Found } 477} \text{m/e } 530$$

A peak at m/e 517 (20%) can be attributed to the loss of $(CH_2NHCOCH_3)^+$ unit from the molecular ion.

A peak at m/e 558 (22.5%) is formed by the direct loss of 31 mass units (there is no M-1 ion peak). This is probably due to the loss of methoxyl group from the carbomethoxyl group situated ortho to a methoxyl group at position 6 on ring A of MLA III. This is supported by the fragmentation of the methyl ether-ester of salicyclic acid on electron impact, giving rise to a base peak due to the loss of a

CHART-6

MODE OF FRAGMENTATION OF MLA III



methoxyl group. A formation of a base peak by the loss of 31 mass units is possible by the cleavage of methoxyl from carbomethoxyl group, as the direct loss of methoxyl by the aryl-oxygen fission is not a preferred pathway.

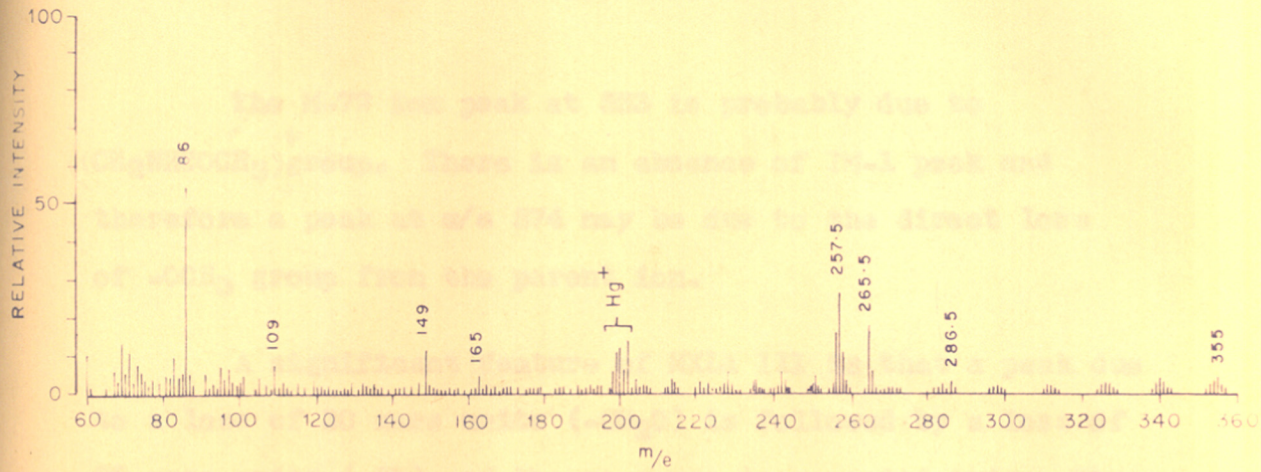
Another striking feature of the mass spectrum of MLA III, is the formation of peaks at masses 470, 441, 412 and 383, probably due to the expulsion of four formyl radicals in succession. This is in agreement with the fact that the mass spectrum of 1,4-dimethoxyanthraquinone which has been studied[35] in this laboratory, also shows peaks of significant intensity due to loss of 29 mass units (CHO) four times in succession from the molecular ion. Process operating in the formation of $(M-CHO)^+$ peaks may be the same as has been suggested by Bowie and Williams[25] for the fragmentation of methoxynaphthaquinones.

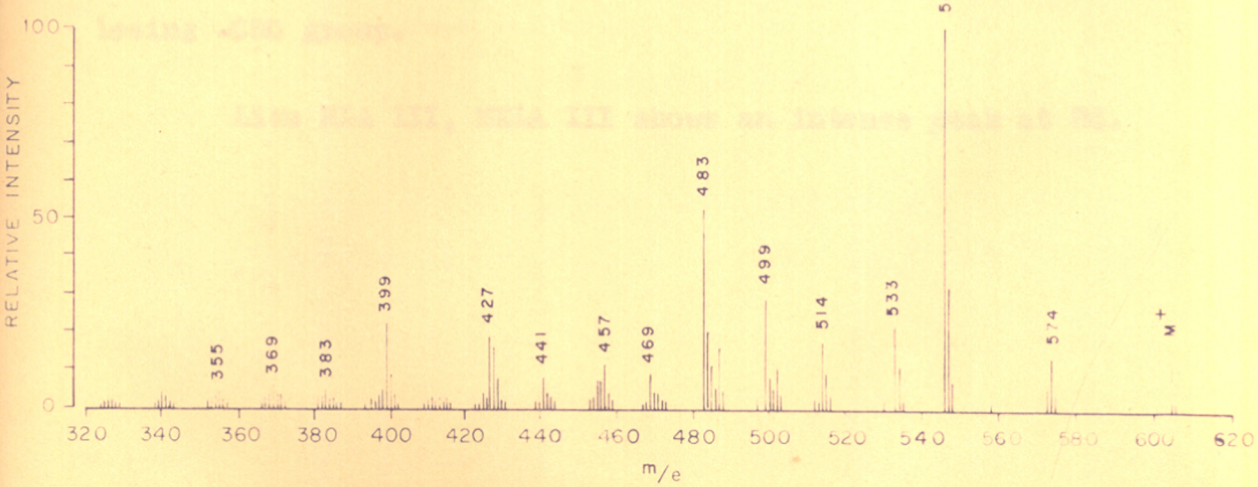
Chart 6, shows a probable mode of fragmentation of MLA III. Thus a peak at m/e 383 and 368 may contain biphenylene unit and a benzofuran unit fused together.

The genesis of the ions at m/e 542 (16%) and 483 (72.5%) is uncertain.

Mass spectrum of MXLA III

The mass spectrum of MXLA III (Fig 12), shows a molecular ion at 605 (14%) and a base peak at m/e 546 is formed by the loss of 59 mass units. The presence of a metastable peak at m/e 492


RELATIVE INTENSITY
100
50
0
86
109
149
165
Hg+
257.5
265.5
286.5
355
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
m/e


RELATIVE INTENSITY
100
50
0
355
369
383
399
427
441
457
469
483
499
514
533
546
574
M+
320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620
m/e

supports the formation of a peak at m/e 546 by the direct loss of 59 mass units from the parent ion.

$$\text{m/e 605} \xrightarrow[\text{Cal. 492.7}]{\text{Found 492}} \text{m/e 546}$$

The M-72 ion peak at 533 is probably due to $(CH_2NHCOCH_3)^+$ group. There is an absence of M-1 peak and therefore a peak at m/e 574 may be due to the direct loss of $-OCH_3$ group from the parent ion.

A significant feature of MXLA III is that a peak due to a loss of 30 mass units ($-CH_2O$) is followed by a loss of 28 mass units (-CO) and the sequence is repeated twice. Thus peaks at m/e 499, 469 and 441 show a loss of $-CH_2O$ followed by the loss of a molecule of carbon monoxide, the same sequence is shown by the peaks at m/e 457, 427 and 399. A peak at m/e 428 is probably formed from an ion at m/e 457 by losing -CHO group.

Like MLA III, MXLA III shows an intense peak at 86.

Work on the water-soluble lac pigments (laccaic acids) carried out in this laboratory was first described[12] in 1963. A new method of isolation of the mixed laccaic acids was developed and the laccaic acids were shown to be purpurin derivatives reducible to xantholaccaic acids with loss of an α-hydroxyl group in an anthraquinone nucleus. Attempts to prepare an analytically pure homogeneous laccaic acid were not successful at that time, but it was found that the ether-esters of the laccaic acids and xantholaccaic acids were separable into pure homogeneous constituents (MLA I to V, methylated laccaic acids; and MXLA I to III, methylated xantho-laccaic acids). Mainly on the basis of NMR and mass spectral data, structures were assigned to two of the ether-esters MXLA I and MLA V. They were identified as derivatives of 2-phenylanthraquinones, and the laccaic acids were therefore pigments of a type not encountered earlier among natural products. The correct molecular formulae of MLA III and MXLA III (derived from the nitrogen-containing laccaic acid A) and the presence of a C-methyl group in these compounds were reported.[36] In a paper[37] communicated in July 1966 the complete structure of laccaic acid A was demonstrated.

While our work on lac pigments was nearing completion, the first paper on the subject by Schofield *et al*[38] appeared in November 1965; in the separation of crude laccaic acid by

by cellulose powder chromatography into four constituents, laccaic acid A_1, A_2, B and C, was described.

Laccaic acid A appears to be identical with Schofield's laccaic acid A_1. We are greatly indebted to Professor Schofield for sending us a sample and for informing us that in a communication to Tetrahedron Letters he has reported evidence for formulating the nitrogen function of laccaic acid A_1 as $CH_2CH_2NHCOCH_3$. As stated by him in his letter dated June 14, 1966, "this specimen was very slightly contaminated with laccaic acid A_2." It was found that by TLC of a butanolic hydrochloric acid solution on polycaprolactam powder, or of a benzene-ethanol solution on silica gel impregnated with oxalic acid, Schofield's laccaic acid A_1 has the same R_f value as our laccaic acid A. In this laboratory records our nitrogen-containing laccaic acid was designated LA III because it corresponded to MLA III, but in order to avoid confusion we now propose to call it laccaic acid A. Schofield's laccaic acid A_2 is probably similar to our non-nitrogenous laccaic acid obtained as the second major fraction in the butanolic acid-polycaprolactam procedure. Methylation by the acetone-potassium carbonate method of the small sample of laccaic acid A_1 available to us gave an ether-ester which had the same Rf value as MLA III on silica gel and a benzene-acetone solvent system. Dr. A.P.B. Sinha

kindly examined the X-ray powder diffraction patterns (taken with Cu-K_α radiation) of our laccaic acid A and the sample obtained from Professor Schofield. Our product was more crystalline in character, giving prominent diffraction lines arising from crystalline material with a faint halo pattern of a minor amorphous constituent. Laccaic acid A_1 gave a broad, dark halo pattern, indicating amorphous material as a major portion, and a faint diffraction line pattern of a crystalline phase as a minor fraction. Unfortunately it was therefore not possible to prove the identity of laccaic acid A and A_1 by this method; but there is little doubt that they are indeed identical.

Schofield[38] suggested the molecular formula $C_{26}H_{21}NO_{12}$ for laccaic acid A_1 and concluded that laccaic acid A_1 "is a quinone-carboxylic acid containing four acetylable hydroxyl groups (three arranged as in purpurin), one C-Me group and a non-basic nitrogen atom which can be estimated by the van Slyke method. The chromophore of laccaic acid A1 is probably that of an anthraquinone modified by further conjugation."

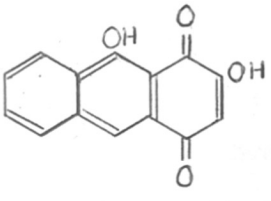
In the second paper by Schofield and co-workers,[39] the molecular formula for laccaic acid A_1 was modified to $C_{26}H_{17}NO_{11},H_2O$, and the evidence for the presence of the arrangement $ArCH_2CH_2NHCOCH_3$ was presented. Schofield[39] believes that the van Slyke value obtained for laccaic acid A_1

is due to the hydrolysis of the N-acetyl group under the conditions of van Slyke estimation. However, it has been shown in the present work that this value is anomalous and is obtained because of the polyphenolic nature of the compound.

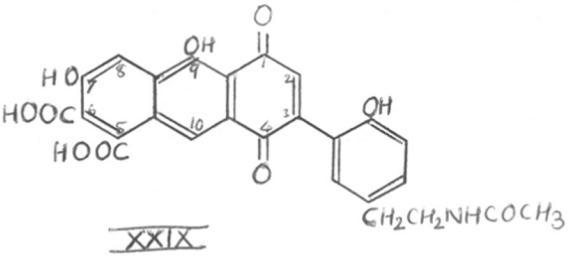
A complete structure for laccaic acid A was presented in the third paper (J.Chem.Soc. 842, 1967; date of receipt October 21, 1966) by Schofield et al.[40] The molecular formula of laccaic acid A_1 was again revised to $C_{26}H_{19}NO_{12}$ and the structure (XXV) was assigned, mainly based on the NMR spectra of the methylated derivatives. The orientation of the two carboxyl groups in ring A was decided by the comparison of pK values of laccaic acid A_1, anthraquinone 1,2-dicarboxylic acid and carminic acid. This is not very convincing, because the pK value of anthraquinone 1,4-dicarboxylic acid has to be considered to rule out structure (VIII).

The relative orientation of rings A and B in laccaic acid A and B was decided in the present work on biogenetic grounds. Schofield made a spectral study of the products obtained by hydrogenation of purpurin and laccaic acid A_1 in presence of Adam's catalyst in methanol, followed by air oxidation. From the ultraviolet spectra it was shown that both of the products are derivatives of 9- or 10-hydroxy-1,4-anthraquinone. The infrared spectra of both compounds showed

strong bands at 1667 cm^{-1} attributable to an unchelated carbonyl group indicating the presence of hydroxyl groups at 2- and 9-positions of 1,4-anthraquinone. Based on this evidence structures (XXVIII) and (XXIX) were formulated for the two products of purpurin and laccaic acid A_1 respectively.



XXVIII



XXIX

The alternative arrangement in which the carboxyl and hydroxyl groups of ring A are at 8, 7,6 positions respectively was ruled out as its NMR spectrum showed signals at 1.25 and 2.09 which can be assigned to protons at positions 10 and 8 respectively.

The assignments made by Schofield for some of the methoxyl groups in methylation products of laccaic acid A_1 and xantholaccaic acid A_1 have to be revised. The signal at 5.67 in laccaic acid A_1 methyl ether-ester (his LC and our MLA III) has been assigned to one of the carbomethoxyl groups, while it has been shown conclusively later in this part of the thesis that this signal has to be assigned to the methoxyl group

adjacent to the furan oxygen. Similarly in the case of xantholaccaic acid A_1 methyl ether-ester (X.L.C.), the signal at 6.51 which is assigned to a carbomethoxyl group should be assigned to the methoxyl group in the 1-position of anthraquinone. Further, the assignments of some of the aromatic protons also require revision; e.g. in the ether-ester LC (MLA III), desacetyl laccaic acid A_1 methyl ether-ester D(DID), and the 'methylated Hofmann product', the lowest signal (═) which was assigned to the α-proton of ring A of anthraquinone, should be assigned to the proton in the 6-position of the 2-phenyl group since this proton clearly shows meta-coupling, as discussed earlier in this part of the thesis.

No.	Compound	Solvent	value	Multiplicity	No. of protons	Assignment
1	Laccaic acid A	DMSO	2.22	s	1	5-H
			2.7 to 3.3	m	3	3',4',6'-H
		NaOD	2.42	s	1	5-H
			2.7 to 3.1	m	3	3',4',6'-H
			6.5	t	2	N-CH_2-
			7.2	t	2	Ar-CH_2
			8.0	s	3	-N-Ac
		Pyridine	6.34	t	2	N-CH_2-
			7.0	t	2	Ar-CH_2-
			7.91	s	3	N-Ac
2	Xantholaccaic acid A	DMSO	2.42	s	1	5-H
			2.67	s	1	4-H
			2.9 to 3.2	m	3	3',4',6'-H
		Pyridine	6.35	t	2	N-CH_2
			7.09	t	2	Ar-CH_2
			7.99	s	3	N-Ac
3	MLA-III	$CDCl_3$	2.22	s	1	5-H
			1.98	d,J=2.5 cps	1	6'-H
			2.43	d	1	3'-H
			2.64	q	1	4'-H
			4.2	t(broad)	1	-NH-
			5.91	s	3	-COOMe
			5.93	s	3	-COOMe
			6.07	s	3	6-OMe
			6.00	s	3	1-OMe
			5.68	s	3	4-OMe
			6.47	t	2	N-CH_2
			7.02	t	2	Ar-CH_2
			8.02	s	3	N-Ac

No.	Compound	Solvent	value	Multiplicity	No. of protons	Assignment
4	MLA III hydrolysis product	Pyridine	5.57	s	3	4-OMe
			5.67	s	3	1-OMe
			6.05	s	3	6-OMe
			6.22	t	2	N-CH_2
			6.83	t	2	Ar-CH_2
			7.89	s	3	N-Ac
5	HBr demethylated product of MLA III	Pyridine	5.84	s	3	-COOMe
			6.02	s	3	-COOMe
			6.05	s	3	6-OMe
			6.27	t	2	-NCH_2-
			6.93	t	2	Ar-CH_2
			7.91	s	3	N-Ac
6	MXLA III	$CDCl_3$	2.25	s	1	5-H
			2.38	s	1	4-H
			2.83	q	1	4'-H
			3.05	d (J=2.5 cps)	1	6'-H
			3.14	d	1	3'-H
			4.2	t (broad)	1	-NH-
			5.98	s	3	COOMe
			6.08	s	3	COOMe
			6.1	s	6	2',6-$(OMe)_2$
			6.25	s	3	3-OMe
			6.48	s	3	1-OMe
			6.57	t	2	NCH_2
			7.25	t	2	Ar-CH_2
			8.08	s	3	N-Ac
7	MXLA III hydrolysis product	Pyridine	6.05	s	3	6-OMe
			6.14	s	3	2'-OMe
			6.23	s	6	1,3-$(OMe)_2$
			6.3	-	2	-NCH_2
			7.03	t	2	Ar-CH_2
			7.93	s	3	N-Ac

No.	Compound	Solvent	value	Multiplicity	No. of protons	Assignment
8	Demethylation product of MXLA III (BF_3-etherate -Ac_2O reaction)	Pyridine	6.02	s	3	-COOMe
			6.08	s	3	-COOMe
			6.11	s	3	6-OMe
			6.26	s	3	2'-OMe
			6.3	s	3	3-OMe
			6.43	t	2	N-CH_2
			7.11	t	2	Ar-CH_2
			8.0	s	3	N-Ac
9	Laccaic acid A heptamethyl ether-ester	$CDCl_3$	2.22	s	1	5-H
			2.72	q	1	4'-H
			2.95	d (J=2.5 cps)	1	6'-H
			3.02	d	1	3'-H
			4.4	t (broad)	1	-NH-
			6.53	t	2	N-CH_2
			7.22	t	2	Ar-CH_2
			5.95	s	3	-COOMe
			5.98	s	3	-COOMe
			6.05	s	6	4,6-$(OMe)_2$
			6.20	s	6	3,2'-$(OMe)_2$
			6.45	s	3	1-OMe
			8.05	s	3	N-Ac

EXPERIMENTAL

Laccaic acid A

It was isolated from the crude laccaic acid by the method,[14] described in the introduction, and was crystallized from methanol, to yield bright red needles. It charred at 230° and charred as the temperature was increased. Found: C, 57.9; H, 4.0; N, 2.5. $C_{26}H_{19}NO_{12}$ requires: C, 58.1; H, 3.5; N, 2.6%.

Xantholaccaic acid A

To a solution of laccaic acid A (1.0 g) in 2% aqueous sodium hydroxide (100 ml) was added sodium hydrosulphite (0.5 g) at room temperature. The purple solution turned yellowish red. After shaking for 2 hours, the reaction mixture was air oxidized and then acidified (Congo Red) with conc. hydrochloric acid. After scratching the solution with glass rod, a reddish orange product separated. It was filtered, washed with little ice-cold water and air dried (0.8 g). On crystallization from water, orange microscopic crystals (0.7 g) were obtained, m.p. 209-210° (dec.). Found: C, 57.5; H, 3.3; N, 2.1. $C_{26}H_{19}NO_{11}, H_2O$ req. C, 57.9; H, 3.8; N, 2.5%.

Hydrogenation of purpurin

To a solution of purpurin (1.0 g), in 2% sodium hydroxide (100 ml) was added palladium charcoal (0.1 g; 10%) and hydrogenated at atmospheric pressure. The hydrogen uptake

(2 moles) was complete in 8 hours. The colour of the reaction mixture changed from purple to yellowish red at the end of this period. It was filtered and the solution air-oxidized. On acidification with conc. hydrochloric acid (Congo Red), a brownish orange product separated. It was filtered, washed with water and air-dried. On crystallization from glacial acetic acid, yellow crystals were obtained (0.75 g), m.p. 264° The mixed m.p. with an authentic sample of xanthopurpurin was undepressed.

Hydrogenation of laccaic acid A

Laccaic acid A (1.0 g) was dissolved in 2% aqueous sodium hydroxide, palladium charcoal (0.1 g, 10%), was added and the mixture was hydrogenated at atmospheric pressure. Hydrogen uptake became very slow after 8 hours, and the colour of the reaction mixture changed from violet to yellowish red. The solution was filtered and air-oxidized. On acidification (Congo Red) with conc. hydrochloric acid a yellowish orange product separated. It was filtered, washed with little cold water and dried (0.8 g). On crystallization from water, orange crystals were obtained (0.7 g), m.p. 209-210° (dec). On methylation, it gave the same methylated derivative (MXLA III) as was obtained by the methylation of xantholaccaic acid A, prepared by the action of alkaline dithionite on laccaic acid A.

Methylation of laccaic acid A

Laccaic acid A (1.0 g) and anhydrous potassium carbonate (5.0 g) was taken in dry acetone (500 ml), and

dimethyl sulphate (2 ml) was added to it. The reaction mixture was refluxed for 15 hours. Acetone was removed and the residue was treated with cold water. After 6 hours, a brownish yellow solid separated. It was filtered, washed with water and air-dried (0.9 g). The product was dissolved in methanol, filtered and concentrated, when a yellow solid separated. It was filtered (0.35 g). The yellow solid was chromatographed on a silica gel column (10 x 2 cm) using benzene-acetone (8:2) mixture as an eluant. The eluate, on concentration gave a residue, which after crystallisation from methanol, gave yellow microscopic needles (0.7 g), m.p. 242-243°, mixed m.p. with the previously obtained[5] sample was undepressed. Found: C, 63.2; H, 4.9; N, 2.6. $C_{31}H_{27}NO_{11}$ requires: C, 63.1; H, 4.6; N, 2.4%.

The mother liquor, which remained behind after removal of the yellow solid (0.35 g) was concentrated to obtain a brown sticky residue. It was dissolved in benzene and chromatographed on ten silica gel plates (20x20x1 cm^3), using benzene-acetone (8:2), a mixture as a solvent system. After three developments, a yellow band (R_f value 0.3) overlapping the band of MLA III (R_f value 0.27) separated. It was scraped, extracted, and the residue obtained after removal of the solvent from the extract, was crystallised from methanol to yield yellow microscopic needles (0.050 g), m.p. 115-116°. Found: C, 61.8; H, 4.6; N, 2.0. $C_{33}H_{33}NO_{12}$ requires: C, 62.2; H, 5.1; N, 2.2%.

Hydrolysis of methylated laccaic acid A (MLA III)

MLA III (0.15 g) was refluxed with methanolic potassium hydroxide (5%; 15 ml) for 3 hours. The solution was concentrated to half and diluted with water (20 ml) and acidified (Congo Red) with concentrated hydrochloric acid. The reddish precipitate, thus obtained, was filtered, washed with cold water and dried (0.13 g). One crystallization from methanol, orange crystals (0.1 g) were obtained, m.p. above 300°. It gave no colouration with ethanolic ferric chloride. Found: C, 61.2; H, 4.3. $C_{29}H_{23}NO_{11}$ requires: C, 62.0; H, 4.3%.

Action of hydrogen bromide on MLA III

MLA III (0.075 g), was taken in dry methylene chloride (4 ml). To the clear yellow solution was added a saturated solution of hydrogen bromide in glacial acetic acid (1 ml). Within 10 minutes, a crystalline product separated. After keeping at room temperature, for about 24 hours, the reaction mixture was poured on water (20 ml) and warmed on water bath to remove methylene chloride. Excess HBr was destroyed by adding a small amount of sodium bisulphite to it. The reddish brown precipitate was filtered, washed with water and air dried (0.070 g). It gave dark brown colouration with ethanolic ferric chloride.

On crystallization from acetic acid, orange needles were obtained (0.06 g). It does not melt up to 300°. Found: C, 61.6; H, 4.4; N, 2.5. $C_{29}H_{23}NO_{11}$ requires: C, 62.0; H, 4.3; N, 2.5%.

Methylation of xantholaccaic acid A

Xantholaccaic acid A (0.5 g) was dissolved in dimethylformamide (100 ml). Silver oxide (3.5 g) and methyl iodide (3 ml) were added and the reaction mixture was kept shaking at room temperature for 24 hours. It was then filtered, and the solution was concentrated under reduced pressure (water pump). A brown semi-solid was obtained, which was extracted with chloroform. Concentration of chloroform extract, gave brownish yellow semi-solid, which on crystallisation from methanol yielded yellow plates (0.4 g), m.p. 168-169°, mixed m.p. with the previously obtained[5] sample of MXLA III was undepressed.

Isolation of methylated xantholaccaic acid A (MXLA III) from a mixture of methylated xantholaccaic acids

Xantholaccaic acid (1.0 g) was methylated with methyl iodide (6 ml), and silver oxide (1.0 g) in dimethylformamide (200 ml) at room temperature, for 24 hours. The reaction mixture was filtered and dimethyl formamide was removed on water bath under reduced pressure. The residue was extracted with benzene and the benzene extract was

chromatographed on neutral alumina (Brockmann grade I). The column was first eluted with benzene when MXLA I and MXLA II were obtained. The column was then eluted with chloroform, yellow solution was obtained which on removal of the solvent gave a residue (0.3 g). On crystallization from methanol, yellow plates (0.28 g) were obtained, m.p. 168-169°, mixed m.p. with the previously obtained[6] sample of MXLA III was undepressed.

Demethylation of methylated xantholaccaic acid A

To a clear melt obtained by heating a mixture of anhydrous aluminium chloride (8.0 g) and sodium chloride (2.0 g), methylated xantholaccaic acid A (0.5 g) was added with stirring at 140°. The reaction mixture became deep violet in colour. After heating for 10 minutes at 140-150°, the reaction mixture was poured hot into a mixture of ice (15.0 g) and conc. hydrochloric acid (15 ml). After 3 hours, an orange solid separated. It was filtered, washed with little cold water, and air dried (0.35 g). Crystallization from water gave orange prisms (0.3 g), m.p. 210° (dec.). Remethylation of this compound with methyl iodide and silver oxide in dimethyl formamide, followed by crystallization from methano, yielded a compound, m.p. 168-169°, mixed m.p. with MXLA III, undepressed.

Hydrolysis of methylated xantholaccaic acid A

Methylated xantholaccaic acid A (0.5 g) was taken in 5% methanolic potassium hydroxide (50 ml) and refluxed for 3 hours. Methanol was removed, the residue was cooled and diluted with water (10 ml) to form a red solution. On acidification (Congo Red) with conc. hydrochloric acid, a yellow solid separated. It was filtered, washed with cold water, and dried under suction (0.4 g). The product crystallised from dilute acetic acid in yellow prisms (0.35 g), m.p. 202-203°. Found: C, 61.8; H, 5.2; N, 2.0. $C_{30}H_{27}NO_{11}$ requires: C, 62.3; H, 4.7; N, 2.4%.

Action of diazomethane on the product from the hydrolysis of methylated xantholaccaic acid A

The product (0.2 g) obtained in the above experiment was taken in a 250 ml conical flask and an ethereal solution of diazomethane (2.5 g, 100 ml) was added to it at room temperature. After keeping overnight in refrigerator, a drop of glacial acetic acid was added and the ether was removed from the reaction mixture. The greenish yellow residue was washed with hexane and then crystallised from methanol to yield yellow plates (0.18 g), m.p. 168-169°, mixed m.p. with methylated xantholaccaic acid was undepressed.

Action of boron trifluoride etherate and acetic anhydride on methylated xantholaccaic acid

Methylated xantholaccaic acid (0.25 g) was taken in acetic anhydride (5 ml) and boron trifluoride etherate(3 ml) was added to it. The red solution, was kept at room temperature, for 18 hours and then poured on ice. After 3 hours, an orange solid separated, it was filtered, washed with cold water, and air dried. On crystallization from methanol, orange needles were obtained (0.2 g), m.p. 185-186°. Found: C, 61.3; H, 4.4; N, 2.1. $C_{31}H_{29}NO_{11}$ requires: C, 62.8; H, 4.9; N, 2.5%.

Attempted condensation of 2,4-dinitrochlorobenzene with methylated xantholaccaic acid A.

(a) MXLA III (0.025 g) and 2,4-dinitrochlorobenzene (0.028 g) were taken in dimethylformamide (2.5 ml). Triethylamine (0.025 g) was added and the reaction mixture was kept shaking at room temperature for 17 hours. It was poured on ice cold water (5 ml). The precipitated yellow solid was filtered, washed with cold water and air-dried. The crude product (0.020 g) after crystallizing twice from methanol yielded yellow plates (0.019 g), m.p. 168-169°, mixed m.p. with MXLA III was undepressed.

(b) MXLA III (0.025 g), 2,4-dinitrochlorobenzene (0.025 g) and anhydrous potassium carbonate (0.1 g) were taken in dry acetone (5 ml) and kept shaking at room temperature for 24 hr.,

and then refluxed for 1/2 hr. The solvent was removed and water was added to the reaction mixture. The precipitated yellow solid was filtered, washed with water, dried and crystallized from methanol, when starting material (0.020 g) was recovered, m.p. 168-169°, mixed m.p. with MXLA III undepressed.

Oxidation of methylated laccaic acid A (MLA III)

MLA III (0.8 g) was taken in nitric acid (30 ml;35%) and refluxed for 1 hour. Nitric acid was then removed under reduced pressure (water pump) on water bath. Water (10 ml) was added when a yellow sticky solid was obtained. It was collected by decanting the excess water, and dried under vacuum. It was taken up in dry acetone (50 ml), and methylated with dimethyl sulphate (3 ml) in presence of dry potassium carbonate (10.0 g), by refluxing for 12 hours. Acetone was removed, the residue was treated with water and then extracted with ethylacetate. The ethylacetate extract was washed with water and dried over anhydrous sodium sulphate. The product obtained after removal of ethylacetate, showed on TLC (silica gel, 9.5:0.5) benzene-acetone system), two major spots, one of which was corresponding with that of tetramethyl anisole-2,3,4,5-tetracarboxylate (an authentic sample). After PLC of the product on five plates (20x20x1 cm^{-2}) using the above solvent system, a band corresponding to the

above spot was collected and extracted with acetone. The residue (0.3 g) obtained after removal of acetone was crystallised from methanol, to yield white crystalline compound (0.090 g), m.p. 124-126°. It was recrystallised from benzene (0.035 g), m.p. 128-129°. Mixed m.p. with an authentic sample of tetramethyl anisole-2,3,4,5-tetracarboxylate was undepressed.

1,2-Benzobrazanquinone

2,3-Dichloro-1,4-naphthaquinone (4.54 g., 0.02 mole) and β-naphthol (2.88 g., 0.02 mole) were taken in dry pyridine (50 ml) and refluxed for 2 hours. After cooling overnight, the product separated from the reaction mixture. It was filtered, washed with boiling water till the filtrate was colourless. The residue was air dried and crystallised from pyridine to yield glistaning brown crystals (0.65 g), m.p. 270-271°, lit. m.p. for 1,2-benzobrazanquinone is 270-271°.

Reductive methylation of 1,2-benzobrazanquinone

1,2-Benzobrazanquinone (0.4 g) was dissolved in 1% aqueous sodium hydroxide (100 ml), to form a blue solution. Sodium hydrosulphite (0.1 g), was added when an orange vat was obtained. Dimethyl sulphate (10 ml) was added in portions of 0.5 ml, at an interval of 5 minutes. A presence of excess alkali and hydrosulphite was checked after every addition of dimethyl sulphate. After addition of dimethyl sulphate was

over, the reaction mixture was shaken at room temperature for 1 hour and then filtered. A residue on the filter paper was washed with water and dried. The crude product (0.14 g) was crystallised from ethanol to yield pale yellow needles (0.12 g), m.p. 141-142°. Found: C, 79.9; H, 4.9. $C_{22}H_{16}O_3$ requires: C, 80.3; H, 4.9%.

Hydrolysis of 1,2-benzobrazanquinone

1,2-Benzobrazanquinone (0.4 g) was refluxed with 0.5% ethanolic sodium hydroxide (100 ml) for 15 hours. The solution was concentrated under vacuum, diluted with water and acidified (Congo Red) with conc. hydrochloric acid, when a pink precipitate was obtained. It was filtered, washed with water, dried and crystallized from chloroform to yield pink needles (0.3 g), m.p. 154°. After heating on water bath for 2 hours, under vacuum, the product became orange brown, m.p. lit.* m.p. for 2-hydroxy-3 (2'-hydroxynaphthyl)-1,4-naphthaquinone 222°.

Methylation of 2-hydroxy-3(2'-hydroxynaphthyl)-1,4-naphthaquinone

A compound (0.2 g), dimethyl sulphate (1 ml, 6 moles), and anhydrous potassium carbonate were taken in dry acetone (20 ml) and refluxed for 6 hours. Acetone was removed, and cold was added to the reaction mixture. Water insoluble solid was filtered, washed with water, and air dried. Crude product (0.15 g) was crystallised from pyridine to yield brown needles

* Glemo G. R. & Spence R., J. Chem. Soc. 2811-9 (1928)

(0.12 g), m.p. 269-270°. Mixed m.p. with 1,2-benzobrazanquinone was undepressed.

Acetylation of 2-hydroxy-3-(2'-hydroxynaphthyl)-1,4-naphthaquinone

Compound (0.1 g) was taken in acetic anhydride (5 ml) and perchloric acid (1 drop) was added. A clear solution was obtained. After keeping for 3 hours, it was poured on ice. Yellow solid separated after 1 hour. It was filtered, washed with cold water and dried (0.09 g). On crystallization from ethanol, yellow needles were obtained (0.07 g), m.p. 166-167°. Lit.* m.p. 166-167°.

Reductive methylation of 3-methoxybenzobrazanquinone

3-Methoxybenzobrazanquinone (0.5 g) was dissolved in aqueous sodium hydroxide (100 ml; 1%). To the intense blue solution, so formed, was added sodium hydrosulphite (10 g., portionwise), when the colour of the reaction mixture became orange. Dimethyl sulphate (10 ml) was added in the portions of 1/2 ml, at the interval of 5 minutes. Excess of alkali and hydrosulphite was checked from time to time. After one hour, the solution was warmed and filtered. The precipitate was washed with water, dried and crystallized twice from alcohol, m.p. 162-163°. Found: C, 74.2; H, 5.4. $C_{19}H_{16}O_3$ requires C, 74.0; H, 5.2%.

* See p. 77.

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PART II

A STUDY OF MASS SPECTRA OF N-ACETYL-β-PHENYLETHYLAMINE AND ITS DERIVATIVES.

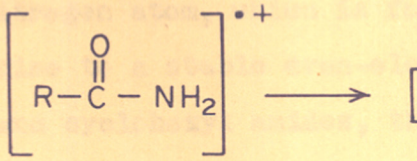
***

INTRODUCTION

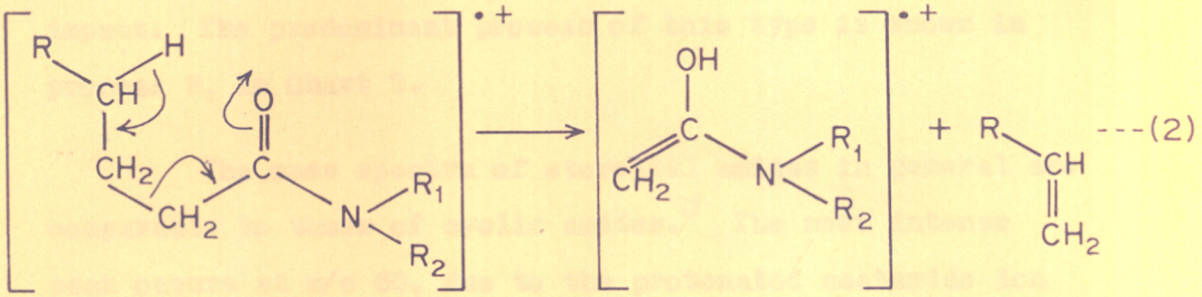
An exhaustive study of the mass spectra of a wide variety of aliphatic amides was first carried out by Gilpin.[1] He found that on ~~xx~~ electron impact, formamide, acetamide, propionamide and isobutyramide undergo an α-cleavage to form a m/e 44 ion peak (Process (1), Chart 1). The amides (primary, secondary and tertiary), which possess an alkyl side chain having at least three carbon atoms attached to an amide carbonyl group, undergo a McLafferty rearrangement[2] resulting in a β-cleavage with the transfer of a γ-hydrogen atom as shown in process (2)[1] in Chart 1. In the absence of such a three-carbon chain, secondary amides give rise to $(H_2\overset{+}{N}{=}CH_2)$ ion at m/e 30. From the comparative study of n-butylacetamide and its deuterated analogues, Djerassi et al[3], have shown that the formation of m/e 30 ion, involves a fission of a carbon-carbon bond next to nitrogen and a transfer of a hydrogen atom from the acyl group to the nitrogen atom resulting in a loss of ketene (process 3, Chart 1). Besides m/e 30 ion peak, peaks due to $(CH_3C{=}\overset{+}{O})$, $(\overset{+}{N}H_2CH_2CH_2)$, $(CH_3CONH_3)^+$ and $(CH_3CONHCH_2)^{++}$ at m/e 43, 44, 60 and 72 respectively, were also observed in the spectra of aliphatic secondary amides.[3]

In the spectra of aliphatic tertiary amides the predominant processes are the same as those operative in secondary amides.[3]

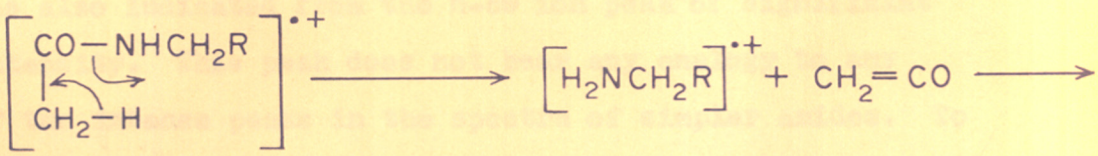
CHART-1



--- (1)



---(2)



---(3)

m/e 30

In case of cycloalkyl amines and their amides, an original step in the fragmentation, is a single cleavage β- to nitrogen atom, which is followed by a rearrangement giving rise to a stable even-electron ion. In both cyclopentyl and cyclohexyl amides, this is further followed by a loss of ketene, to give a more stable amine-type ion,[3] (process (4), Chart 2). Moreover, a tendency to lose the side chain is also shown by these amides on electron impact. The predominant process of this type is shown in process 5, in Chart 2.

The mass spectra of steroidal amides in general are comparable to those of cyclic amides.[3] The most intense peak occurs at m/e 60, due to the protonated acetamide ion ($CH_3C\overset{+}{O}NH_3$). The formation of this ion involves a double transfer from positions 1,2,3,4,5 and 19. A fragmentation which involves a transfer of a single hydrogen atom, mainly from C-2, C-4 and C-5 and to a small extent from C_1 and C_3, was also indicated from the M-59 ion peak of significant intensity. This peak does not bear any analogy to any of the intense peaks in the spectra of simpler amides. To explain the single proton transfer from C_2 and C_4 atoms, the mechanism as shown in the Chart 3 (process 6) was proposed.[3]

A study of the mass spectra of N,N-diphenylphenylacetamide and its analogues has been reported in which an

CHART-2

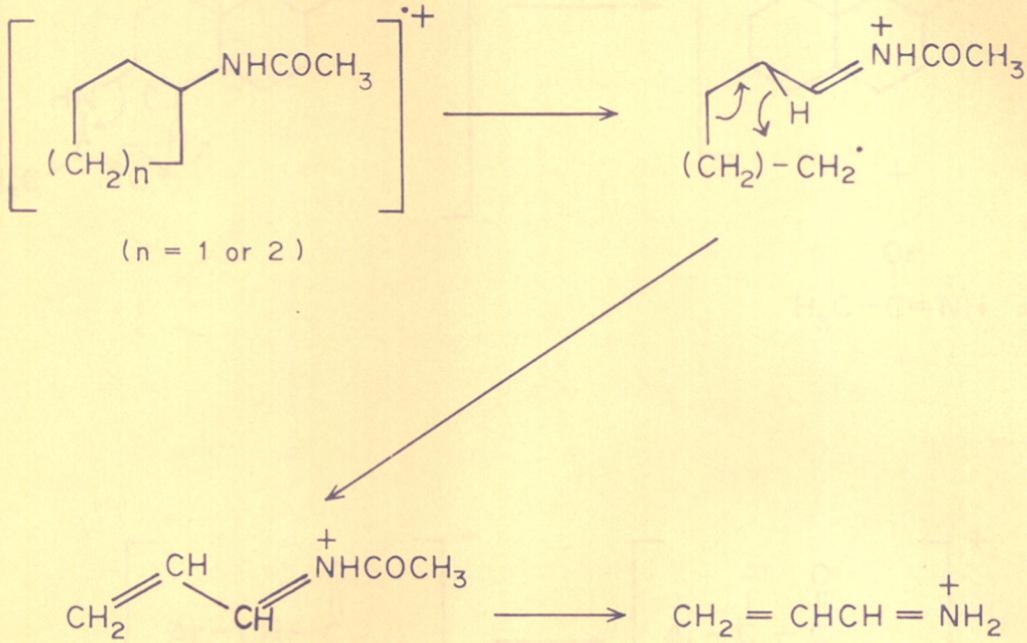
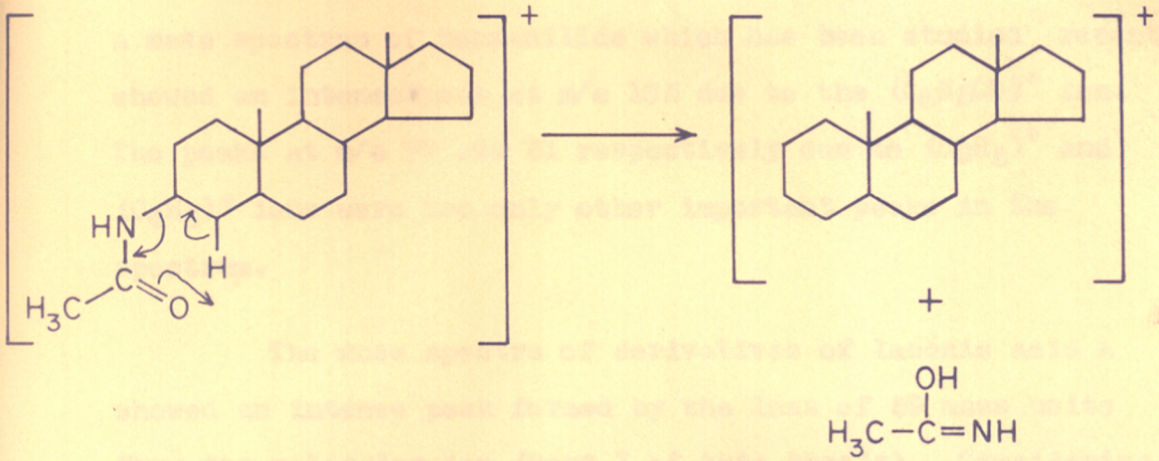
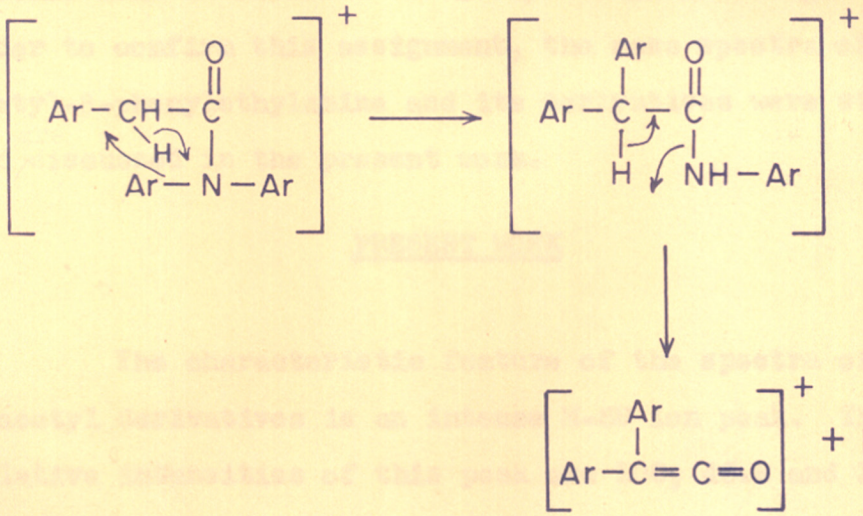

NHCOCH3
(CH2)n
(n = 1 or 2)
+NHCOCH3
H
(CH2)-CH2
CH
CH2
CH
+NHCOCH3
CH2 = CHCH = +NH2
---(4)
H
H
N+
CH3
C
O
H
+
H2N+
CH3
C
OH
H2N
CH3
C
OH
+
---(5)
m/e 60

CHART-3


HN
H
C
H3C
O
+
OH
H3C–C=NH

---(6)


O
Ar–CH–C
H
Ar–N–Ar
+
Ar
O
Ar–C–C
H
NH–Ar
Ar
Ar–C=C=O
+ H2N–Ar

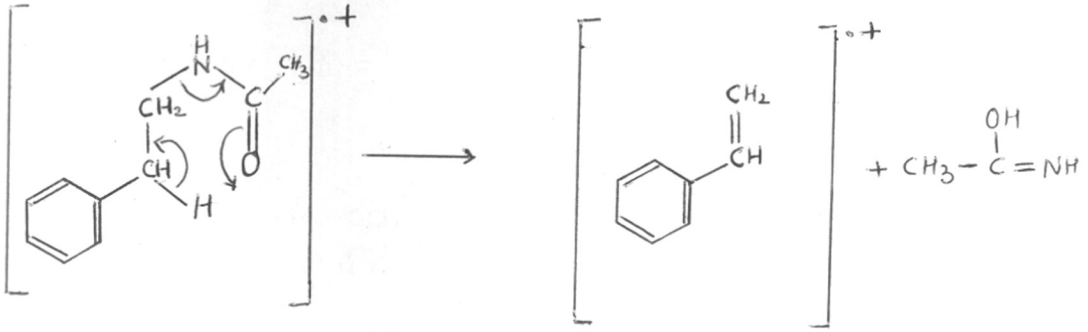
---(7)

aryl migration as shown in process 7 (Chart 3) was observed.[4] A mass spectrum of benzanilide which has been studied[5] recently showed an intense peak at m/e 105 due to the $(C_6H_5CO)^+$ ion. The peaks at m/e 77 and 51 respectively due to $(C_6H_5)^+$ and $(C_4H_3)^+$ ions were the only other important peaks in the spectrum.

The mass spectra of derivatives of laccaic acid A showed an intense peak formed by the loss of 59 mass units from the molecular ion (Part I of this thesis). Considering all the possibilities for the origin of this peak, it was concluded that the fragment lost was CH_3CONH_2, and that laccaic acid A contained the group $ArCH_2CH_2NHCOCH_3$. In order to confirm this assignment, the mass spectra of N-acetyl-β-phenylethylamine and its derivatives were studied and are discussed in the present work.

PRESENT WORK

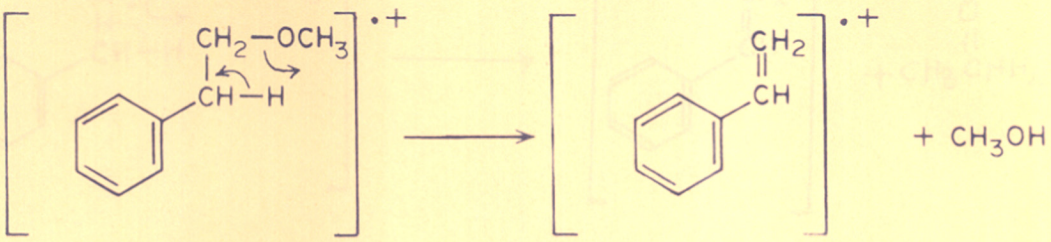
The characteristic feature of the spectra of the N-acetyl derivatives is an intense M-59 ion peak. The relative intensities of this peak are 100, 45.9 and 100% respectively for N-acetyl-β-phenylethylamine (Fig.1), its p-nitro (Fig.2) and 3,4-dimethoxy derivatives (Fig.3). The mass spectrum of N-benzoyl-β-phenylethylamine (Fig.4) showed a base peak at m/e 104, formed by the loss of $C_6H_5CONH_2$ from the molecular ion. The fragementation mode can be explained by a mechanism involving a six-membered ring transition state.



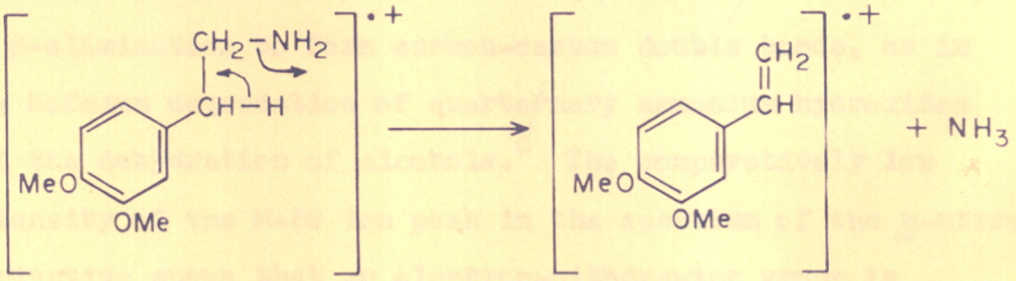
An M-59 ion in the spectrum of 3β-N-acetylamino-5α-androstane was explained by Djerassi[3] as resulting from a similar mechanism in which a hydrogen is transferred to the CH_3CONH group, leaving the positive charge with the steroid nucleus; but by far the most intense peak occurred at m/e 60, a protonated acetamide ion ($CH_3C\overset{+}{O}NH_3$).

The possibility of a four-membered ring transition state cannot be ruled out, because it is indicated from the spectra of methyl β-phenylethyl ether (Fig.5) and β-(3,4-dimethoxyphenylethylamine (Fig.6) (Chart 4). The mass spectrum of methyl β-phenylethyl ether shows a peak at m/e 104 (11%), resulting from the loss of methanol from the molecular ion. The spectrum of dimethoxyphenylethylamine shows a peak at m/e 164 (3.5%), formed by the loss of ammonia from the molecular ion.

CHART-4

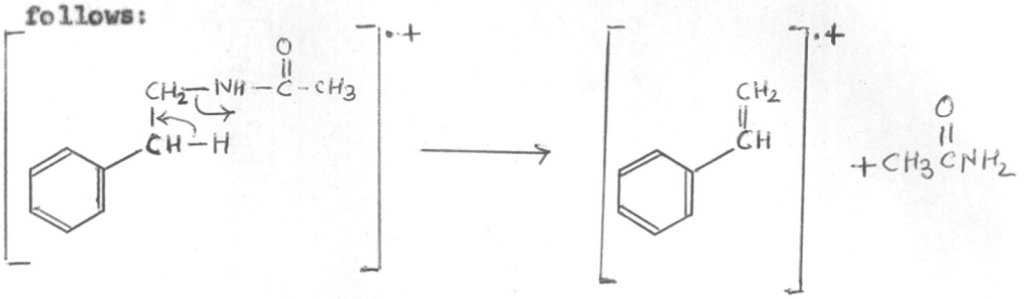


m/e 104



m/e 164

Based on the analogy of these compounds, the process operating in N-acetyl-β-phenylethylamine can be written as follows:

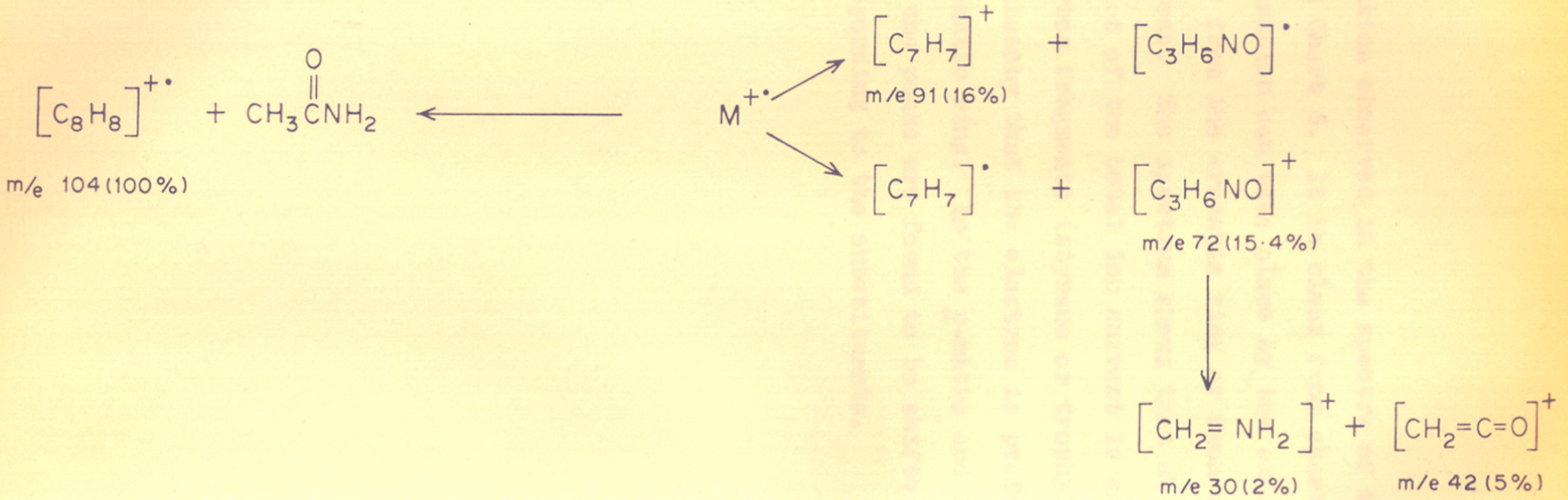


The driving force for the hydrogen transfer appears to be the ready loss of a stable molecule from the parent ion, resulting in the formation of a conjugated cation which is able to stabilize the positive charge. The chemical analogy is β-elimination to form carbon-carbon double bonds, as in the Hofmann degradation of quaternary ammonium hydroxides and the dehydration of alcohols.[6] The comparatively low intensity of the M-59 ion peak in the spectrum of the p-nitro-derivative shows that an electron-withdrawing group is unfavourable for this mode of fragementation and facilitates the cleavage of the amide bond to form a base peak of the $(CH_3CO)^+$ ion at m/e 43.

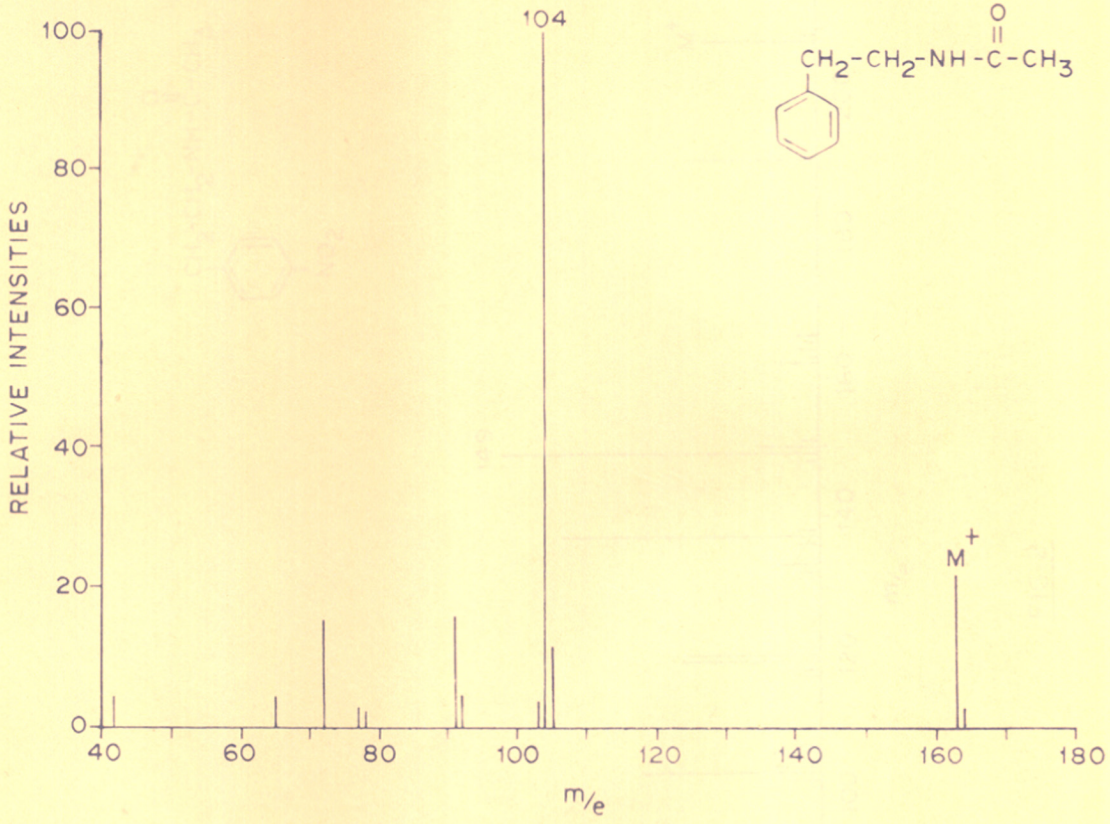
The peak at m/c 30 due to $(NH_2{=}CH_2)^+$ ion which is generally intense in the mass spectra of aliphatic amides is found to be insignificant in N-acetyl-β-phenylethylamine and its p-nitro and 3,4-dimethoxy derivatives. Various modes

CHART-5

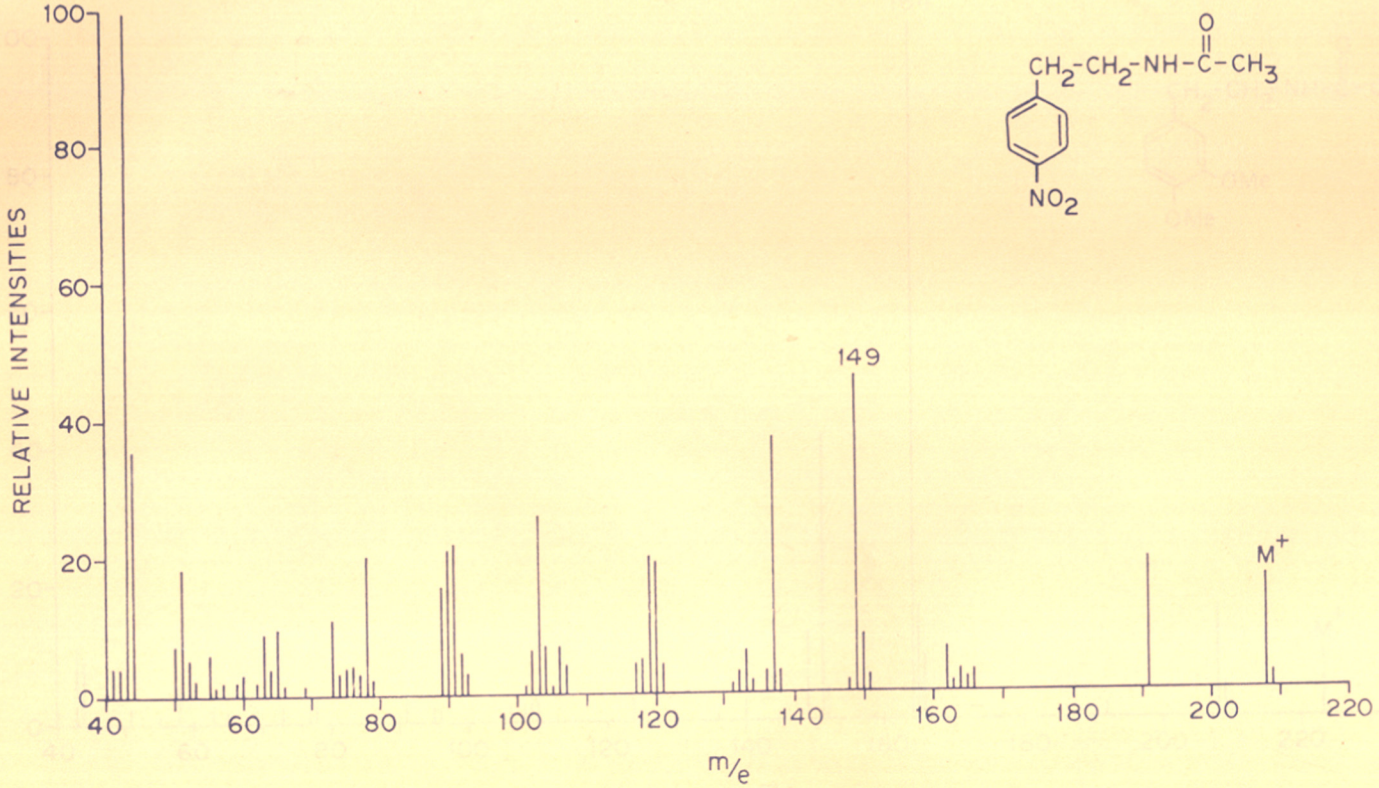
GENERAL MODES OF FRAGMENTATION OF N-ACETYL-β-PHENYLETHYLAMINE



of fragmentation observed in the spectra of these amides are shown in Chart 5. It is clear from chart 5, that the initial ionization can take place by the expulsion of electron either from the aromatic ring or from the nitrogen or oxygen atoms. The spectrum shows that at 70 e.v. the major fraction of the total ion current is carried by the hydrocarbon fragments (styrene or trophylium ions) indicating thereby that the electron is preferentially lost from the aromatic ring. In the p-nitro and 3,4-dimethoxy derivatives the peaks are found to be shifted by the mass units corresponding to the substituents.


104
M+
RELATIVE INTENSITIES
100
80
60
40
20
0
40
60
80
100
120
140
160
180
m/e
CH2-CH2-NH-C-CH3
O

FIG·1


RELATIVE INTENSITIES
100
80
60
40
20
0
40
60
80
100
120
140
160
180
200
220
m/e
149
M+
CH2-CH2-NH-C-CH3
O
NO2

FIG·2

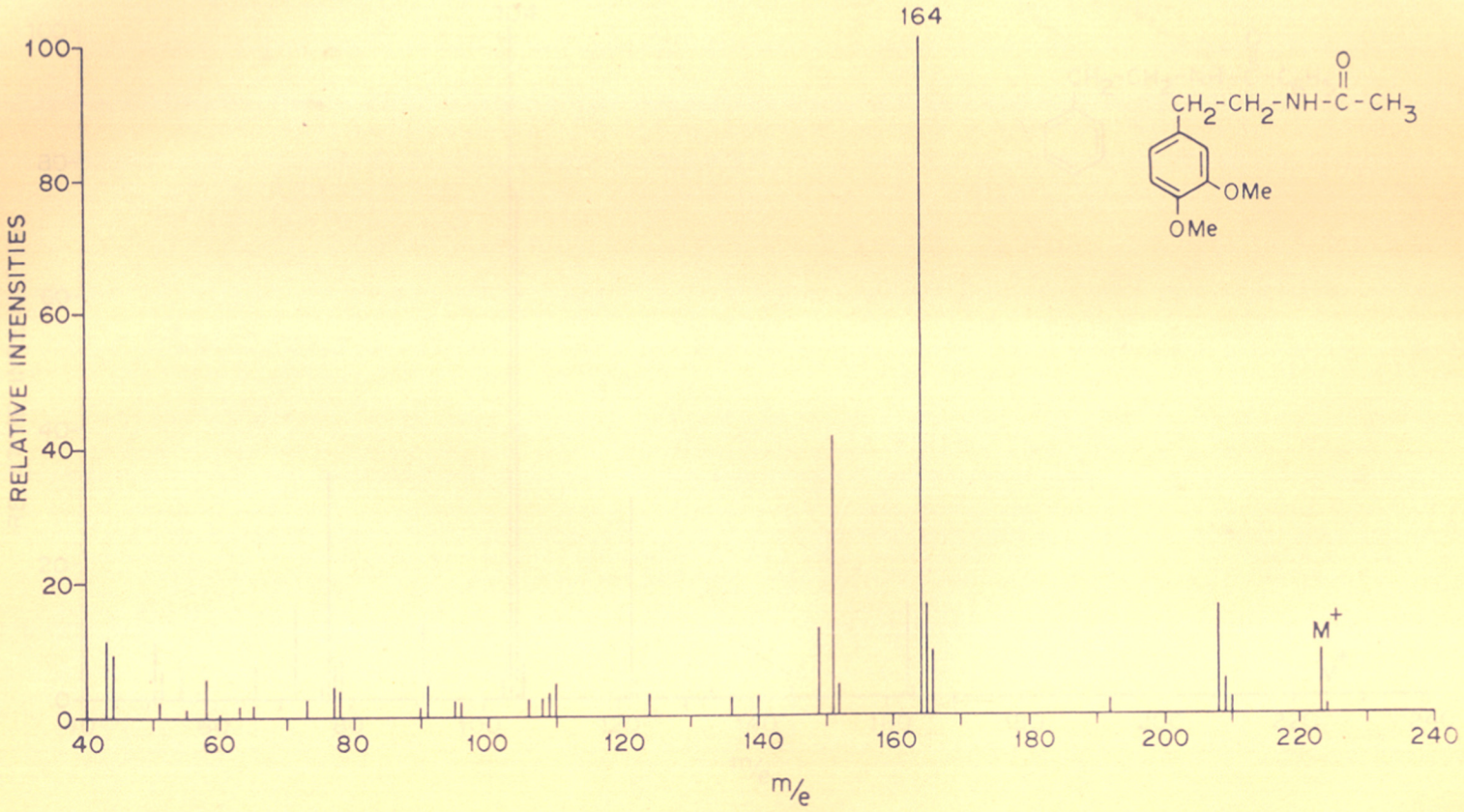
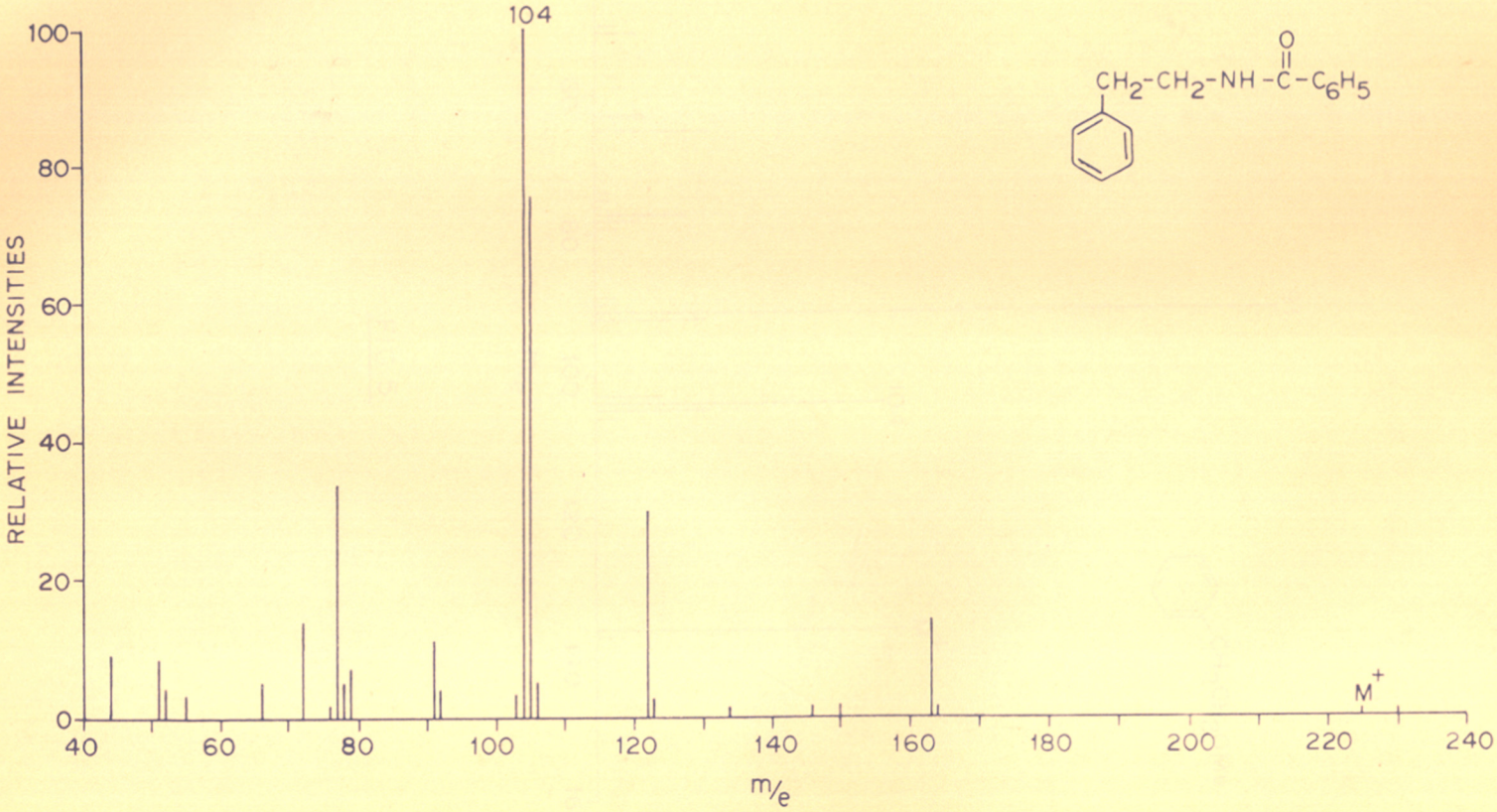
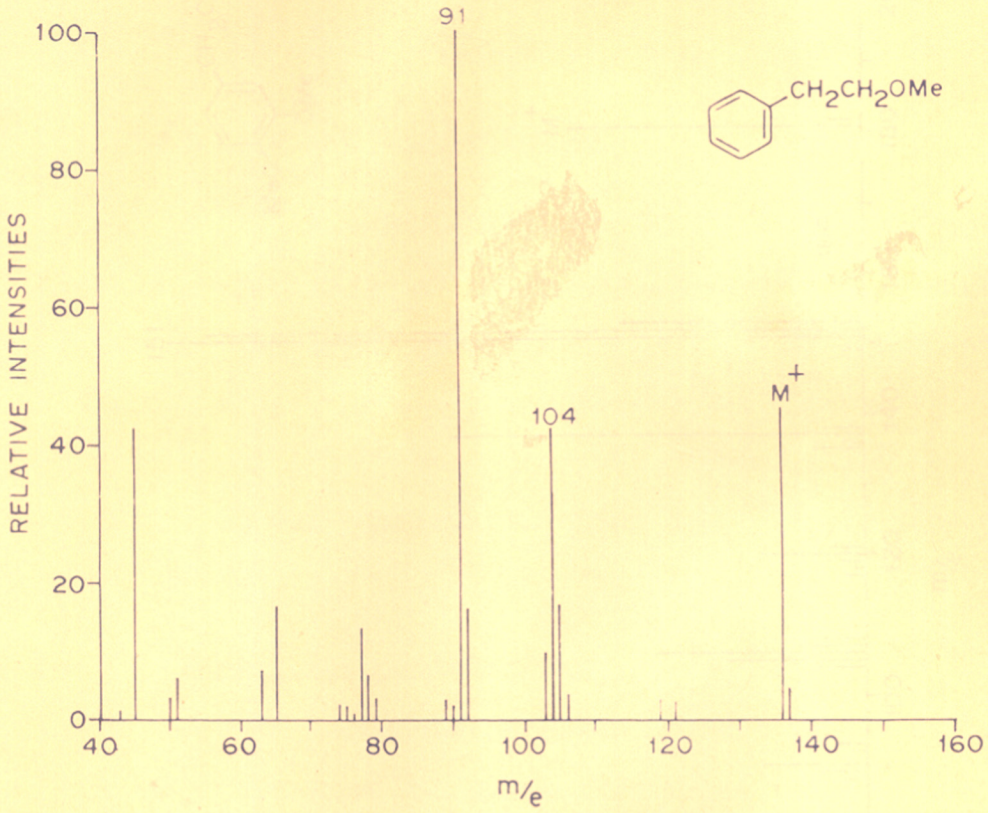

164
100
80
60
40
20
0
RELATIVE INTENSITIES
40
60
80
100
120
140
160
180
200
220
240
m/e
M+
CH2-CH2-NH-C-CH3
O
OMe
OMe

FIG. 3


104
100
80
60
40
20
0
RELATIVE INTENSITIES
40
60
80
100
120
140
160
180
200
220
240
m/e
M+
CH2-CH2-NH-C-C6H5
O

FIG·4


91
100
80
60
40
20
0
RELATIVE INTENSITIES
104
M+
CH2CH2OMe
40
60
80
100
120
140
160
m/e

FIG·5

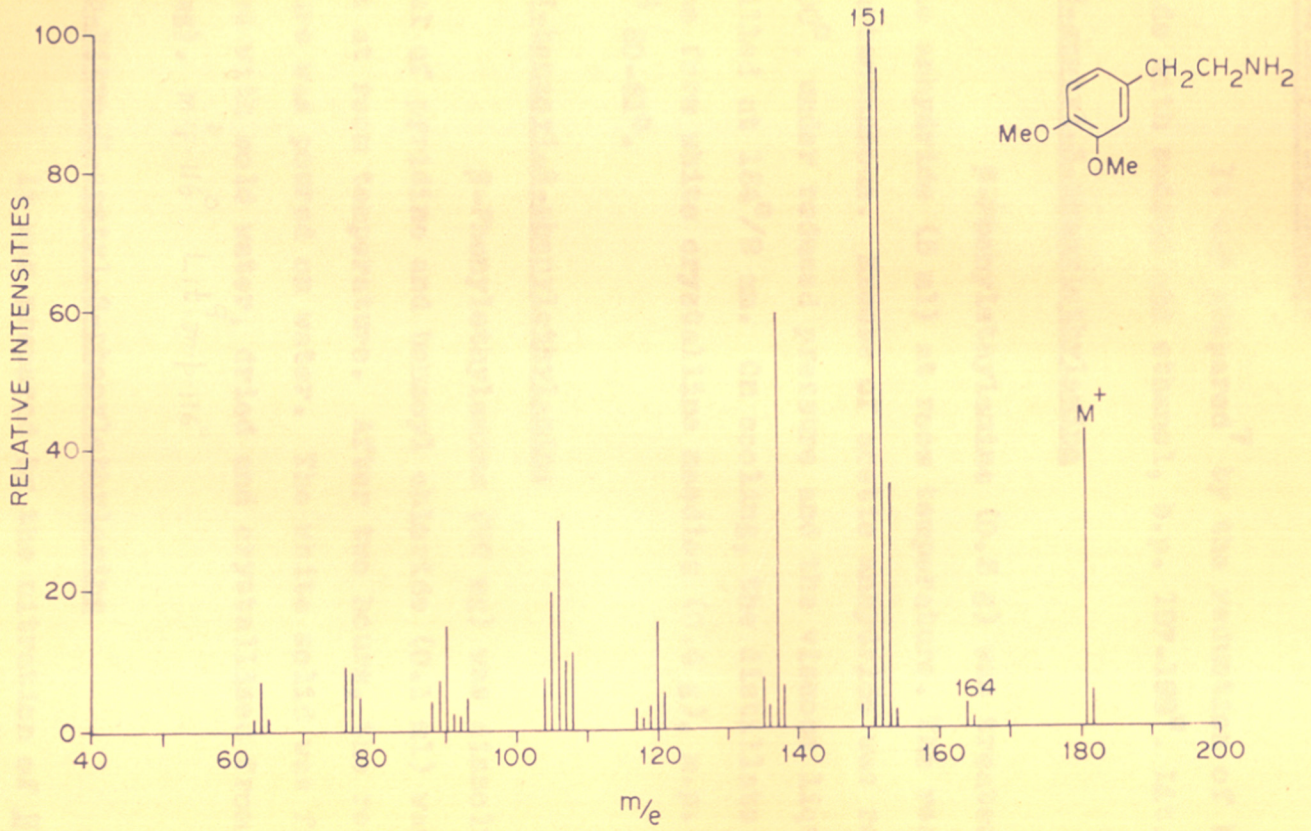

RELATIVE INTENSITIES
100
80
60
40
20
0
40
60
80
100
120
140
160
180
200
m/e
151
164
M+
CH2CH2NH2
MeO
OMe

FIG. 6

EXPERIMENTAL

1. β-Phenylethylamine.

It was prepared[7] by the reduction of benzyl cyanide with sodium and ethanol, b.p. 197-198°. Lit.[7] 197-198°.

2. N-acetyl-β-phenylethylamine

β-Phenylethylamine (0.5 g) was treated with acetic anhydride (5 ml) at room temperature. The reaction is instantaneous. Excess of acetic anhydride was removed at 100°, under reduced pressure and the viscous liquid was distilled at 154°/2 mm. On cooling, the distillate solidified in the form white crystalline needles (0.4 g), m.p. 50-51°. Lit.[8] 50-51°.

3. N-benzoyl-β-phenylethylamine

β-Phenylethylamine (20 mg) was dissolved in 0.2 ml of pyridine and benzoyl chloride (0.1 ml) was added to it at room temperature. After two hours, the reaction mixture was poured on water. The white solid was filtered, washed with cold water, dried and crystallised from ethanol (10 mg). m.p. 116° Lit.[9] m.p. 116°

4. p-Nitro-N-acetyl-β-phenylethylamine

It was prepared by the nitration of N-acetyl-β-phenylethylamine with fuming nitric acid (d, 1.5), m.p. 142°. Lit.[10] 142°.

5. N-acetyl-(3,4-dimethoxy)-β-phenylethylamine

It was prepared from commercially available sample of (3,4-dimethoxy)-β-phenylethylamine, by the method described in 2, m.p. 94-95°. Lit.[11] 94-95°.

6. Methyl β-phenylethyl ether.

It was prepared by the methylation of β-phenylethanol with methyl iodide and silver oxide in dimethyl formamide at room temperature for 24 hours, b.p. 194-196°. Lit.[12] 190-195°.

The spectra were recorded on CEC 21-110 double focussing mass spectrometer using a direct inlet system at 70 ev. and 20 μa, and at temperature below the melting point of the compound.

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PART III

STUDIES IN DEMETHYLATION OF METHOXYANTHRAQUINONES

***

Protection of hydroxyl groups by methylation of a polyhydric phenol and partial or complete demethylation are commonly used in synthetic as well as structural organic chemistry. Various methods of methylation and demethylation have been reported in the chemistry of hydroxyanthraquinones.

Methylation of hydroxyanthraquinones

Methylation with dimethyl sulphate in aqueous sodium hydroxide was often employed for methylation of hydroxyanthraquinones. However, the use of dimethyl sulphate in presence of anhydrous potassium carbonate in boiling acetone usually gives better yields. For compounds such as the laccaic acids, which contain both carboxyl and hydroxyl groups, the use of anhydrous conditions during methylation was found to be advantageous, because the saponification of the ester so formed was minimized. Purpurin was converted into its trimethyl ether in 80% yield by using the dimethyl sulphate-anhydrous potassium carbonate-acetone method.[1] Nitrohydroxyanthraquinones cannot be easily methylated by this method. 3-Nitroalizarin dimethyl ether[2] was prepared (about 50% yield) by heating the dry potassium salt of 3-nitroalizarin with dry sodium carbonate and dimethyl sulphate at 140°.

Diazomethane is a well-known methylating agent which can be employed under neutral conditions for methylation

of carboxylic acids, enols and phenols. Using diazomethane, Ali and Haynes[3] converted carminic acid into methyl 1,3,4,6-tetramethyl carminate, the hydroxyl groups of the glucose residue being unaffected. 2-Acetoxy-1-methoxyanthraquinone[4] was prepared by treatment of 2-acetylalizarin with diazomethane.

Methylation using methyl iodide in presence of silver oxide in dimethyl formamide at room temperature was found to be useful for methylation of xantholaccaic acid.[5]

Demethylation of methoxyanthraquinones

Sulphuric acid,[6,7] anhydrous aluminium chloride[5,9] and a mixture of conc. hydrobromic acid and acetic acid[8] are three reagents which have been employed for the demethylation of methoxyanthraquinones.

Demethylation with sulphuric acid

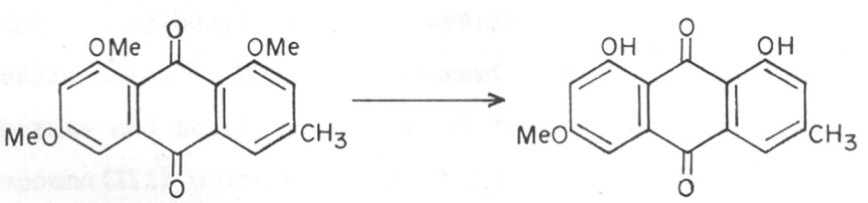
At 80-110° only α-methoxyl groups are demethylated. Thus alizarin dimethyl ether gave the 2-methyl ether of alizarin in 89% yield by heating with 78% sulphuric acid at 100°[6]; and quinizarin dimethyl ether the monomethyl ether of quinizarin by heating with conc. sulphuric acid at 110° for 10 minutes.[7] At higher temperature (above 140°) β-methoxyl groups are also attacked.[4a,6a] The drawback of the method is that at high temperatures there is always a

possibility of sulphonation and other side reactions. Thus 2,3-dimethoxyanthraquinone on heating with sulphuric acid at 205° gave a mixture of 2,3-dihydroxyanthraquinone (hystazarin) and alizarin.[6a]

Demethylation with aluminium chloride

In this method, a methoxyanthraquinone is heated with anhydrous aluminium chloride or preferably with the melt prepared from a mixture of aluminium chloride and sodium chloride. Complete demethylation of the compound is achieved in 15 minutes in almost quantitative yield. In this laboratory, 1,2,5,7-tetramethoxy-4-methylanthraquinone (erythrolaccin tetramethyl ether) was demethylated with aluminium chloride-sodium chloride melt to yield erythrolaccin quantitatively.[5]

It has been reported[9] that on treatment with anhydrous aluminium chloride in boiling benzene emodin trimethyl ether (I) is converted into physcion (II) by the demethylation of the ∝-methoxyl groups.



I II

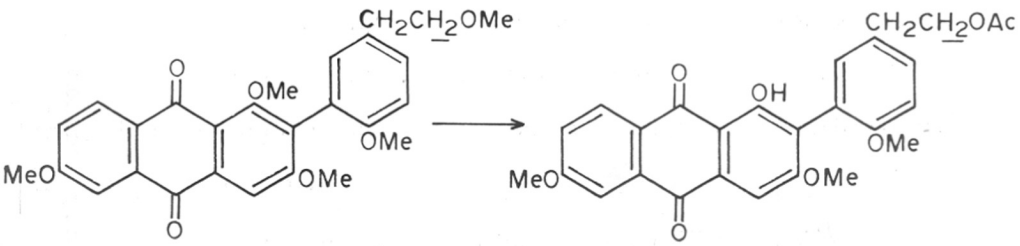
Demethylation with hydrobromic acid in acetic acid

When a methoxyanthraquinone is refluxed with a mixture of conc. hydrobromic acid and glacial acetic acid, demethylation of both α- and β-methoxyl groups takes place to give the hydroxyanthraquinone in 70 to 80% yields.[8]

The use of hydrobromic acid at different temperature and concentrations,[10] hydrochloric acid[11] at 150°, and glacial acetic acid saturated with hydrogen chloride[12] has been reported.

Meerwein and Maier-Huser[13] observed in 1932 that if equivalent quantities of ethyl ether, acetic anhydride and boron trifluoride react at room temperature for 15 hours ethyl acetate is formed in 14.8% yield. It was recently reported that on treatment with boron trifluoride etherate and acetic anhydride at 0° for 14 hours steroidal methyl ethers can be cleaved and converted into their acetates; allylic and homoallylic ethers gave the corresponding acetates in over 90% yield; completely saturated ethers gave the acetate with retention of configuration as the main product, but the epimeric acetate and elimination products were also obtained.[14a,b] This reagent was employed successfully for determining the presence of an aliphatic methoxyl group in certain methylated derivatives of laccaic acid.[15] Thus on treatment with boron trifluoride etherate and acetic anhydride at room temperature for 18 hours, compound (III) which was a derivative of laccaic acid, was converted to (IV). NMR spectrum of compound (IV) showed the disappearance of

the signal at 6.72 , due to the aliphatic methoxyl group which was seen in NMR spectrum of (III) and the downfield shift of the indicated CH_2 protons because of the conversion of $-CH_2OMe$ to $-CH_2OAc$.



(III) (IV)

PRESENT WORK

In order to know more about the action of this reagent on polymethoxyanthraquinones, the present work was carried out. Besides boron trifluoride etherate, the action of hydrogen bromide in glacial acetic acid was also found to be useful in dealing with the chemistry of lac pigments; and the demethylating action of this reagent has also been studied.

Various methoxyanthraquinones were subjected to the action of (a) boron trifluoride etherate and (b) a saturated solution of hydrogen bromide in glacial acetic acid. The results are summarised in Table 1.

TABLE 1

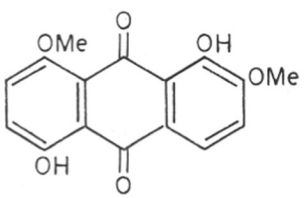
(a) Action of BF_3-etherate + Ac_2O at room temperature on methoxyanthraquinones.

(b) Action of HBr in acetic acid at room temperature on methoxyanthraquinones.

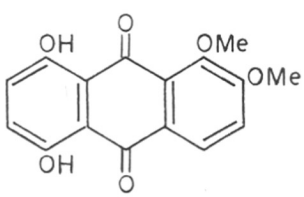
		Substitution in anthraquinone			
No.	Reactant	a	Yield	b	Yield
1	1,2-$(OMe)_2$	1-OH-2-OMe	75%	1-OH-2-OMe	80%
2	1,3-$(OMe)_2$	1-OH-3-OMe	70%	1-OH-3-OMe	80%
3	1,4-$(OMe)_2$	1-OH-4-OMe	73%	1-OH-4-OMe	40%
4	1,5-$(OMe)_2$	1-OH-5-OMe*		1-OH-5-OMe	
5	1,8-$(OMe)_2$	1-OH-8-OMe*		1-OH-8-OMe	
6	1,2,4-$(OMe)_3$	1-OH-2,4-$(OMe)_2$	70%	1,4-$(OH)_2$-2-OMe	90%
7	1-OH-2,4-$(OMe)_2$	1-OH-2,4-$(OMe)_2$	99%	-	
8	1,3-$(OMe)_2$-2-Me	1-OH-2-OMe-3-Me	70%	-	
9	1,2,5,8-$(OMe)_4$	1,5-(OH_2)-2,8-$(OMe)_2$	70%	-	

* The products were not isolated in pure form.

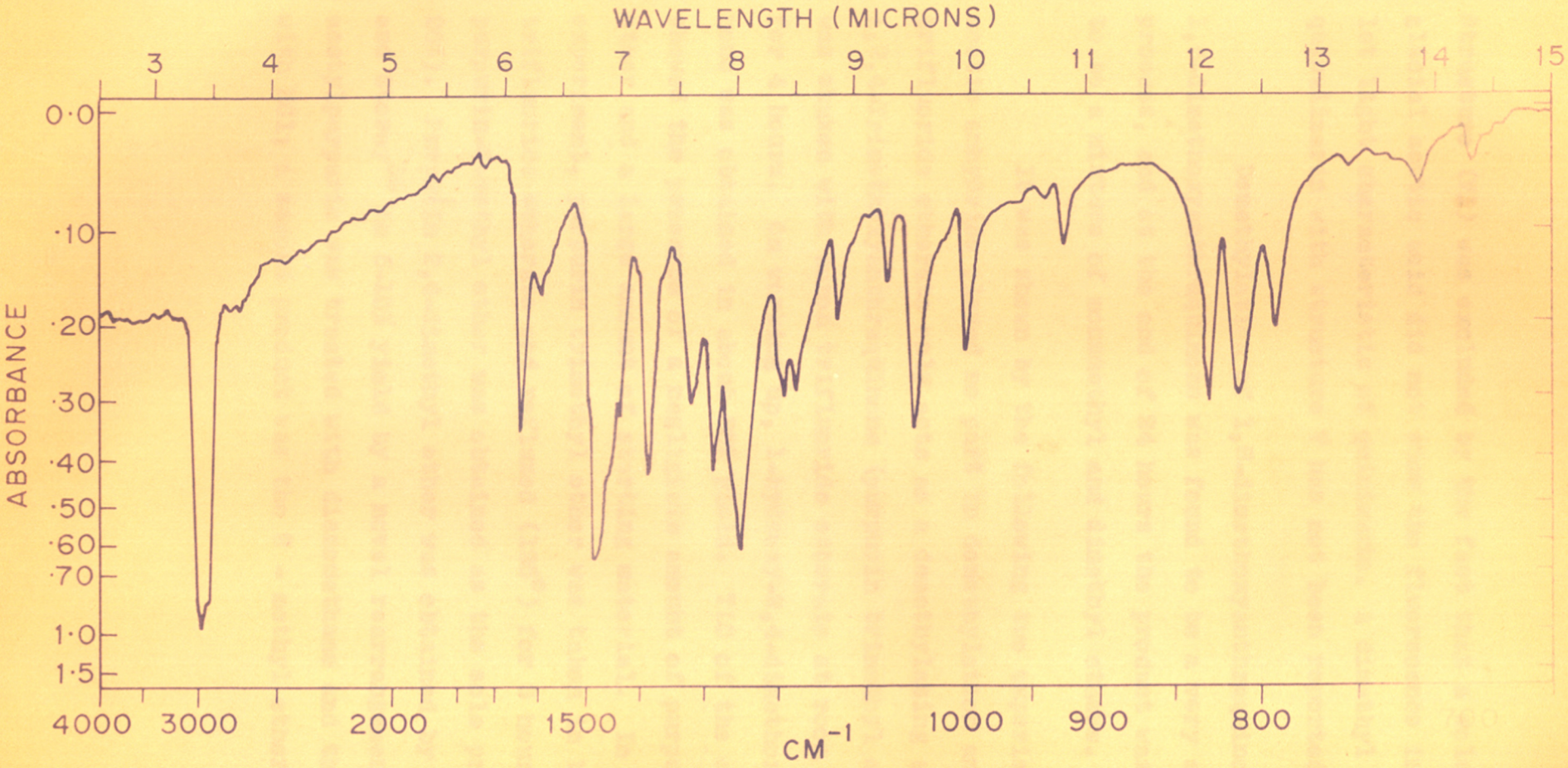
Most of the experiments with boron trifluoride etherate were carried out under the set of conditions used in the conversion of (III) into (IV). A methoxyanthraquinone was taken in acetic anhydride, boron trifluoride etherate (excess) was added and the reaction mixture kept at room temperature for 18-24 hours. The product was isolated by pouring the reaction mixture on ice. It was found that there was no replacement of methoxyl by acetoxyl groups, but demethylation of only one of the ∝-methoxyl groups took place, the only exception being 1,2,5,8-tetramethoxyanthraquinone (giving quinalizarin tetramethyl ether) in which two out of three ∝-methoxyl groups were demethylated. The infrared spectrum showed in the carbonyl region a single peak at 1630 cm^{-1} (Fig. 1), both the carbonyl groups are therefore chelated and the product of demethylation can be assigned structure (V) or (VI).



V



VI


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

FIG·1

Structure (VI) was excluded by the fact that a solution in glacial acetic acid did not show the fluorscence in ultraviolet light characteristic of quinizarin. A dimethyl ether of quinalizarin with structure V has not been reported so far.

Demethylation of 1,8-dimethoxyanthraquinone and 1,5-dimethoxyanthraquinone was found to be a very slow process, and at the end of 24 hours the product was found to be a mixture of monomethyl and dimethyl ethers.

It was shown by the following two experiments that acetic anhydride played no part in demethylation and boron trifluoride etherate only acts as a demethylating agent. 1,2,4-Trimethoxyanthraquinone (purpurin trimethyl ether) was shaken with boron trifluoride etherate at room temperature for 4 hours. On working up, 1-hydroxy-2,4-dimethoxyanthraquinone was obtained in about 30% yield. TLC of the crude product showed the presence of a negligible amount of purpurin-2-methyl ether and a large amount of starting material. In the second experiment, purpurin trimethyl ether was taken in boron trifluoride etherate and refluxed (126°) for 3 hours, when purpurin-2-methyl ether was obtained as the sole product (yield 90%). Purpurin 2,4-dimethyl ether was obtained by Perkin- and Storey[16] in 5-10% yield by a novel rearrangement when 2-O-acetylpurpurin was treated with diazomethane and then hydrolysed with HCl; a second product was the 2 - methyl ether.

The latter was prepared by Graebe[17] by heating the dry trisodium salt of purpurin with dimethyl sulphate at 150-160° for an hour, and by Perkin[18] by heating the mono-K salt with methyl iodide in benzene in a sealed tube at 230°.

Demethylation with hydrogen bromide in glacial acetic acid.

The methoxyanthraquinone was dissolved in the minimum amount of dry methylene chloride, an excess of a saturated solution of HBr in glacial acetic acid was added. After keeping for 18-24 hours at room temperature, the product was isolated by pouring the reaction mixture on ice. Under these experimental conditions, demethylation of only α-methoxyl groups took place. Thus the dimethyl ethers of alizarin and xanthopurpurin and the trimethyl ether of purpurin were converted quantitatively into alizarin 2-methyl ether, xanthopurpurin-3-methyl ether and purpurin-2-methyl ether respectively. Demethylation of quinizarin dimethyl ether was slow and gave at the end of the above mentioned reaction period, quinizarin mono-methyl ether (about 30%) and quinizarin (about 20%). Demethylation of 1,5-dimethoxy and 1,8-dimethoxyanthraquinone was slow, and the product was found to be a mixture of the mono- and dimethyl ethers.

The required methoxy anthraquinones were prepared by methylation of the hydroxyanthraquinones with dimethyl sulphate and anhydrous potassium carbonate in boiling acetone,

The compounds were obtained in pure form by chromatography on alumina, Brockmann grade I, followed by crystallization from ethanol.

Mechanism of demethylation

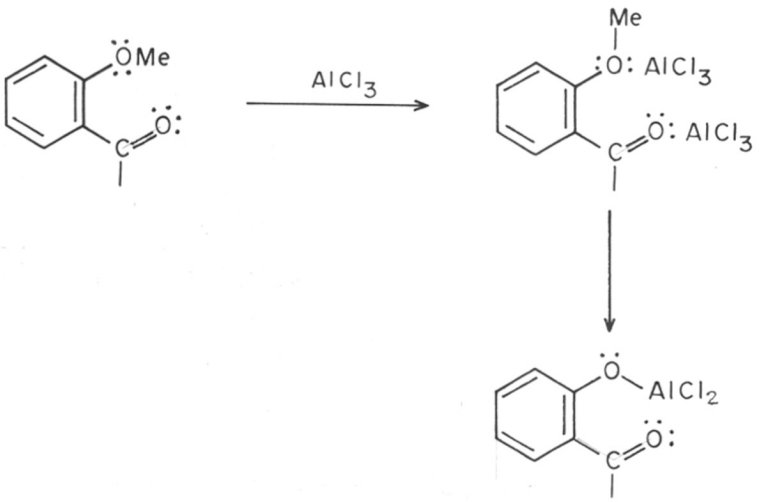
Cleavage of aryl alkyl ethers with reagents such as hydrogen iodide and aluminium chloride proceeds as follows:[19,20]

1) $Ar\text{-}\ddot{O}\text{-}R \underset{}{\overset{H^+}{\rightleftharpoons}} Ar\text{-}\overset{+}{\ddot{O}}\text{-}R \xrightarrow{I^-} Ar\text{-}OH + RI$

2) $Ar\text{-}\ddot{O}\text{-}R + AlCl_3 \rightarrow ArOAlCl_2 + RCl$

$ArOAlCl_2 + 3H_2O \rightarrow ArOH + Al(OH)_3 + 2HCl.$

In 1899 Kostanecki and Tambor[21] found that by heating phloracetophenone trimethyl ether with aluminium chloride at 110° the 2,4-dimethyl ether was produced, a methoxyl adjacent to the carbonyl group undergoing demethylation. Bharadwaj and Venkataraman[22] applied this selective demethylation to the synthesis of 5-hydroxyflavones from polymethoxyflavones containing a methoxyl in the 5-position, using aluminium chloride in nitrobenzene at 100°.[23] It is obvious that the facile demethylation of o-methoxyacetophenone, 5-methoxyflavone, and similar ethers is dependent on the formation of a metal chelate such as (VII), specially in a six-membered ring system.



(VII)

+ (MeCl + $AlCl_3$ ⇌ MeCl : $AlCl_3$)

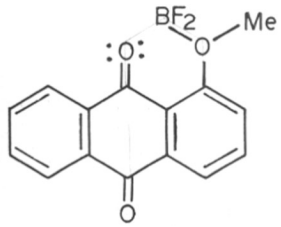
Similar considerations indicate that in a polymethoxyanthraquinone the α-methoxyl groups will undergo demethylation more readily than β-methoxyl groups.

Brossi[23a] has recently shown that a methoxyl group *meta* to a carbonyl function undergoes selective demethylation by Lewis acids. With one equivalent of $AlCl_3$ veratraldehyde yields isovanillin, because the most electron-rich ethereal oxygen co-ordinates first; but with excess $AlCl_3$ the aldehyde group also complexes with $AlCl_3$ and vanillin is obtained.[23b]

For use in the Friedel-Crafts, Fries and Nencki reactions, demethylations and other purposes, boron trifluoride has several advantages in comparison with aluminium chloride or zinc chloride.[24] It has the least volume, the atoms being very

densely packed; because fluorine has the highest electronegativity, boron trifluoride has strong electropolar character and is a powerful electron acceptor; it is soluble in many organic solvents, and can also be used conveniently as the etherate, the acetic acid complex or other appropriate co-ordination compound. Ethers have been cleaved at room temperature by boron trichloride[25a] and boron tribromide.[25b] Dean *et al.*[25c] have recently claimed advantages for selective demethylation by boron trichloride which appear to us to be very doubtful. Use of diborane with iodine[26a] and boron triiodide[26b] at room temperature for the cleavage of ethers has also been reported recently.

As mentioned earlier, the action of boron trifluoride etherate and acetic anhydride at room temperature on 1,8-, 1,4-, and 1,5-dimethoxyanthraquinones leads to demethylation of only one methoxyl group. When the chelate compound (VIII) is formed, there is no possibility of a second boron trifluoride molecule forming a chelate with an 8-methoxyl group. Secondly, the coordination complex (VIII) causes a powerful electron pull, which renders the formation of a coordination complex at the second quinone carbonyl group more difficult. Hence, the demethylation of the second α-methoxyl group in the 4- or 5-position gets retarded.



VIII

In the case of purpurin trimethyl ether, demethylation occurs at the ∝-methoxyl group which is ortho to a β-methoxyl group. This is probably because of the electron-releasing effect of the β-methoxyl group, which facilitates the attack of an electrophilic agent at the ∝-position. Demethylation occurring in the 1- and 5-positions of quinalizarin tetramethyl ether can be explained by the consideration that the formation of a boron trifluoride complex between the 1-methoxyl and 9-carbonyl groups inactivates the 8-methoxyl for attack by a second boron trifluoride molecule; the 5-methoxyl and 10-carbonyl groups are then relatively more susceptible to the formation of a boron trifluoride complex and subsequent demethylation.

EXPERIMENTAL

Demethylation of methoxyanthraquinones by boron trifluoride etherate in acetic anhydride

1. Demethylation of 1,2-dimethoxyanthraquinone

1,2-Dimethoxyanthraquinone (0.27 g; 0.001 mole) was taken in acetic anhydride (5 ml), and boron trifluoride etherate (3 ml; 0.025 mole) was added. The reaction mixture became homogeneous and deep red in colour. After keeping for 18 hours at room temperature, the reaction mixture was poured on ice and kept standing for 2 hours. A reddish yellow solid separated. It was filtered, washed with cold water and dried (0.2 g). The product was insoluble in aqueous sodium carbonate, soluble in hot sodium hydroxide giving a red solution, and in conc. sulphuric acid giving a cherry red solution. It gave a dark brown colour with ethanolic ferric chloride. Crystallization from ethanol yielded orange-yellow needles (0.18 g; yield 75%. It was obtained alongwith dimethyl ether by the action of dimethyl sulphate and sodium hydroxide (10%) at 100°, m.p. 230-231° (lit.[6] m.p. for 1-hydroxy-2-methoxyanthraquinone 230-231°). Found: OMe 12.2. $C_{15}H_{10}O_4$ requires for one OMe 12.2%.

2. Demethylation of 1,3-dimethoxyanthraquinone

1,3-Dimethoxyanthraquinone (0.29 g; 0.001 mole) was taken in acetic anhydride (5 ml) and boron trifluoride etherate

(3 ml; 0.025 mole) was added to it. After keeping for 18 hours, at room temperature, the reaction mixture was worked up as in (1). A brown coloured crude product (0.18 g) was insoluble in aqueous sodium carbonate, soluble in aqueous sodium hydroxide and gave dirty brown colour with ethanolic ferric chloride. On crystallization from glacial acetic acid, golden yellow needles of 1-hydroxy-3-methoxyanthraquinone were obtained (0.17 g; 70% of theory), m.p. 190-191°, lit.[27] m.p. 193°. Found: OMe 11.8%. $C_{15}H_{10}O_4$ requires 12.2%.

3. Demethylation of 1,4-dimethoxyanthraquinone

1,4-Dimethoxyanthraquinone (0.27 g; 0.001 mole) was taken in acetic anhydride (5 ml) of boron trifluoride etherate (3 ml; 0.025 mole) was added to it. Further experiment was carried out as in (1), when an orange red product (0.2 g) was obtained. It showed brownish black ferric colour. It gave violet red solution in aqueous sodium hydroxide and casin red solution in conc. sulphuric acid. A solution in glacial acetic acid showed no fluorscence, when exposed to ultraviolet light, (a test characteristic of 1,4-dihydroxyanthraquinones). On crystallization from ethanol, 1-hydroxy-4-methoxyanthraquinone was obtained in orange needles (0.185 g; 73% of theory), m.p. 168-169°; lit.[7] m.p. 167-168°. Found OMe 12.0. $C_{15}H_{10}O_4$ requires 12.2% for one OMe.

4. Demethylation of 1,8-dimethoxyanthraquinone

1,8-Dimethoxyanthraquinone (0.27 g; 0.001 mole) was taken in acetic anhydride (5 ml) and boron trifluoride etherate (3 ml; 0.025 mole) was added to it. The compound went partially in solution and even after further addition of acetic anhydride (10 ml) and boron trifluoride etherate (5 ml), the reaction mixture remained heterogeneous. After shaking the reaction mixture at room temperature for about 24 hours, it was worked up as in (1), to yield a yellowish black product (0.21 g), which gave greenish black ferric colour. TLC (silica gel, benzene + acetone (7:3) showed the absence of 1,8-dihydroxyanthraquinone and presence of approximately equal amounts of 1-hydroxy-8-methoxy and 1,8-dimethoxy-anthraquinones.

5. Demethylation of 1,5-dimethoxyanthraquinone

A mixture of 1,5-dimethoxyanthraquinone (0.27 g; 0.001 mole), acetic anhydride (10 ml) and boron trifluoride etherate (6 ml; 0.01 mole) was kept shaking at room temperature for about 24 hours. After working up as in (1), the crude product (0.2 g) was obtained, which gave dirty brown colour with ethanolic ferric chloride. TLC (silica gel, benzene + acetone 7:3) showed the absence of 1,5-dihydroxy-anthraquinone, and presence of approximately equal amounts of 1,5-dimethoxyanthraquinones, and probably 1-hydroxy-5-methoxy-anthraquinone.

6. Demethylation of 1,2,4-trimethoxyanthraquinone

1,2,4-Trimethoxyanthraquinone (0.3 g; 0.001 mole) was taken in acetic anhydride (10 ml) and boron trifluoride etherate (5 ml; 0.04 ml) was added to it. The resultant deep red solution was kept overnight at room temperature and worked up as in (1), to yield a crude product (0.21 g) which was insoluble in aqueous sodium carbonate but soluble in hot aqueous sodium hydroxide. It gave brown ferric colour and showed no fluorscence when dissolved in glacial acetic acid and exposed to UV light. Crystallization from ethanol yielded 1-hydroxy-2,4-dimethoxyanthraquinone, reddish orange needles (0.19 g; 70% of theory), m.p. 188-189°; lit.[16] m.p. 186-189°.

7. Demethylation of 1-hydroxy-2,4-dimethoxyanthraquinone

A mixture of 1-hydroxy-2,4-dimethoxyanthraquinone (0.1 g; 0.0005 mole), acetic anhydride (3 ml), and boron trifluoride etherate (2 ml; 0.015 mole) was kept at room temperature for about 18 hours and worked up as in (1). TLC of the crude product showed a very faint spot of 1,4-dihydroxy-2-methoxyanthraquinone, and that of the starting material. Crystallization from ethanol, 95% of the starting material was obtained (0.9 g), m.p. 188-189°. Mixed m.p. with the product from (6), was undepressed. Found: OMe 20.5. $C_{16}H_{12}O_5$ requires 21.0%.

8. Demethylation of 1,2,4-trimethoxyanthraquinone at water bath temperature

Using the same quantities of reactants as in (6), the reaction was carried out on boiling water bath for 2 hours and worked up as in (1). TLC of the crude product (0.25 g) showed a very faint spot corresponding to 1,4-dihydroxy-2-methoxyanthraquinone, and a major spot corresponding to 1-hydroxy-2,4-dimethoxyanthraquinone. Crystallization from ethanol, yielded 1-hydroxy-2,4-dimethoxyanthraquinone (0.18 g; 65-70% of theory), m.p. 186-188°, mixed m.p. with the product from (6), was undepressed.

9. Demethylation of 1,2,4-trimethoxyanthraquinone with boron trifluoride etherate in the absence of acetic anhydride

After shaking at room temperature for 4 hours, a mixture of 1,2,4-trimethoxyanthraquinone (0.3 g) and boron trifluoride etherate (5 ml), it was worked up as in (1). The crude product showed on TLC (silica gel benzene-acetone 6:4) a faint spot corresponding to 1,4-dihydroxy-2-methoxy-anthraquinone, and two spots of nearly equal intensity corresponding to 1-hydroxy-2,4-dimethoxyanthraquinone and the starting material.

10. Demethylation of 1,2,4-trimethoxyanthraquinone at the reflux temperature of boron trifluoride etherate

1,2,4-Trimethoxyanthraquinone (0.2 g; 0.007 mole) was taken in boron trifluoride etherate (10 ml) and refluxed

(125°) for 4 hours. After cooling the reaction mixture was poured on ice. A brick red solid separated. It was filtered, washed with cold water and dried (0.18 g). TLC showed a presence of single spot. On crystallization from benzene, orange red prisms (0.19 g) of 1,4-dihydroxy-2-methoxyanthraquinone was obtained, m.p. 238-239°. lit.[11,18] m.p.238-240°.

11. Demethylation of 1,3-dimethoxy-2-methylanthraquinone

A mixture of 1,3-dimethoxy-2-methylanthraquinone (0.1 g), acetic anhydride (2 ml) and boron trifluoride etherate (1 ml) was kept overnight at room temperature and worked up as in (1) to yield 0.18 g of brownish yellow product, soluble in aqueous sodium hydroxide but insoluble in aqueous sodium carbonate. It gave brown ferric colour. Crystallization from methanol, yielded yellow plates of 1-hydroxy-3-methoxy-2-methylanthraquinone (0.07 g; 70% of theory), m.p. 188-189°, lit.[28] m.p. 188-189°.

12. Demethylation of 1,2,5,8-tetramethoxyanthraquinone

1,2,5,8-Tetramethoxyanthraquinone (0.2 g) was taken in acetic anhydride (15 ml), and boron trifluoride etherate (2.5 ml; 0.02 mole) was added to it. After keeping overnight at room temperature, the reaction mixture was worked up as in (1) The crude product (0.17 g) was insoluble in aqueous sodium carbonate, but soluble in hot sodium hydroxide solution. It gave reddish brown ferric colour and showed no fluorscence

in glacial acetic acid, when exposed to UV light. Crystallization from ethanol, yielded crimson needles (0.15 g; 70% of theory), m.p. 234-236°. Found: OMe, 19.0. $C_{16}H_{12}O_6$ requires 19.5% for OMe). Infrared spectrum showed a peak at 1630 cm^{-1} indicating that the compound was 1,5-dihydroxy-2,8-dimethoxyanthraquinone.

Demethylation of methoxyanthraquinones by a saturated solution (38%) of hydrogen bromide in glacial acetic acid

13. Demethylation of 1,2-dimethoxyanthraquinone

1,2-Dimethoxyanthraquinone (0.25 g; 0.001 mole) was dissolved in dry methylene chloride (3 ml), and 38% solution of hydrogen bromide gas in glacial acetic acid (2 ml) was added to it. The reaction mixture (deep red solution) was kept overnight and then poured on ice cold water. The mixture was warmed for 5 minutes at 50° to remove methylene chloride. The brown solid separated. It was filtered, washed with water and dried in air. The crude product(0.22 g) was insoluble in aqueous sodium carbonate, but soluble in hot aqueous potassium hydroxide. It gave cherry red solution with sulphuric acid. Crystallization from ethanol yielded orange yellow needles (0.2 g); 80% of theory), m.p. 230-231°. Mixed m.p. with 1-hydroxy-2-methoxyanthraquinone, obtained by the demethylation of 1,2-dimethoxyanthraquinone by boron trifluoride etherate-acetic anhydride was undepressed.

14. Demethylation of 1,3-dimethoxyanthraquinone

1,3-Dimethoxyanthraquinone (0.1 g) was dissolved in dry methylene chloride (2 ml) and the reagent (HBr/HAc) (1 ml) was added. The resultant deep red solution was kept overnight at room temperature and worked up as in (13). A crimson red product (0.09 g) behaved as a single spot on TLC (silica gel, benzene-acetone (7:3) system) and on crystallization from acetic acid gave 1-hydroxy-3-methoxyanthraquinone (0.08 g; 80% yield), m.p. 190-192°; mixed m.p. with the authentic sample undepressed.

15. Demethylation of 1,2,4-trimethoxyanthraquinone

1,2,4-Trimethoxyanthraquinone (0.1 g) was dissolved in 2 ml of dry methylene chloride and the reagent (HBr/HAc) (1 ml) was added to it. The reaction mixture (deep red solution) was kept overnight at room temperature and worked up as in expt. no. 13. A crimson red product (0.09 g) was obtained, which was corresponding to 1,4-dihydroxy-2-methoxyanthraquinone, on TLC (silica gel, benzene + acetone 7:3; R_f value 0.3). On crystallization from benzene, orange red prisms (0.085 g; 90% of theory) were obtained, m.p. 238-239° mixed melting point with 1,4-dihydroxy-2-methoxyanthraquinone was undepressed.

16. Demethylation of 1,4-dimethoxyanthraquinone

1,4-Dimethoxyanthraquinone (0.25 g) was dissolved in 5 ml. of dry methylene chloride and the reagent (HBr/HAc) (1 ml) was added to it. After working up reaction as in (13) a orange coloured crude product (0.18 g) was obtained. TLC (silica gel, benzene + acetone 7:3), showed that it was a mixture of 1-hydroxy-4-methoxyanthraquinone and the starting compound. There was no formation of 1,4-dihydroxyanthraquinone. Chromatography on silica gel column (10 g., 10xl cm^{-1}) using benzene-acetone mixture, 1-hydroxy-4-methoxyanthraquinone (0.1 g; 40% of theory) was obtained, m.p. 167-168°, mixed m.p. with a sample of 1-hydroxy-4-methoxyanthraquinone was undepressed.

17. Demethylation of 1,5-dimethoxyanthraquinone

1,5-Dimethoxyanthraquinone (0.25 g) was taken in dry methylene chloride (10 ml), and the reagent (HBr/HAc) (1 ml) was added to it. The red coloured heterogeneous mixture was shaken for 18 hours. After this period, the reaction mixture became black in colour with yellowish tinge. It was worked up as in (13). The crude product (brownish black) (0.2 g), showed on TLC (silica gel, benzene + acetone 7:3) a spot (R_f value 0.1) corresponding to 1,5-dimethoxyanthraquinone, and another one with R_f value 0.8, which was probably 1-hydroxy-5-methoxyanthraquinone. There was no spot corresponding to 1,5-dihydroxyanthraquinone (R_f value 0.2). The crude product gave a positive (greenish black colour) ferric chloride reaction.

18. Demethylation of 1,8-dimethoxyanthraquinone

1,8-Dimethoxyanthraquinone (0.25 g) was taken in dry methylene chloride (5 ml) and the reagent (HBr/HAc)(1 ml) was added to it. The red coloured reaction mixture was kept shaking for 18 hours and worked up as in (13). The reaction product (0.21 g) showed on TLC (silica gel, benzene + acetone; 7:3), two spots (R_f values 0.82 and 0.7). The slow moving spot was corresponding to that of 1,8-dimethoxyanthraquinone. The fast moving spot was probably due to 1-hydroxy-8-methoxy-anthraquinone. There was no spot corresponding to 1,8-dihydroxyanthraquinone (R_f value 0.2).

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SUMMARY

Part I. Constitution of laccaic acid A.

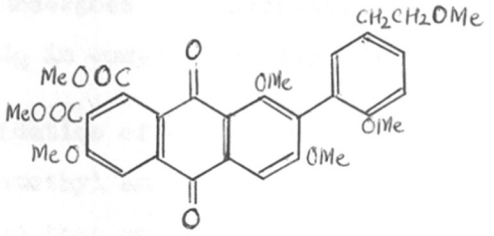
Lac pigments occur in the resinous material called stick lac secreted by the insect, Laccifer lacca, which thrives on certain Indian trees. The water-soluble lac pigments can be separated into laccaic acids A, B, C and D by chromatography on polyamide powder using butanol saturated with 0.2 N hydrochloric acid, a method developed by I.N. Shaikh from this laboratory.

Laccaic acid A crystallizes from water or methanol in bright red needles. The molecular formula is $C_{26}H_{19}NO_{12}$. The UV-visible spectra of laccaic acid A and the product of its reduction by alkaline dithionite show the presence of an anthraquinone chromophore, probably in conjugation with an aromatic ring. Laccaic acid A gives colour reactions characteristic of purpurin (1,2,4-trihydroxyanthraquinone). The IR spectrum shows bands for chelated quinone carbonyls, carboxyl groups, and an additional carbonyl group. On treatment with aqueous alkaline sodium dithionite, laccaic acid A underwent the purpurin → xanthopurpurin reduction to form xantholaccaic acid A. The same reduction can also be accomplished by the hydrogenation of laccaic acid A in aqueous alkali, in presence of palladized charcoal.

On methylation with dimethyl sulphate and anhydrous potassium carbonate in boiling acetone, laccaic acid A gave a crystalline product, MLA III. From the molecular weight (589, mass spectrum) and the elemental analysis, the molecular formula for MLA III was assigned as $C_{31}H_{27}NO_{11}$. The IR spectrum shows bands for ester and quinone carbonyl groups, as well as an -NH- function. The NMR spectrum of MLA III shows the presence of a C-methyl group, five methoxyl groups, one of which appears at unusually low field (5.67), two methylene groups which are adjacent to each other, and an NH proton. One of the methylene groups is benzylic and the other attached to an N-C(=O)- group. The aromatic region shows the presence of the anthraquinone nucleus and the other three exhibit a typical ABC pattern in a benzene ring. Alkaline hydrolysis of MLA III gave a dibasic acid, indicating the presence of two carbomethoxyl groups in MLA III. From the remaining three methoxyl groups, two were found to be α-methoxyls, as they were demethylated on treatment with hydrogen bromide in glacial acetic acid at room temperature. It is found that hydrogen bromide in glacial acetic acid at room temperature attacks only α-methoxyl and not β-methoxyl groups in anthraquinone.

On methylation with methyl iodide and silver oxide in dimethylformamide at room temperature, xantholaccaic acid A

was converted into a yellow crystalline compound, MXLA III. The molecular weight by mass spectrum was found to be 605 and the molecular formula $C_{32}H_{31}NO_{11}$. The IR spectrum shows bands for ester and quinone carbonyls an -NH- group, probably as an amide. Kuhn-Roth estimation indicated the presence of a C-methyl group. The NMR spectrum of MXLA III shows the presence of a C-methyl group, six methoxyl groups, two methylene groups (Ar-CH_2 and N-CH_2) adjacent to each other and an NH proton. The aromatic region shows the presence of five aromatic protons; two are assigned to α-protons of the anthraquinone nucleus and the remaining three protons show an ABC pattern in a benzene ring corresponding to 1,2,4-substitution. MXLA III, on hydrolysis with methanolic potassium hydroxide, gave a compound which was soluble in aqueous sodium carbonate and could be reconverted to MXLA III by treatment with diazomethane in ether. The dibasic acid showed a phthalein test, similar to anthraquinone-1,2-dicarboxylic acid. On treatment with boron trifluoride etherate and acetic anhydride at room temperature (a reaction, the significance of which is discussed in Part III), MXLA III gave a product, which gave a positive ferric chloride test and showed in its NMR spectrum the presence of five methoxyl groups. The absence of an acetoxyl signal indicated that there is no aliphatic methoxyl group in MXLA III, unlike MXLA I(Ia),



Ia

a derivative of nitrogen-free laccaic acid. A signal for one methoxyl group which comes at high-field (6.48) in MXLA III is absent in the demethylated product. The demethylation experiment showed that this high field signal in the NMR spectrum of MXLA III is due to an α-methoxyl group which is subject to some kind of shielding influence.

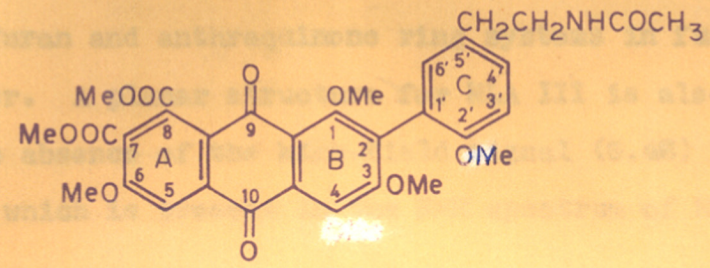
Taking into account an additional band for a carbonyl group in the IR spectrum of laccaic acid A and the signals for $ArCH_2\underline{CH_2}$-, $\underline{NH}$, and $\underline{C}$-CH_3 groups in the NMR spectra of MXLA III and MLA III, it was concluded that these compounds contain an $ArCH_2CH_2NHCOCH_3$ group. This is further supported by the NMR spectrum of N-acetyl-β-phenylethylamine (Varian NMR Catalogue, Sp. No. 265), which has a resemblance to the spectra of MLA III and MXLA III, as far as the signals for $ArCH_2\underline{CH_2}\underline{NH}COCH_3$ are concerned. The correct acetyl value was obtained only under drastic conditions. During the course of this study it was found that the NH proton in N-acetyl-β-phenylethylamine and its

derivatives undergoes a diamagnetic shift of more than 1 ppm in $CDCl_3$ in comparison with the value in CCl_4.

Oxidation of MLA III with dilute nitric acid yielded tetramethyl anisole-2,3,4,5-tetracarboxylate. This clearly showed that one of the rings of the anthraquinone nucleus of laccaic acid A and its derivatives carries two carboxyl groups and a hydroxyl adjacent to one another.

Taking into account (a) the purpurin → xanthopurpurin reduction shown by laccaic acid A, (b) the oxidative degradation of MLA III, (c) three aromatic protons showing an ABC pattern and an $ArCH_2CH_2NHCOCH_3$ group shown in the NMR spectra of MLA III and MXLA III, and (d) the absence of a signal for a β-proton of anthraquinone in the NMR spectrum of MXLA III, the structure (I) is assigned to MXLA III.

Although MLA III and MXLA III are derived from the same purpurin derivative, MXLA III contains one methoxyl group more than MLA III, and not less as one would expect from a normal purpurin → xanthopurpurin change. It has been established that during methylation with dimethyl sulphate and potassium carbonate in acetone, laccaic acid A undergoes a cyclization, which involves displacement of a β-methoxyl group from the anthraquinone nucleus by the phenolate ion from the attached phenyl ring, resulting in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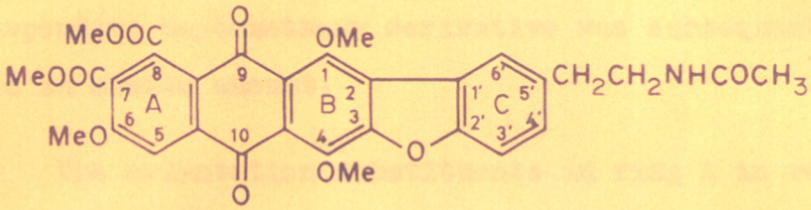
O

I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OMe
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B
C
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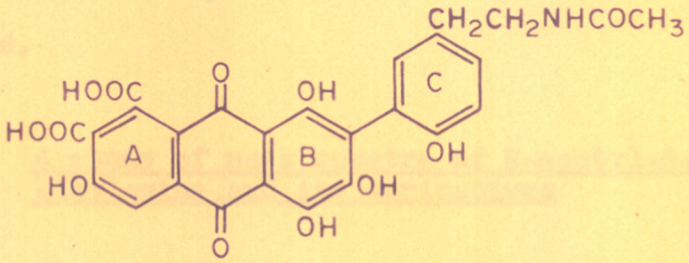
O
O

II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HOOC
HOOC
HO
OH
OH
OH
A
B
C
O
O

III

the formation of a furan structure. Thus MLA III contained benzofuran and anthraquinone ring systems in fusion with one another. A planar structure for MLA III is also supported by the absence of the high-field signal (6.48) for a methoxyl group which is present in the NMR spectrum of MXLA III.

Based on the above evidence, structures II and III are assigned to MLA III and laccaic acid A. After careful chromatography of the methylation product of laccaic acid A, a corresponding heptamethoxy derivative was subsequently obtained in minute amount.

The orientation of substituents in ring A in relation to those in ring B is based on biogenetic grounds.

The mass spectra of MLA III and MXLA III are discussed.

Part II. A study of mass spectra of N-acetyl-β-phenylethylamine and its derivatives

The mass spectra of N-acetyl-β-phenylethylamine and its derivatives were studied and it was found that the formation of an M-59 ion peak, by the loss of neutral acetamide molecule is a major mode of fragmentation. A probable mechanism for the process operating in this fragmentation is suggested.

Part III. Studies in demethylation of methoxyanthraquinones

Boron trifluoride etherate and acetic anhydride, and hydrogen bromide in glacial acetic acid, were found to be useful as demethylating agents in structural elucidation. Several methoxyanthraquinones were subjected to the action of these reagents and it was found that only α-methoxyls are affected. Boron trifluoride etherate at room temperature demethylates one α-methoxyl group preferentially, and the attack on a second α-methoxyl group is very slow. In no case, the demethylation was followed by acetylation.

A probable mechanism of the demethylation is discussed.

ACKNOWLEDGMENT

I am greatly indebted to Professor K. Venkataraman, National Chemical Laboratory, Poona, for suggesting the problem and guidance during the progress of the work.

My thanks are due to Drs. P.M. Nair, A.V. Rama Rao, C.I. Jose and K.G. Das, for fruitful discussions. Thanks are also due to Mr. I.N. Shaikh for sincere cooperation during the work.

I am also thankful to Professor B. D. Tilak, Director, National Chemical Laboratory, Poona, for allowing me to submit this work in the form of a thesis and for his interest in the progress of the work.

E. D. Pandhare

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