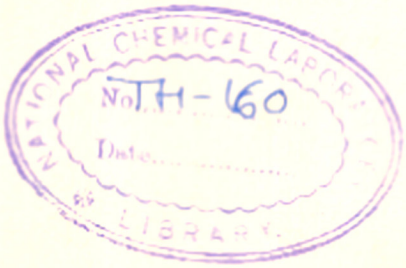

NATURALLY OCCURRING 2,2-DIMETHYLCHROMENES AND A NEW TYPE OF FLAVONOID PIGMENT FROM *GARCINIA MORELLA*

A THESIS

SUBMITTED BY

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Statement required to be submitted under Rule 0.413 of the University of Bombay

No part of this thesis has been submitted for a degree or diploma or other academic award. The literature concerning the problems investigated has been surveyed, and all the necessary references are given in the thesis. The present work has been clearly indicated separately. The experimental work has been carried out entirely by me. In accordance with the usual practice, due acknowledgement has been made wherever the work presented is based on the results of other workers.

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CANDIDATE

Poona, May 1967

K Venkataraman

(K. Venkataraman)
RESEARCH GUIDE

C O N T E N T S

PART I

PIGMENTS OF GARCINIA SPECIES

(A) PIGMENTS OF GAMBOGE

INTRODUCTION

The dried latex of different species of Garcinia is commonly known as gamboge. The latex is obtained by making incisions in the lower parts of the stems and collecting the exudate in bamboo tubes. This gamboge was used in mixtures for its mild laxative action. It was also observed that the gamboge of Garcinia hanburryi had the capacity to damage sarcoma 37.[1]

Gambogic acid is the principal acidic component of the pigment gamboge[2], which has been a subject of analytical and chemical study for over hundred and fifty years[3]. It was shown that most of the acidic components of the gamboge were quantitatively precipitated from the ether solution by saturating it with ammonia[4]. Pure gambogic acid is a yellow, optically active acidic resin which could not be crystallized. It was Furer[5], who first obtained a crystalline acetate of gambogic acid, by acetylating the acidic fraction of the gamboge. Gambogic acid was however easily characterized as a crystalline pyridine salt, m.p. 147° and a dimethyl derivative, m.p. 129° [6-7].

β-Guttiferrin[8], the acidic component of the gamboge of Garcinia morella gave a crystalline

pyridine salt, m.p. 148° and dimethyl derivative, m.p. 128°-129°. These properties of β-guttiferrin indicated its identity with gambogic acid.

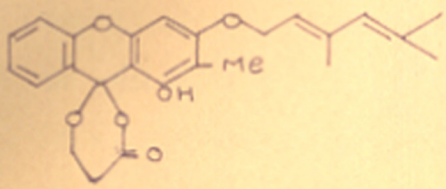
A number of molecular formulae were proposed for gambogic acid. The molecular formula suggested on the basis of the results of X-ray crystallographic examination[8] of the two derivatives of gambogic acid was later found to be incorrect[9-10]. The revised molecular formula ($C_{38}H_{44}O_8$) was arrived at from the equivalent weight of gambogic acid determined potentiometrically, the analytical data and also from the proton integrals corresponding to the NMR spectra of its crystalline derivatives. The potentiometric titrations also revealed that it was a monocarboxylic acid.

It was observed that gambogic acid absorbed five mols of hydrogen. Its octahydro-derivative is a crystalline compound, m.p. 144-145° [9] gambogic acid takes up a molecule of methanol in the Michael addition reaction. Potassium hydroxide fusion[4] of gambogic acid for 20 minutes at 220° gave, n-butyric acid, isovaleric acid, methyl succinic acid, homophthalic acid, phloroglucinol and 6-methyl-5-heptane-2-ol. Pure gambogic acid on heating with 10% ethanolic potassium hydroxide for two minutes and cooling gave a di-potassium salt[4-11]. This compound with two carboxyl

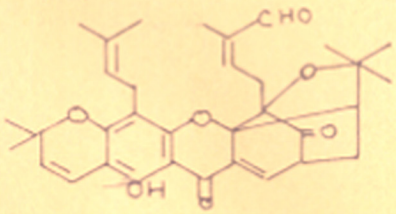
groups was called garciniolic acid. Initially gambogic acid was represented by the structure (I) corresponding to the molecular formula $C_{29}H_{34}O_6$. This was unacceptable as it did not account for the acidity, colour and stability of gambogic acid. Later Dyson and Rigby[12] referred to the molecular formula $C_{38}H_{44}O_8$ for gambogic acid on the basis of their unpublished work.

It was around this time that an important paper appeared on the structure of morellin[13], a pigment of Garcinia morella, the structure of which was established on the basis of chemical, IR, UV, NMR and X-ray crystallographic data. It was now clear that morellin (II; $C_{33}H_{36}O_7$) and gambogic acid ($C_{38}H_{44}O_8$) were very closely related.

The suspicion that gambogic acid and morellin contained the same skeleton (III) was fully confirmed by a comparison of the NMR spectra of gambogic acid and its derivatives with those of morellin, and the signals could be assigned with certainty to all the protons indicated in the structure (IV) suggested by P. Yates et al.[9] for gambogic acid. The NMR spectrum of gambogic acid (Fig. 6) showed the presence of an additional isoprene unit. This additional isoprene unit was placed on one of the methyl groups of the 2,2-dimethylchromene system of the molecule. This



I



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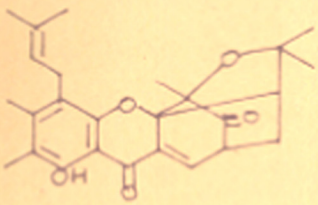

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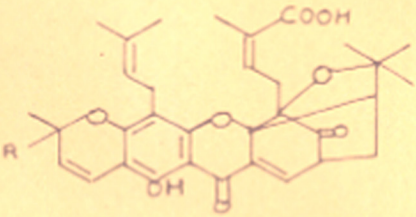
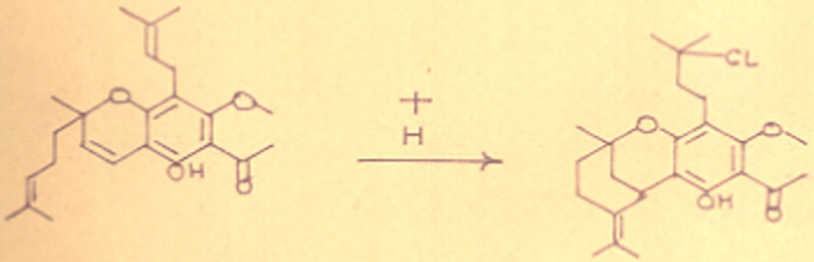
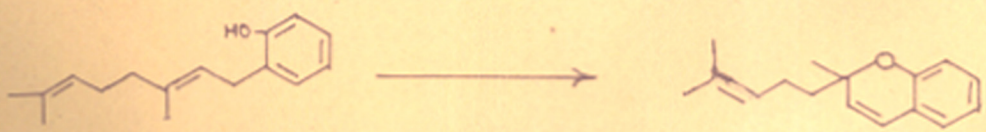


IV

IV R = $Me_2C=CH-CH_2-CH_2-$



V VI



VII VIII

observation was confirmed by the acid catalyzed cyclisation of the side-chain of gambogic acid (V) to gamboginic acid (VI)[10]. In this cyclization there is a loss of three vinyl protons. The alkylation of phenols by isopentenyl pyrophosphate to give ortho-isopentenyl phenols and the oxidative transformation of these to 2,2-dimethylchromenes are plausible biosynthetic processes[14]. It could therefore be expected that natural phenolic compounds such as represented by partial structure (VII) and (VIII) which contain C_{10} substituents could be similarly derived biosynthetically from geranyl pyrophosphate. Several such compounds are known including geranyl ethers like Collinin[15], 5-geranyloxy 7-methoxycoumarin[16] and bergamottin[17]. No natural product has been described previously containing the 2-methyl-2-(4-methylpent-3-enyl)-chromene (VIII) residue.

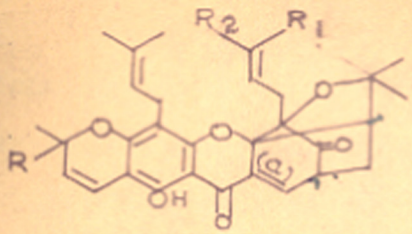
Regarding the stereochemistry of the side-chain carrying the carboxyl group the earlier workers[9-10] were unable to come to any definite conclusion.

PRESENT WORK

With the accumulation of NMR data on morellin and allied pigments like desoxymorellin (IX)[18], isomorellin (X)[19] and dihydroisomorellin (XI)[18] (single bond at a) isolated from the seed coat of Garcinia morella, it was shown that morellin and isomorellin were cis-trans isomers, the methyl and the methylene groups of the aldehyde side chain having the trans configuration around the double bond in morellin and the cis configuration in isomorellin[19].

α-Gambogic acid was isolated by Furrer[5] in the form of its O-acetyl derivative from the dry latex of G. morella. Yates et al[9] obtained acetyl α-gambogic acid from the same source. The gross structure suggested by Yates et al[9] for gambogic acid was confirmed by Ollis et al[10], mainly as the result of the acid catalysed cyclisation of the side-chain (CH_2-CH_2-CH=CMe_2) of the 2,2-dimethylchromene with the chromene ring to form gamboginic acid. Their source of gambogic acid was G. hanburryi, formerly regarded as a variety of G. morella.

As part of our study of the pigments of G.morella, the latex obtained from Mangalore in South India was examined. The latex was dark brownish-yellow in colour, and sticky in nature. This was subjected to



IX Desoxymorellin ; $R = R_1 = R_2 = -CH_3$

X Isomorellin ; $R = CH_3$, $R_1 = CH_3$, $R_2 = CHO$

XI Dihydroisomorellin ; $R = CH_3$, $R_1 = CH_3$, $R_2 = CHO$, single bond at (a)

XII Morellic acid ; $R = CH_3$, $R_1 = COOH$, $R_2 = CH_3$

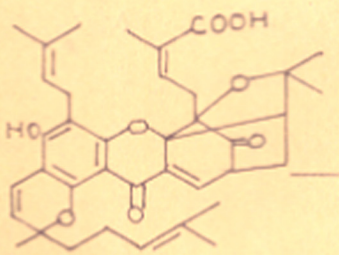
XIII Isomorellic acid ; $R = CH_3$, $R_1 = CH_3$, $R_2 = COOH$

XIV Gambogic acid ; $R = CH_2 - CH_2 - CH = C(CH_3)_2$

$R_1 = COOH$, $R_2 = CH_3$

XV Isogambogic acid ; $R = CH_2 - CH_2 - CH = C(CH_3)_2$

$R_1 = CH_3$, $R_2 = COOH$



→ allogambogic acid

XVI

functional separation. The gamboge did not give an appreciable amount of bicarbonate soluble fraction. About 70% of the gamboge was carbonate soluble. The crude carbonate soluble extract on acidification with dilute sulphuric acid gave an amorphous yellowish solid. Expecting this to be mostly gambogic acid the amorphous yellow solid was dissolved in pyridine, the pyridine solution was diluted with a few drops of water, but it was not possible to get a crystalline pyridine salt. Using similar conditions gambogic acid very readily gave a pyridine salt m.p. 148°. This observation led us to investigate further the pigments of this gamboge.

Thin layer chromatography (TLC) on silica-gel (benzene: ethyl acetate 9:1) of these pigments further confirmed the earlier observation about the total absence of gambogic acid in the gamboge of G. morella, that we were examining. This gamboge instead revealed the presence of two new pigments. The vulnerability of most of the pigments occurring in G. morella to chromatographic separation on silica-gel and other adsorbents, leading to their isomerization prompted us to avoid the use of any chromatographic procedure for the separation of these pigments.

It was observed that one of the two pigments

occurring in the carbonate soluble fraction of the gamboge was slightly more soluble than the other in cold hexane. This observation was very useful for the separation of these two pigments from each other, without the use of chromatographic technique. The hexane soluble fraction was named isomorellic acid and the hexane insoluble component morellic acid. The nomenclature of these two substances was based on the later observations made on their structures.

They were both amorphous and resisted crystallization from most of the solvents. As mentioned earlier they did not give a crystalline pyridine salt like gambogic acid, when subjected to similar treatment. Both gave a dark green ferric colour, which indicated a chelated hydroxyl group. The presence of a carboxyl group, indicated by the solubility in carbonate was confirmed by the iodide-iodate test, although neither of the acids (as well as gambogic acid) responded to the hydroxamic acid test.

On heating with acetic anhydride and fused sodium acetate these acids gave acetyl derivatives which crystallized from methanol in yellow plates m.p. 240° and 174° corresponding to morellic and isomorellic acids respectively. It could be mentioned here that during the acetylation of morellic acid a part of it was converted to acetyl isomorellic acid.

Acetyl estimation showed the presence of only one acetyl group in each case. Both the acids gave crystalline dimethyl derivatives, m.p. 160° and 154° corresponding to morellic and isomorellic acids respectively. It was also observed that the acetyl derivative of isomorellic acid was more soluble in methanol than the acetate of morellic acid. Elemental analysis and mass spectral molecular weights led to the same molecular formula $C_{35}H_{38}O_9$ for both the acetates.

Morellin can be isomerised to isomorellin by running a benzene solution through a column of silica gel or fuller's earth, or by treatment at room temperature with traces of hydrochloric acid in acetone. By the same procedure morellic acid was converted to isomorellic acid, showing that morellic acid and isomorellic acids were probably related to each other in the same way as morellin and isomorellin.

The infrared spectra of the acetyl derivatives (Figs. 1 and 2) of these two acids were very much similar and showed four bands in the carbonyl region.

Morellic acid (acetate)	Isomorellic acid (acetate)
1760	1770
1730	1735
1700	1705
1640	1640

The band at ~1760 could be assigned to the carbonyl of the acetate (1760, 1770) and that at ~1730 to the carbonyl of the bycyclooctenone ring. The carbonyl due to the carboxyl group could be easily assigned to the band at 1700, [redacted] and the band at (1635, [redacted] to the carbonyl of the chromanone.

Morellic and isomorellic acids absorbed four mols of hydrogen in presence of 10% palladium charcoal in alcohol indicating the presence of four double bonds.

The NMR spectra* of the acetates of morellic and isomorellic acids (Figs. 3 and 4) in comparison with those of morellin and isomorellin readily showed that morellic and isomorellic acids are carboxylic acids (XII) and (XIII) respectively corresponding to morellin and isomorellin. In the spectra of the two acids the aldehyde signals of morellin and isomorellin were replaced by broad hydroxyl signals at 1.35 and 1.42 The NMR spectrum of both the acetates showed a total proton count of 38.

In the vinyl region there were three doublets at 4.33, 3.59 and 2.5. The positions of the vinyl doublets in the spectra of morellic and isomorellic acids were identical. The correspondence between the two pairs (morellin, isomorellin and morellic and isomorellic acids) of spectrawas very close.

Seven C-methyl groups, a 2,2-dimethylchromene

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*Chemical shifts are cited on τ scale.

system, a prenyl side-chain attached to an aromatic ring and the bicyclooctenone part of the morellin skeleton were all readily recognizable from the NMR spectra of morellic and isomorellic acids.

The vinyl proton (H_v) of the α,β-unsaturated carboxylic acid side-chain of morellic acid acetate appeared at 3.85 and the corresponding absorption of the isomorellic acid acetate was at 3.25, the downfield shift indicating the isomerization of the carboxyl group from trans to cis configuration as discussed earlier in connection with morellin and isomorellin.

Ollis et al[10] have stated that "the stereochemistry of the side-chain" carrying the carboxyl group "is probably as shown (IV: R_1 = Me R_2 = COOH) but the NMR results are not conclusive". Yates et al[9] have not presented the NMR spectrum of acetyl α-gambogic acid and have made no reference in their discussion to the chemical shift of the vinyl proton (H_v). Ollis et al[10] did not examine the acetyl α-gambogic acid, but they recorded the NMR spectra of the pyridine salt of gambogic acid, dimethyl gambogate (the ether-ester) and monomethyl gamboginate; H_v appeared at 3.88, 4.02 and 4.12 respectively in the three compounds.

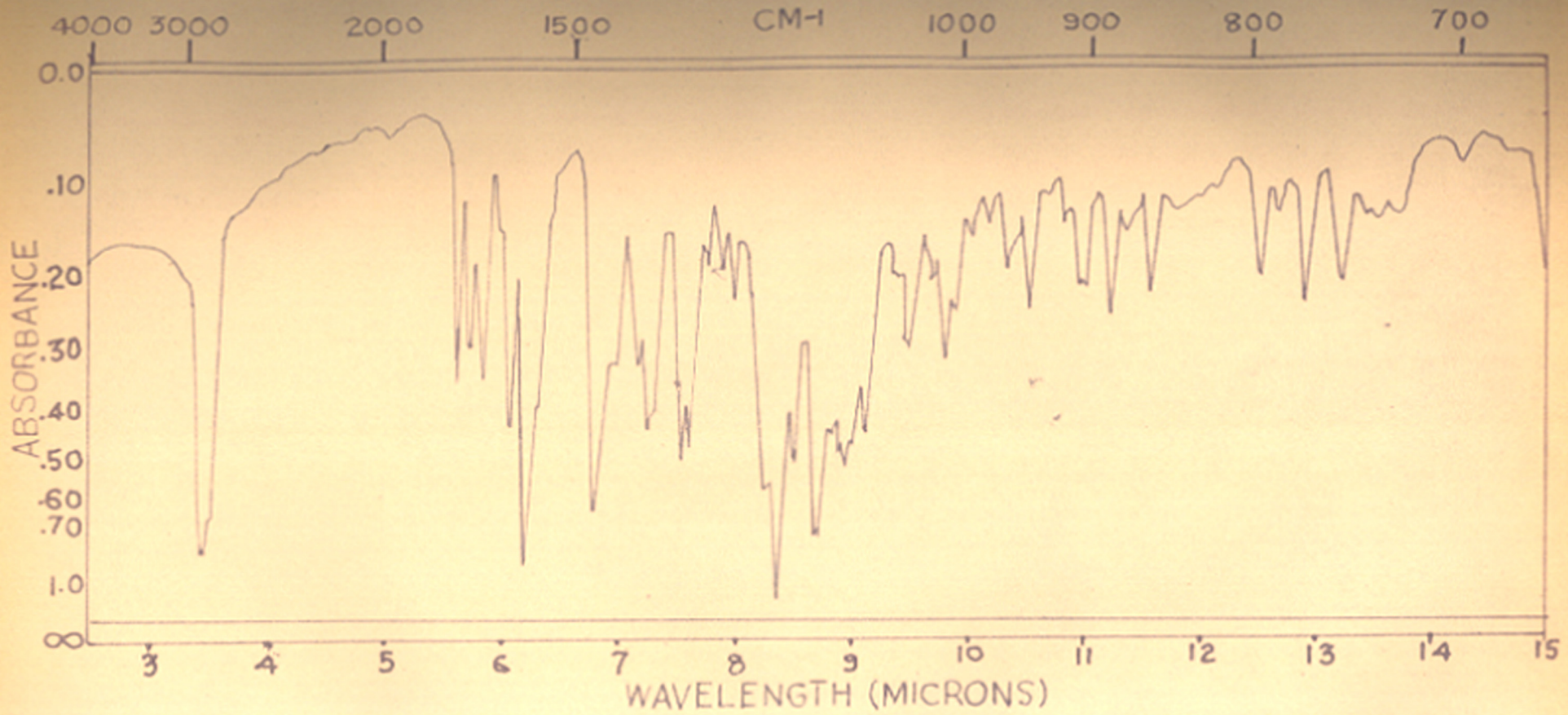
We have examined the NMR spectrum of gambogic acid (Fig. 6) kindly supplied by Professor H. Auterhoff

who prepared it through the pyridine salt from a commercial 'Harzdrog', which was believed to originate from G. morella. In the spectrum of gambogic acid H_v appeared at 3.88 and in the light of the data cited for morellins and the morellic acids it may be concluded that the carboxyl or the carbomethoxyl and H_v have the trans configuration in gambogic acid, dimethyl gambogate and methyl gamboginate; gambogic acid therefore corresponds to morellic acid. In the NMR spectrum of the acetyl derivative of isogambogic acid (Fig. 7) (which we prepared from the gamboge of G. hanburryi procured from Bangkok) H_v appeared at 3.35 , from which it follows that the acetate is derived from isogambogic acid corresponding to isomorellic acid. Isomerization from the unstable angelic to the more stable tiglic acid configuration takes place during the treatment with sodium acetate and acetic anhydride. To confirm this observation about the stereochemistry of the side-chain carrying carboxyl group a number of derivatives like the ester of the acetyl isogambogic acid and monomethyl isogamboginate were prepared and their NMR spectra recorded.

In a more recent paper on the constitution of gambogic acid by Rigby et al.[20], it has been reported that when gambogic acid was boiled in pyridine or

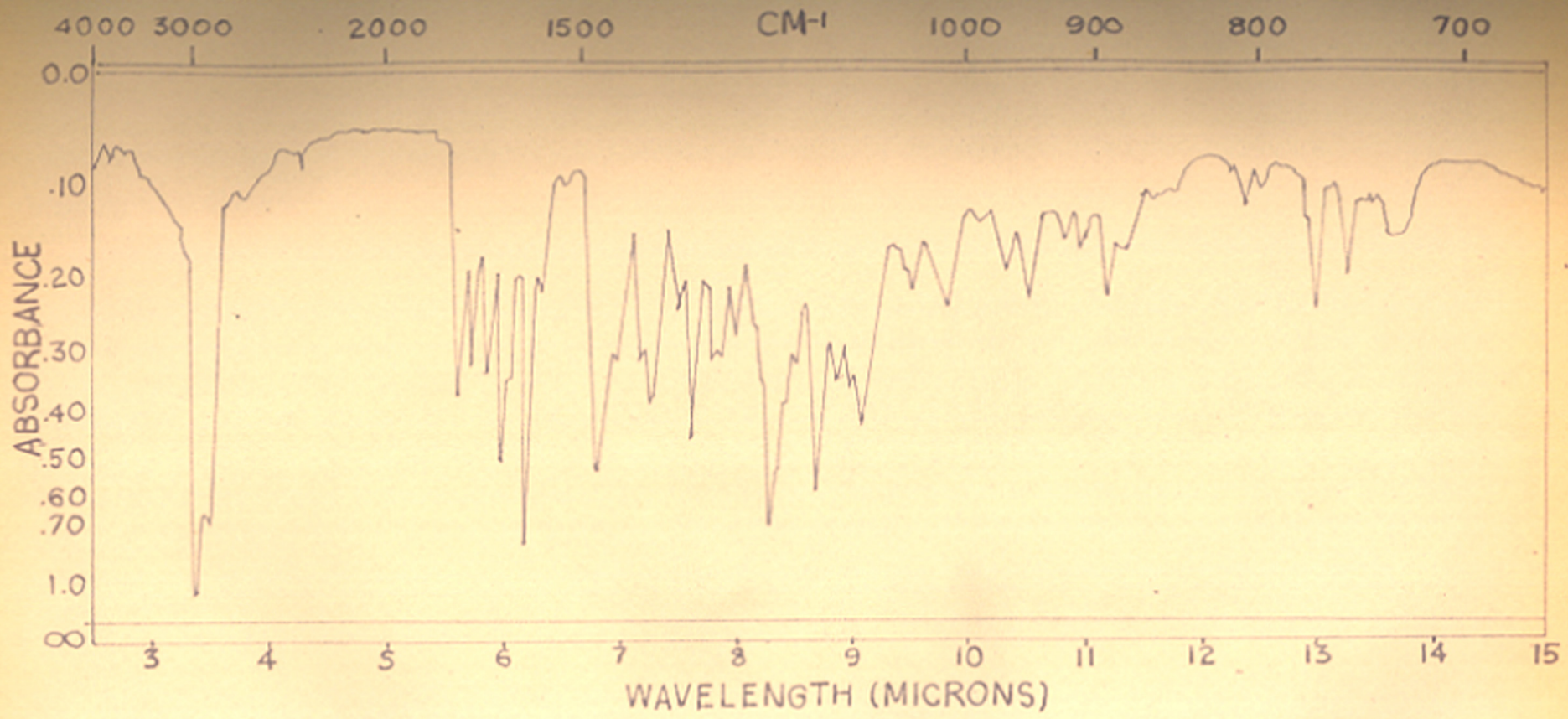
toluene an equilibrium was set up between approximately equal amounts of gambogic acid and a second, apparently very similar substance to which they have referred to as allogambogic acid. In the NMR spectrum of the latter they have observed a slight down-field shift of 2.5 c/sec. from 4.67 and a shift of the vinyl triplet representing the vinyl proton of the carboxyl side-chain by 6 c/sec. (3.88 to 3.25). They were unable to isolate the "allogambogic acid" in pure form. It has been suggested by these workers that the acetate of the gambogic acid m.p. 204° does not have the structure of gambogic acid but that of allogambogic acid. Rigby[20] also feels that the new substance is not formed by cis-trans isomerization at the double bond α,β to the carboxyl group. The isomerisation presumably involves the opening of the chromene ring with reclosure at the chelated hydroxyl, the analogous reaction occurring in rottlerin to give isorottlerin. These workers have also prepared an acetate of gambogic acid under mild conditions m.p. 145°. This acetate they could not convert to the acetate m.p. 204° by boiling with pyridine and hence suggested structure (XVI). Assuming that the acetate 204° of gambogic acid has the allogambogic acid structure, the allogambogic acid obtained by the treatment of this acetate with dilute ammonia to

hydrolyse the acetyl group should not show any ferric colour as there is no chelated hydroxyl group in the allogambogic acid structure, but surprisingly enough it showed a dark bluish-green ferric colour. The presence of the chelated hydroxyl group in the hydrolysed product was confirmed by NMR. Moreover these workers did not isolate the allogambogic acid, and the slight shift reported of one of the chromene doublets is very negligible. On the contrary the shift of the vinyl triplet of the carboxyl side-chain is appreciable and is in agreement with the structure suggested here for acetate of isogambogic acid.



IR spectrum of acetyl morellic acid in Nujol

FIG 1



IR spectrum of acetyl isomorellic acid in NUJol

FIG 2

NMR spectrum of acetyl morellic acid

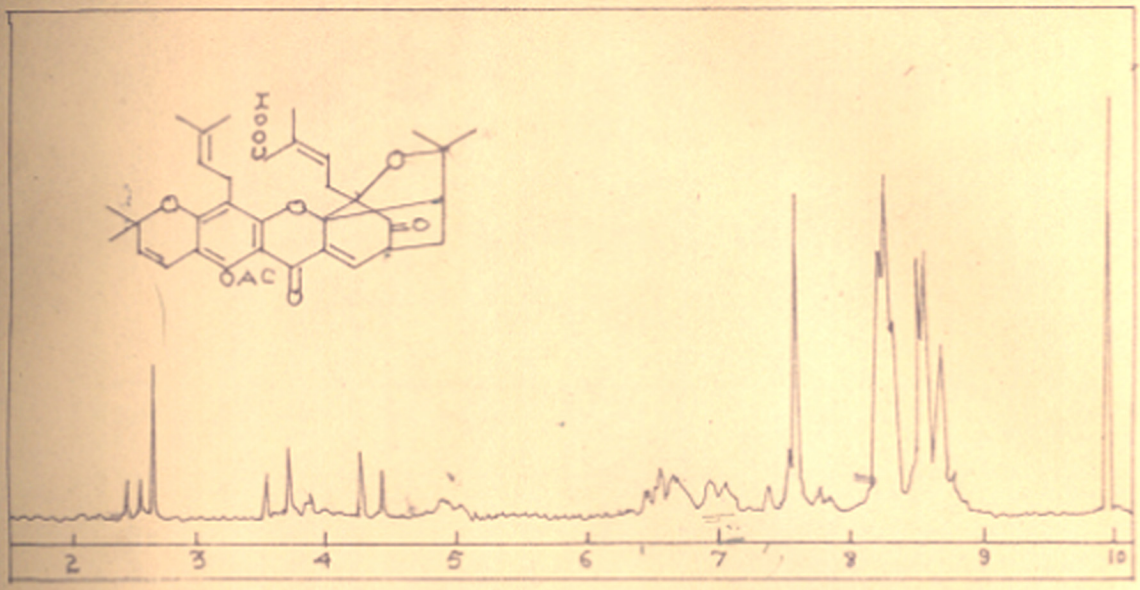


FIG 3 ($CDCl_3$)

NMR spectrum of acetyl isomorellic acid

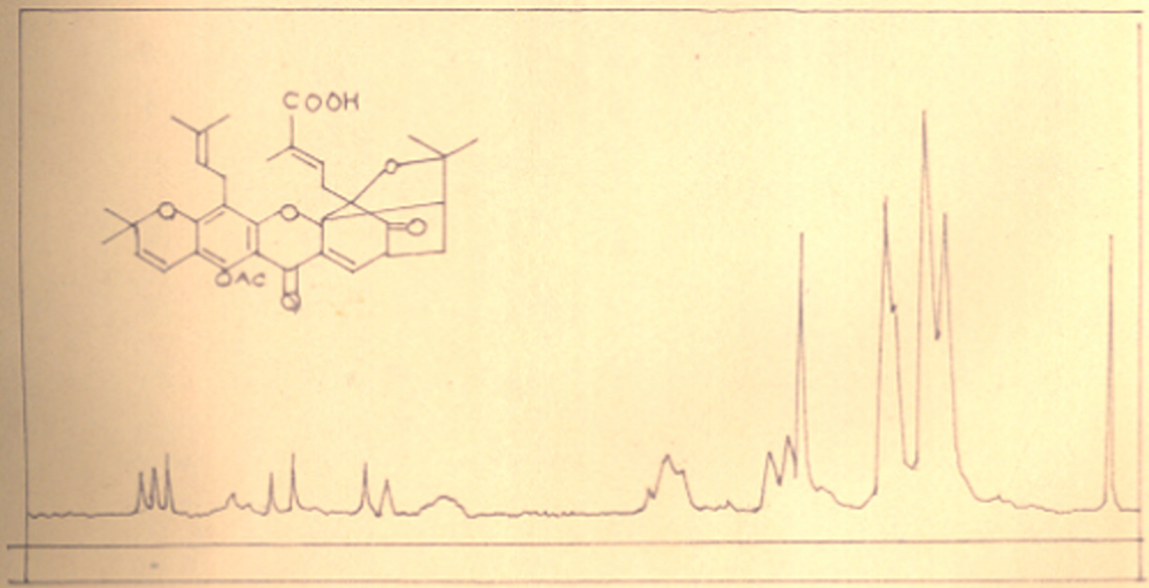


FIG 4 ($CDCl_3$)

NMR data on acetyl morellic acid Fig. 3

Solvent $CDCl_3$

Chemical shift	Multiplicity	No.of H	Assignment
8.7	s	3	Me on tertiary carbon atom
8.5	d = (J = 6 cps)	6	2 Me of the 2,2-dimethyl chromene system
8.33	s	3	Me on a tertiary carbon atom
8.28	s	6	2 Me of the prenyl chain
8.2	s	3	Me of the side-chain bearing the carboxyl group
7.6	s	3	-OCOMe
6.0-7.6	-	6	3 $-CH_2$
4.81	bs	1	Vinyl H of the prenyl chain
4.35	d = (J = 10 cps)	1	Vinyl H of the chromene ring
3.72	bs	1	Vinyl H of the side-chain bearing carboxyl group
3.59	d (J = 10 cps)	1	Benzylic as well as vinyl H
2.5	d (J = 8 cps)	1	Vinyl H of the bicyclo-octenone ring

NMR data on acetyl isomorellic acid Fig.4

Solvent = $CDCl_3$

Chemical shift	Multiplicity	No. of H	Assignment
8.77	s	6	2 Me on a tertiary carbon atom
8.64	s	6	2 Me on a tertiary carbon atom
8.45	s	3	Me of the side-chain bearing a carboxyl group
8.3	s	6	2 Me of the prenyl chain
7.62	s	3	-OCOMe
6.0 - 7.62	-	6	3 $-CH_2$
4.84	bs	1	Vinyl H of the prenyl chain
4.4	d (J = 10 cps)	1	Vinyl H of chromene ring
3.59	d (J = 10 cps)	1	Benzylic as well as vinylic proton of the chromene ring
3.25	s	1	Vinyl H of the side-chain bearing carboxyl group
2.5	d (J = 8 cps)	1	Vinyl H of the bicyclo-octenone ring

NMR spectrum of gambogic acid

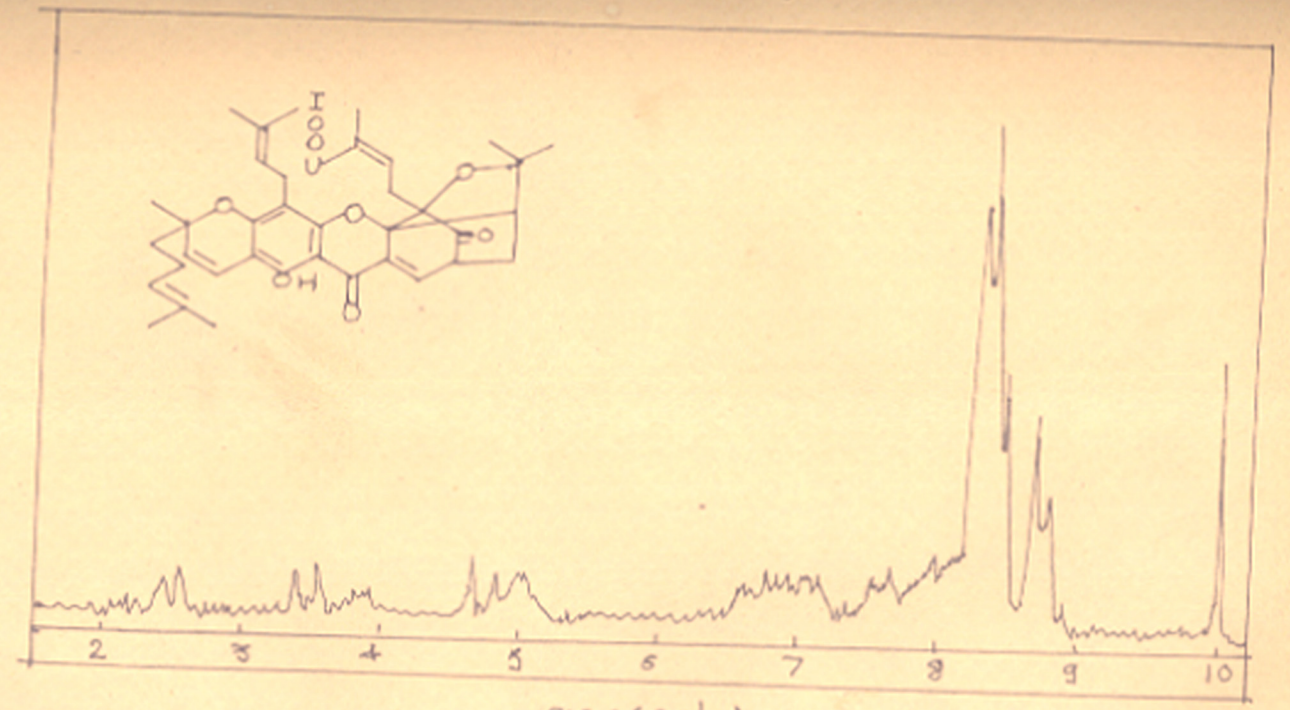


FIG 6(CCl_4)

NMR spectrum of acetyl isogambogic acid

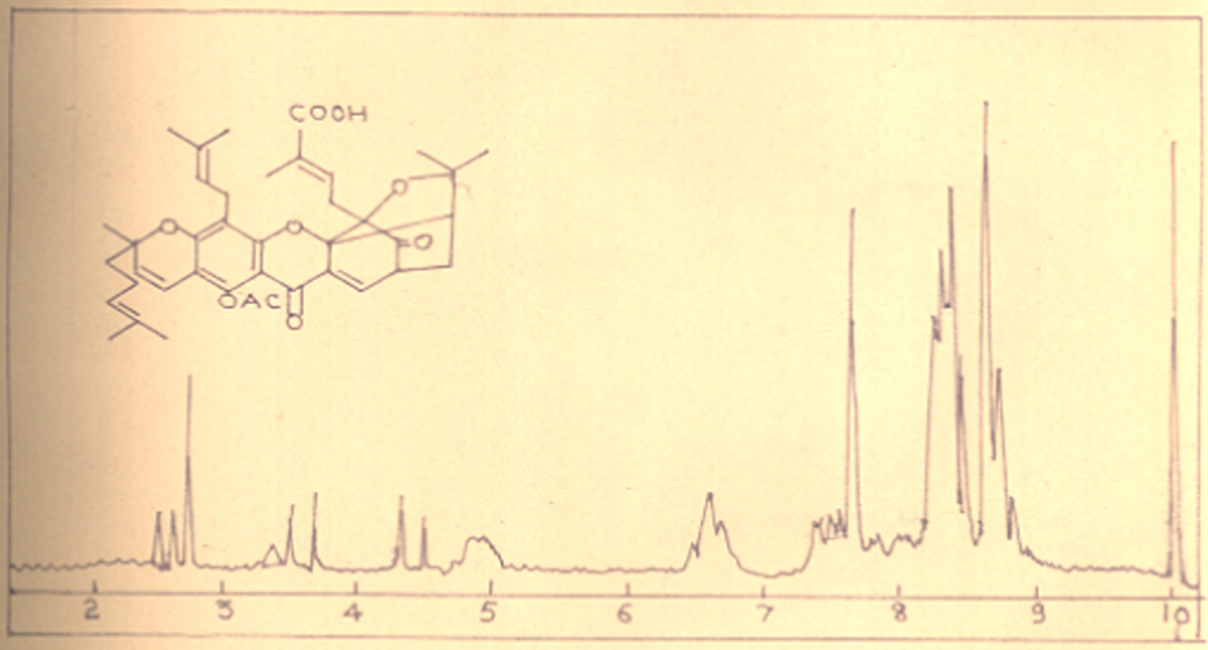


FIG 7 ($CDCl_3$)

NMR data on gambogic acid Fig. 6

Solvent = CCl_4

Chemical shift	Multiplicity	No. of H	Assignment
8.8	d (J = 7 cps)	6	8 - Me's of prenyl chains and on carbon atoms attached to oxygen
8.64	s	3	
8.42	s	9	
8.3	s	6	
6.0 - 8.2	-	12	6 $-CH_2$
5.0	bs	2	Vinyl H_s of two prenyl chains
4.82	d (J = 10 cps)	1	Vinyl H of chrome ring
3.84	bs	1	Vinyl H of the side-chain bearing carboxyl group
3.54	d (J = 10 cps)	1	Benzylic as well as vinylic H of chromene ring
2.5	d (J = 8 cps	1	Vinyl H of the bicyclo-octenone ring

NMR data on acetyl isogambogic acid Fig. 7

Solvent = $CDCl_3$

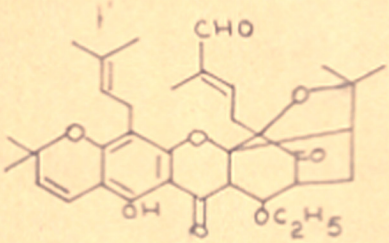
8.9	s	3	8 Me's of prenyl chains and on tertiary carbon atoms
8.82	s	6	
8.54	s	3	
8.42	s	6	
8.34	s	3	
8.2	s	3	
7.6	s	3	-OCOMe
6.0 - 8.2	-	12	6 $-CH_2-$
4.92	bs	2	Two vinyl H of prenyl chain
4.42	d (J = 10 cps)	1	Vinyl H of chromene ring
3.54	d (J = 10 cps)	1	benzylic as well as vinylic H of chromene ring
3.35	bs	1	Vinyl H of the side-chain bearing carboxyl group
2.5	d (J = 8 cps)	1	Vinyl of the bicyclo-octenone ring

PART I

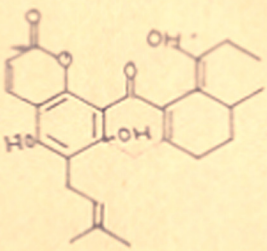
(B) THE PIGMENTS OF THE PERICARP OF GARCINIA MORELLA

INTRODUCTION

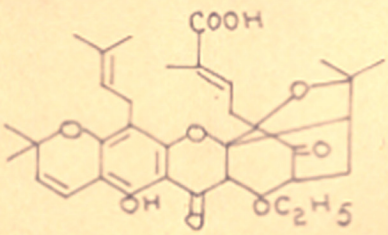
The mother liquor after the crystallization of morellin from the ethanol extract of the colouring matters of the pericarp of the seeds of Garcinia morella yields an orange yellow resinous residue on evaporation. By chromatography of the hexane soluble fraction of the residue on a column of Florex XXX (an extruded Fullers' earth) and development with hexane Bringi et al.[21] obtained from the lower deep orange band a crystalline pigment name desoxymorellin. It was further observed that continued elution of the column with hexane two additional pigments, ethoxy-dihydroisomorellin (m.p. 143°) and dihydroisomorellin[18] were obtained. On the basis of the structure (II) for morellin, chemical and NMR evidence it was possible to assign structures IX, XI and XVII to desoxymorellin, dihydroisomorellin and ethoxydihydro-isomorellin respectively. In addition to these pigments guttiferrin[22] soluble in 10% aq. sodium carbonate forms the major fraction of the alcoholic extract of the seed coat. Guttiferrins are specifically active against Gram-positive bacteria and the bacteriostatic effect against Micrococcus pyrogenes var. aureus (MIC 0.1 - 1 mg/ml) is reversed by methionine[23] albeit to a lesser extent. Further they show similar cross



XVII



XVIII



XIX

reactions. The presence of blood serum on their antibacterial activity is less pronounced and experimental staphylococcal infections in mice are controlled by guttiferrins[23].

∝-Guttiferrin was separated from other guttiferrins, as a sparingly soluble orange yellow crystalline pyridine complex m.p. 115°-117° $C_{33}H_{38}O_8$ was the molecular formula assigned to ∝-guttiferrin[22]. β-Guttiferrin the major acidic component of the gamboge of Garcinia morella was shown to be identical with ∝-gambogic acid. It was observed by these workers that ∝-gambogic acid, ∝-guttiferrin and morellin were closely related, as regards their structures. This conclusion was based upon their similar chemical and biological properties.

P.L.N. Rao et al.[22] made the following observations regarding the chemical nature of ∝-guttiferrin. It was seen that ∝-guttiferrin (XVIII) resisted crystallization from most of the solvents and contained no methoxyl or free carboxylic groups. Its carbonate solubility indicated phenolic groups. They have also reported the presence of a tertiary hydroxyl and a lactone in ∝-guttiferrin. The presence of three double bonds was indicated by catalytic reduction of ∝-guttiferrin to give the corresponding hexahydroderivative. The alkali fusion of

α-guttiferrin gave phloroglucinol, methylheptenol, homophthalic acid and isovaleric acids which were also formed when morellin was subjected to similar treatment[24]. It was observed that α-guttiferrin did not react with diazoaminobenzene and did not couple with diazotized sulphanilic acid, thus indicating the presence of fully substituted phloroglucinol structure.

PRESENT WORK

The mother liquor after the crystallization of morellin from the ethanol extract of the colouring matters of the pericarp of the seeds Garcinia morella yields an orange yellow resinous residue (A) on evaporation. Bringi et al.[21] obtained a crystalline pigment, named desoxymorellin from (A) by chromatographic separation. Bhat et al.[18] observed that after the elution of the lower most deep orange band of desoxymorellin on a column of Florex XXX, continued elution of the column with hexane yielded two additional pigments, ethoxydihydroisomorellin and dihydroisomorellin.

Thin layer chromatographic (TLC) behaviour of(A) on silica-gel using benzene:ethyl acetate (9:1) as solvent, revealed the presence of two new pigments. These would be referred as (B) and (C) hereafter. The pigment (B) was moving faster than the pigment (C) with different solvent systems though both had very close Rf values.

It was observed that a fraction of (A) was soluble in 10% aqueous sodium carbonate. Hence (A) was dissolved in ether, the ether extract was washed repeatedly with 10% aqueous sodium carbonate to remove the carbonate soluble part from (A). The carbonate soluble portion was

acidified with dilute sulphuric acid and again extracted with ether. It was seen by TLC that this carbonate treatment had removed (B) and (C) from (A) and thus facilitated their separation from other pigments. The total carbonate soluble part of (A) was called as guttiferrins by P.L.N. Rao[22].

It was difficult to separate (B) from (C) on silica-gel column as they had very close Rf values with different solvent systems. Hence preparative layer chromatographic technique was made use of to separate them on silica gel using benzene:ethyl acetate (9:1) as solvent. For better separation a very small amount (about 70 mg) was loaded on every plate, which was given multiple runs. Both (B) and (C) were yellow in colour and amorphous in nature, (B) being paler of the two. Both gave a positive iodide-iodate test and resisted crystallization. However they gave crystalline methyl and acetyl derivatives. Methyl ether of (B) m.p. 155° gave needle shaped crystals while the acetate of (C) m.p. 174° was pale yellow in colour and had fine cubical crystalline nature.

As described earlier (B) was a pale yellow amorphous powder and did not crystallize.(B) gave a crystalline methyl ether, creamish yellow needles m.p. 155°. Repeated elemental analysis and molecular

weight of the crystalline methyl ether (626) by Rast method corresponded to the molecular formula $C_{37}H_{44}O_9$. The parent compound gave a green ferric colour. In the presence of palladium on charcoal it absorbed 3 mols of hydrogen. The hydrogenated derivative was not crystalline.

The IR spectrum of the dimethyl ether-ester of (B) did not show any absorption in the hydroxyl region showing the absence of a tertiary OH. There were four absorption bands in the double bond region (1740, 1735, 1685, 1605 cm^{-1}).

The NMR spectrum of the dimethyl ether-ester of (B) (Fig. 5) showed a total proton count of 44 and indicated the presence of eight methyl groups. Seven out of the eight C-methyl absorptions are almost exactly in the same place as in the spectrum of dihydroisomorellin. The additional methyl signal is a triplet (J = 6.5 cps) at 8.86. The triplet methyl absorption shows that this pigment has probably an ethyl group. There is no aldehyde signal in the spectrum of this compound. A 2,2-dimethylchromene system, a prenyl side-chain attached to aromatic ring and bicyclooctenone part of the morellin skeleton were all readily recognizable. The vinyl proton associated with the bicyclooctenone system of morellin is absent and a new single proton doublet appeared at 5.56 (J = 5 cps). The position

of the other four vinyl absorptions are close to isomorellin.

The presence of an ethyl group, the absence of the C_8-C_9 double bond and the other changes in the NMR spectrum of the methyl ether suggest that the pigment m.p. 155° may be the ethanol adduct of isomorellic acid, formed as an artefact in the course of the isolation procedure which involved prolonged treatment of the natural pigments with ethanol. This observation was confirmed by subjecting isomorellin and morellic acids to Michael addition which gave the pigment (B).

Based on these observations it is obvious that pigment (B) has the constitution similar to isomorellic acid but for the double bond in the bicyclooctenone ring which is replaced by an ethoxyl group and a hydrogen and has the structure (XIX).

The pigment (C) as mentioned earlier gave a green ferric colour and the iodide-iodate test for ~~the~~ a carboxylic acid. The positive ferric colour test indicated the presence of a chelated hydroxyl group. (C) gave a crystalline acetate m.p. 174° but did not give a crystalline pyridine salt. The α-guttiferrin as reported by P.L.N. Rao et al.[13] also gave a crystalline acetate m.p. 174° and hence it appears that the pigment (C) probably corresponds to α-guttiferrin.

Elemental analysis and molecular weight determination led to the molecular formula $C_{35}H_{38}O_9$ for the crystalline acetate of (C). Acetyl estimation showed that it was a monoacetate. The presence of a carboxyl group indicated by the carbonate solubility, the iodide-iodate test, was confirmed by the preparation of a methyl ether-ester. This fact was also confirmed by NMR.

Compound (C) absorbed four mols of hydrogen in presence of 10% palladium on carbon and not three mols as reported by earlier workers[22]. NMR studies of the crystalline acetate of (C) showed a total proton count of 38 and indicated the presence of four double bonds. This spectrum when compared with the spectrum of acetyl isomorellic acid[25] shows close resemblance. There are five vinyl protons including the chromene doublet and their positions are identical with the positions of the corresponding vinyl protons in the spectrum of acetyl isomorellic acid. The number of methyl groups (seven) and their respective positions are also identical. In addition to the NMR evidence the infrared spectra of acetyl derivative of (C) and acetyl isomorellic acid are superposable. Their melting points were same and the mixed m.p. was undepressed. All these data confirm the identity of acetyl derivative of (C) with that of acetyl iso-morellic

acid and so has the structure (XIII). It is quite obvious that the compound (C) during the process of isolation undergoes a lot of chromatographic treatment thereby coming in contact with silica-gel for a long time and hence gets transformed to the cis configuration (relation of carboxyl to H_y proton). As present in nature it must be existing in the trans form. This fact was confirmed by directly methylating (C) as it occurs in nature. We got a dimethyl derivative of morellic acid m.p.160°. The mixed m.p. of this compound and the authentic sample of methyl ether-ester of morellic acid was undepressed.

In the light of the structure assigned to α-guttiferrin (morellic acid) it is easy to explain why the earlier workers[23] reported the presence of a lactone ring, as they were able to get an additional carboxyl function when α-guttiferrin was treated with alkali to give guttiferrinic acid. Prof. Auterhoff has reported in his paper on gambogic acid that when gambogic acid was treated with alkali for two minutes, the bicyclooctenone ring in the compound gets opened up giving rise to an additional carboxyl group as shown in the constitution of phenol (A). Gambogic acid and morellic acid or the so called α-guttiferrin have the same basic skeleton and hence morellic acid undergoes a similar change when treated with alkali. Ethoxy-dihydroisomorellic acid and morellic acid

(α-guttiferrin) form an intrinsic mixture and are difficult to separate on the column and probably this was the reason as to why the earlier workers[22] reported that α-guttiferrin has three double bonds.

NMR spectrum of dimethyl ether-ester of ethoxydihydroisomorellic acid

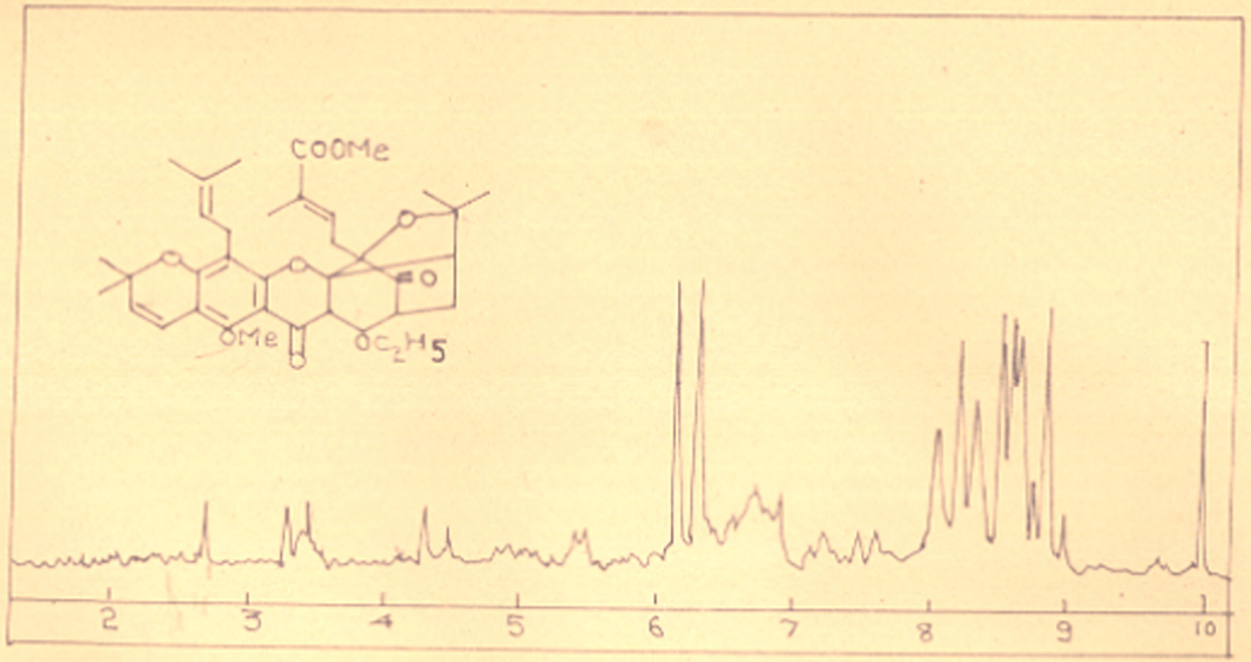


FIG 5 ($CDCl_3$)

NMR data on dimethyl ether-ester of ethoxydihydroisomorellic acid Fig. 5

Solvent = $CDCl_3$

Chemical shift	Multiplicity	No.of H	Assignment
8.9	Tr	3	Me of the ethyl group
8.62	s	6	2 Me on a tertiary carbon atom
8.55	s	6	2 Me on a tertiary carbon atom
8.3	s	3	2 Me of a prenyl chain
8.2	s	3	
8.1	s	3	Me of the side-chain bearing the COOMe group
6.4 - 8.0	-	6	3 $-CH_2-$
6.2 and 6.37	s	6	2 -OMe
5.47	bs	2	$-OCH_2-$
4.95	bs		Vinyl H of prenyl cha
4.45	d (J = 10 cps)	1	Vinyl H of the chromene ring
3.5	d (J = 10 cps)	1	Benzylic as well as vinylic H of the chromene ring
3.35	bs	1	Vinyl H of the side-chain bearing -COOMe group

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

Isolation

Gamboge of Garcinia morella (25 g) obtained from Mangalore (South India) was dissolved in ether (300 ml), filtered and extracted with 10% aqueous sodium carbonate. The carbonate extract was acidified with dil. hydrochloric acid. The acidic constituents liberated during acidification were extracted with ether. The ether extract was washed with water to remove traces of free acid. It was then dried over sodium sulphate, filtered and ether distilled off. The residue was extracted with cold hexane, which dissolved isomorellic acid. Cold hexane extraction was repeated twice. The residue after separating isomorellic acid contained only morellic acid.

Isomorellic acid

Isomorellic acid m.p. 160° was obtained as a resin (6 g) which resisted crystallization from a number of solvents. It showed a green ferric colour and a positive iodide-iodate test, and a negative hydroxamic test for a carboxyl group.

Morellic acid

Morellic acid was also a yellow resin (3 g)

m.p. 172° and had no tendency to crystallize. It gave a green ferric colour and a positive iodide-iodate test, but did not answer the hydroxamic test for the presence of a carboxyl group.

Acetylation of isomorellic acid

Isomorellic acid (1 g), acetic anhydride (10 ml) and sodium acetate (1 g) were refluxed for two hr, and the mixture poured over crushed ice, yellow solid that separated over-night was filtered, washed, dried and crystallized from methanol in fine yellow cubes (700 mg), m.p. 174°. It did not give ferric colour but was soluble in aq. sodium carbonate indicating the presence of a carboxyl group. (Found: C, 70.0, 70.1; H, 6.6, 6.7%. $C_{35}H_{38}O_9$ requires: C, 69.7; H, 6.4%. Acetoxyl estimation showed it to be a monoacetate. MW 602 (mass spec.).

Acetylation of morellic acid

Morellic acid (1 g), acetic anhydride and sodium acetate (1 g) were refluxed for two hr, and the mixture poured over crushed ice, left overnight in contact with water. A yellow solid that separated was filtered, washed and dried. This was observed to be a mixture of two compounds, but its behaviour towards methanol was very helpful in separating the two. One of them was readily soluble in cold methanol (acetyl

isomorellic acid) while acetyl morellic acid could only be dissolved in hot methanol, thus effecting the separation of acetyl morellic acid (200 mg). Recrystallization from the same solvent gave yellow cubes m.p. 240° (Found: C, 69.9, 70.1; H, 6.6, 6.5% MW 602 (mass spec.) $C_{33}H_{38}O_9$ requires: C, 69.7, H, 6.4%. Acetoxyl estimation showed it to be a mono-acetate). It did not give ferric colour, but was soluble in aq. sodium carbonate indicating the presence of a carboxyl group.

Esterification of acetyl isomorellic acid

Acetyl isomorellic acid (100 mg) was dissolved in dry methanol (5 ml) and excess of diazomethane was added. This was kept at 20° for 24 hr, after which excess of diazomethane was destroyed with a few drops of acetic acid. The ethereal solution was washed with water and ether distilled off. The pale yellow residue m.p. 130° was homogeneous but had no tendency to crystallize.

Esterification of acetyl morellic acid

Esterification of the acetyl morellic acid by the above procedure gave the methyl ester of acetyl morellic acid m.p. 200° which was homogeneous and showed no tendency to crystallize.

Isomerization of morellic acid to isomorellic acid

To morellic acid (100 mg) dissolved in acetone (10 ml), was added a drop of conc. hydrochloric acid. The mixture was kept under stirring for 24 hr. Dilution with water gave a residue which was extracted with ether. Removal of ether left a residue which was identical with isomorellic acid.

Conversion of morellic acid to isomorellic acid by passing its solution through a silica-gel column

Morellic acid (100 mg) was dissolved in a mixture of acetone benzene (2:8) and passed through a silica gel column four times, using the same solvent system. Evaporation of the eluent gave isomorellic acid.

Isolation of gambogic acid

The dried gamboge of Garcinia hanburryi (10 g) was powdered, dissolved in ether (400 ml), filtered and extracted with 10% aq. sodium carbonate. The ether extract was alternately washed with water. The carbonate extract and the aqueous washings were mixed together, acidified with dil. hydrochloric acid and taken up in ether. It was washed with water to remove traces of hydrochloric acid and ether removed. The residue was dissolved in minimum amount of pyridine and about ten drops of distilled water were added. Pyridine salt of gambogic acid that separated was filtered, washed with

dilute pyridine and dried. It was recrystallized from methanol m.p. 148°. Pure gambogic acid was obtained by dissolving the pyridine salt in ether and twice washing it with dil. hydrochloric acid, followed by washing with water and removal of ether.

Preparation of acetyl isogambogic acid

The same procedure for acetylating isomorellic acid was adopted for acetylating gambogic acid, but the product obtained was mainly acetyl isogambogic acid m.p. 204°.

Hydrolysis of acetyl isogambogic acid with dilute ammonia

Acetyl isogambogic acid (200 mg) was treated with dilute ammonia (2.5 ml of liq. ammonia diluted to 25 ml with distilled water) and stirred for 1/2 hr. It was neutralized with very dilute hydrochloric acid and taken up in ether. The ether extract was washed with water and ether distilled off. The amorphous residue gave a dark bluish-green ferric colour. This procedure was adopted in order to effect only deacetylation without affecting the other part of the molecule

Hydrogenation of morellic acid

Palladium charcoal (30 mg 10%) was suspended in methanol (15 ml) and saturated with hydrogen. To

this a solution of morellic acid (100 mg) in methanol (10 ml) was added and stirred. It absorbed 4 mols of hydrogen. The resulting solution was filtered to remove catalyst. Removal of methanol left a pale yellow gummy residue which had no tendency to crystallize.

Methylation of morellic acid

A mixture of morellic acid (500 mg), acetone (50 ml), potassium carbonate (5 g) and dimethyl sulphate was refluxed for 6 hr, when a test portion of the reaction mixture did not show ferric colour. Acetone was distilled off from the reaction mixture. The residue was treated with water and left overnight. It was extracted with ether, washed with water and ether distilled off. The residue crystallized from methanol (400 mg) m.p. 160° (Found: C, 71.1; H, 7.2% - OCH_3 estimation 13.8%. $C_{35}H_{40}O_8$ requires: C, 71.4; H, 6.8% - OCH_3 15% for two methoxyls).

Methylation of isomorellic acid

Isomorellic acid was methylated adopting the above procedure. The methylated product so obtained was crystallized from methanol m.p. 154°. (Found: C, 70.0; H, 7.4%. $C_{35}H_{40}O_8$. requires: C, 71.4; H, 6.8%).

Methyl ester of acetyl isogambogic acid

A mixture of acetyl isogambogic acid (500 mg),

acetone (500 ml), potassium carbonate (5 g) and dimethyl sulphate (1 ml) was refluxed for 5 hr, After this acetone was distilled off and the contents of the flask were treated with water and left overnight. It was extracted with ether, the ether extract washed with water, ether distilled off, and the residue crystallized from methanol (450 mg) m.p. 155°. The methyl ester of acetyl isogambogic acid was yellow in colour (Found: C, 71.7; H, 7.2% $C_{41}H_{48}O_9$ requires: C, 71.9; H, 7.0%).

Monomethyl isogambogínate

To the methyl ester of acetyl isogambogic acid (400 mg) dissolved in glacial acetic acid (20 ml) was added conc. hydrochloric acid (2 ml) and the mixture was heated over a water bath for 1 hr. This was cooled, diluted with water (40 ml) and extracted with ether. The ether extract was washed with water to remove traces of acetic acid and hydrochloric acid. Removal of ether gave a residue which crystallized from ethanol (100 mg) This was recrystallized from ether m.p. 200°.

Isolation of the acidic components of the pigments of the pericarp of the seeds of Garcinia morella

The mother liquor after the crystallization of morellin from the ethanol extract of the colouring matters of the pericarp of the seeds of Garcinia morella yielded an orange yellow resinous residue on evaporation.

This resinous residue (20 g) was dissolved in ether (200 ml) and extracted with 10% aqueous sodium carbonate. The ether extract was alternately washed with water. The carbonate extract and the aqueous washings were together acidified with dil. hydrochloric acid. The acidified extract was taken up in ether, the ether extract washed with water. The residue obtained after distilling ether was amorphous. There were two main components in this yellow amorphous powder. Preparative layer chromatography, using silica-gel as adsorbent and acetone-benzene (1:9) was employed as solvent for separating the two constituents. To obtain pure compounds preparative layer chromatography had to be repeated a number of times since the Rf values of these compounds were very close. These were named ethoxydihydroisomorellic acid and isomorellic acid.

Ehoxydihydroisomorellic acid (A)

Compound (A) did not form a crystalline pyridine salt and showed all properties of isomorellic acid. It had no tendency to crystallize.

Methylation of (A)

Compound (A) (500 mg), acetone (40 ml) potassium carbonate (4 g) and dimethyl sulphate (1 ml) were refluxed for 5 hr, when a test portion did not show

ferric colour. Acetone was distilled off, the flask was cooled, water added and kept overnight. This was extracted with ether and washed with water, ether distilled off and the residue crystallized from methanol m.p. 150°. Recrystallization from the same solvent gave colourless crystals m.p. 154°. (Found: C, 69.9; H, 7.3%. $C_{37}H_{46}O_9$ requires: C, 70.0; H, 7.6% - OCH_3 estimation indicated three methoxyls).

Hydrogenation of (A)

Compound (A) (100 mg) was dissolved in methanol (10 ml). Palladium charcoal (30 mg 10%) was suspended in methanol (10 ml) and the catalyst saturated with hydrogen. The solution of (A) was transferred to the saturated catalyst. (A) absorbed 3 mols of hydrogen indicating the presence of three double bonds. This was filtered and washed with methanol. Removal of methanol gave a gummy residue which resisted crystallization though it was homogenous.

Addition of a molecule of ethanol to the molecule of dimethyl isomorellic acid. Michael type of addition

A mixture of dimethyl derivative of isomorellic acid (100 mg), dry ethanol (20 ml) and a drop of piperidine was refluxed for 6 hr. Ethanol was distilled off and the residue was taken in ether, washed with dil. hydrochloric acid, followed with water. Removal of ether gave a residue which crystallized from methanol

(100 mg) m.p. 154°. The mixed m.p. with the authentic sample of dimethyl derivative of ethoxydihydroisomorellic acid was undepressed.

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INTRODUCTION

Morellin is a pigment of Garcinia morella, the structure of which has been established on the basis of chemical, IR, UV, NMR and X-ray crystallographic data[4]. A number of attempts were made by Raghavan[1] to degrade the molecule. The characterization of the degradation products of morellin would have ultimately helped in elucidating its structure. Morellin was highly sensitive to alkali and it was not possible to isolate any crystalline product even when it was subjected to mild treatment with alkali. Unlike morellin, its octahydroderivative was relatively stable and hence was used for further degradative work.

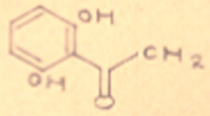
Hydrolysis of octahydromorellin with 15% ethanolic potassium hydroxide on a water-bath for 4 hr gave a crystalline product (A) m.p. 132°, in 60% yield. It was not possible to isolate any other crystalline compounds. Product (A) could be obtained cleaner and in better yield by the hydrolysis of octahydromorellin for 5 hr with 5% ethanolic potassium hydroxide. It was readily soluble in aqueous sodium carbonate and could be isolated conveniently by extracting the ethereal solution of the reaction mixture with 10% aqueous sodium carbonate and subsequent acidification

of the carbonate extract. The compound (A) readily crystallized from methanol.

On the basis of elementary analysis and molecular weight (445, Rast method) (A) was assigned the molecular formula $C_{26}H_{36}O_6$. It exhibited a blue-green ferric colour. The iodide-iodate and the hydroxamic tests were negative. This showed that it was not a carboxylic acid, ester or a lactone. The carbonate solubility and the ferric colour therefore indicated that it contained two phenolic hydroxyl groups. By the lithium aluminium hydride method it was found to contain two active hydrogen atoms, but positive evidence for two hydroxyl groups could not be obtained.

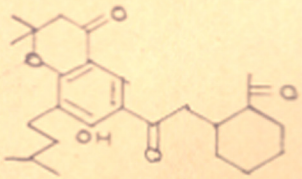
(A) underwent ethyl orthoformate-pyridine-piperidine condensation and the crystalline product obtained m.p. 102°, analysed for $C_{27}H_{34}O_6$, which indicated that a chromone was formed. On heating (A) with acetic anhydride and sodium acetate for 15 hr (Kostanecki reaction) a crystalline compound m.p. 230°, was obtained. The molecular formula $C_{30}H_{40}O_7$, indicated the cyclization of a compound containing the group (I) to a 2-methyl chromone. An acetyl estimation showed the presence of one acetoxyl group.

Since the above evidence indicated the presence of the group (I) in (A), Raghavan attempted to degrade



I

II



III

it to the chromanone corresponding to the C_{16} phenol[3], a product obtainable by alkali fusion of octahydromorellin. By vigorous alkaline hydrolysis with 75 per cent aqueous alkali at reflux (A) was recovered unchanged. Similar stability was observed when hydrolysis was conducted in ethylene glycol at reflux (b.p. 198°). However, alkali fusion with five times the weight of potassium hydroxide at 300° for fifteen minutes gave the C_{16} phenol, the structure of which has been recently elucidated as(II). Phenol (A) was assigned structure (III). Although there were gaps in the evidence, the proposed structure for (A) found support in the infrared (IR) spectrum of (A) (Fig. 1) and IR spectra of its derivatives. (A) showed a hydroxyl band at 3560 cm^{-1} and two bands at 1710 cm^{-1} and 1635 cm^{-1} in the carbonyl region. The 1710 cm^{-1} band due to an unconjugated -CO- group was assigned to the free carbonyl group in the acetyl cyclohexane part. The 1637 cm^{-1} band was assigned to the chelated carbonyl. Only one band at 1635 cm^{-1} was seen, although two distinct chelated carbonyl vibrations would be expected from the structure. However, the synthetic 8-isovaleroyl-5,7-dihydroxy-2,2-dimethyl chromanone showed only one band at 1630 cm^{-1} although it had two chelated carbonyl groups. The structure (III) proposed for (A) agreed with many of its properties. However,

in the work to be described it is shown with the help of NMR and mass spectral data on (A) and its derivatives, that the structure suggested for (A) is untenable and an alternative is proposed.

PRESENT WORK

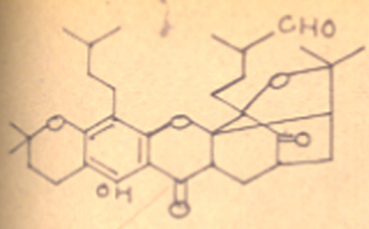
In the light of the established structure of morellin and accumulation of chemical and spectral data on the chemistry of phenol (A), it was felt necessary to revise the structure assigned to it. The molecular formula $C_{26}H_{36}O_6$ for (A) assigned earlier had to be corrected to $C_{33}H_{46}O_8$ in view of the chemical analysis and mass spectral molecular weight (570). This corresponds to the addition of a molecule of water to octahydromorellin ($C_{33}H_{44}O_7$) (IV) during its alkaline hydrolysis.

Phenol (A) (V) was soluble, although sparingly in aqueous sodium bicarbonate, indicated the presence of a carboxyl group, but the iodide-iodate test and hydroxamic test for the same were negative. It gave a crystalline monomethyl derivative m.p. 94°(VI) on treatment with diazomethane in ether and dry methanol. An olive-green ferric colour showed the presence of the chelated hydroxyl. The IR spectrum of (VI) (Fig. 2) indicated the presence of an additional hydroxyl group. It was obvious from this that diazomethane could not be used to get a fully methylated derivative of (A). Prolonged treatment

of (A) with dimethyl sulphate and potassium carbonate in boiling acetone gave a product which resisted crystallization.

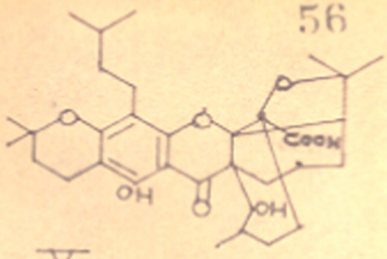
When phenol (A) was methylated by the silver oxide-methyl-iodide-dimethylformamide method it gave a colourless crystalline derivative on chromatography of the crude product over a silica-gel column using ethyl acetate:benzene (1:9) as eluting solvent. This compound was crystallized from methanol m.p. 91°(VII). It did not show any colour with ferric chloride and its IR spectrum (Fig. 3) showed the presence of a hydroxyl group. Methoxyl estimation indicated two $-OCH_3$ groups. It was thus clear that (A) has three hydroxyl functions, only one of which resisted methylation.

Phenol (A) could be dehydrated by heating its solution in phosphorus oxychloride, on a water-bath for 1/2 hr. The dehydrated product was a colourless crystalline compound m.p. 192° (VIII) but the yield was only 10 per cent. It was insoluble in 10 per cent aqueous sodium carbonate and showed a green ferric colour. The IR spectrum of this product (Fig. 4) did not show any absorption in the hydroxyl region. These two observations suggested the formation of a lactone ring during this dehydration.



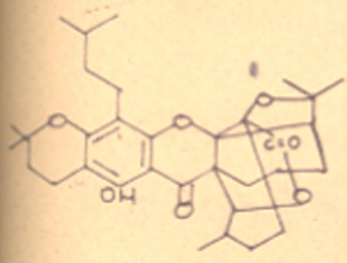
IV

Octahydromorellin



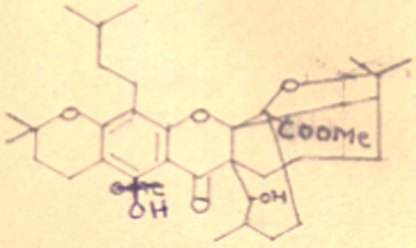
V

Phenol (A)



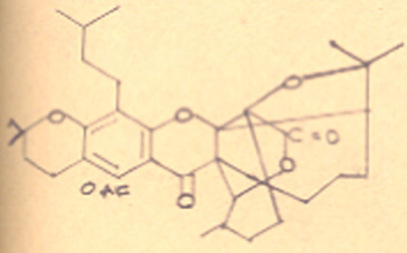
VIII

Lactone of (A)



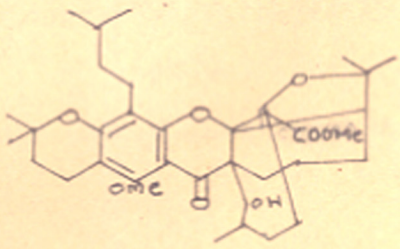
VI

Ester of (A)



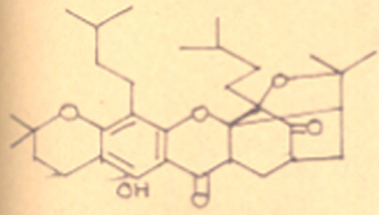
IX

Acetate of (A)



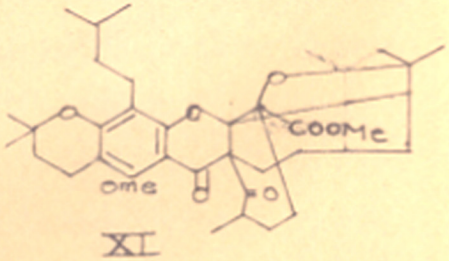
VII

Dimethyl ether-ester of (A)



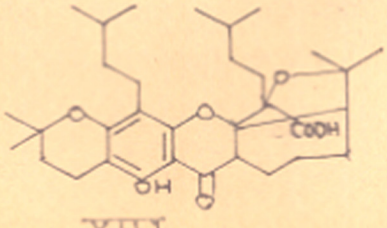
XII

Octahydrodesoxymorellin



XI

Oxidation Product of VII



XIII

The corresponding carboxylic acid

In an attempt to effect decarboxylation of (A), it was refluxed for 1 hr with copper bronze in diphenyl oxide and a drop of quinoline when a colourless crystalline compound m.p. 192° was obtained. This compound was identical with the product obtained by the dehydration of (A) with phosphorus oxychloride. Their IR spectra were superposable and the mixed m.p. was undepressed.

The dehydration product of (A) could easily be hydrolysed by 5 per cent alcoholic potassium hydroxide to give back (A). Similarly when the monomethyl derivative of (A) obtained by diazomethane methylation was subjected to hydrolysis by 5 per cent ethanolic potassium hydroxide, (A) was obtained in quantitative yield.

The formation of a lactone ring, when (A) was dehydrated, the formation of a methyl ester when (A) was treated with diazomethane and their subsequent alkaline hydrolysis to give (A) in quantitative yield, amply indicated the presence of a carboxyl group. From the chemical and spectral data discussed so far it became evident that (A) has three hydroxyl groups and their nature is as follows.

1. A chelated hydroxyl group
2. A carboxyl group
3. An alcoholic hydroxyl group

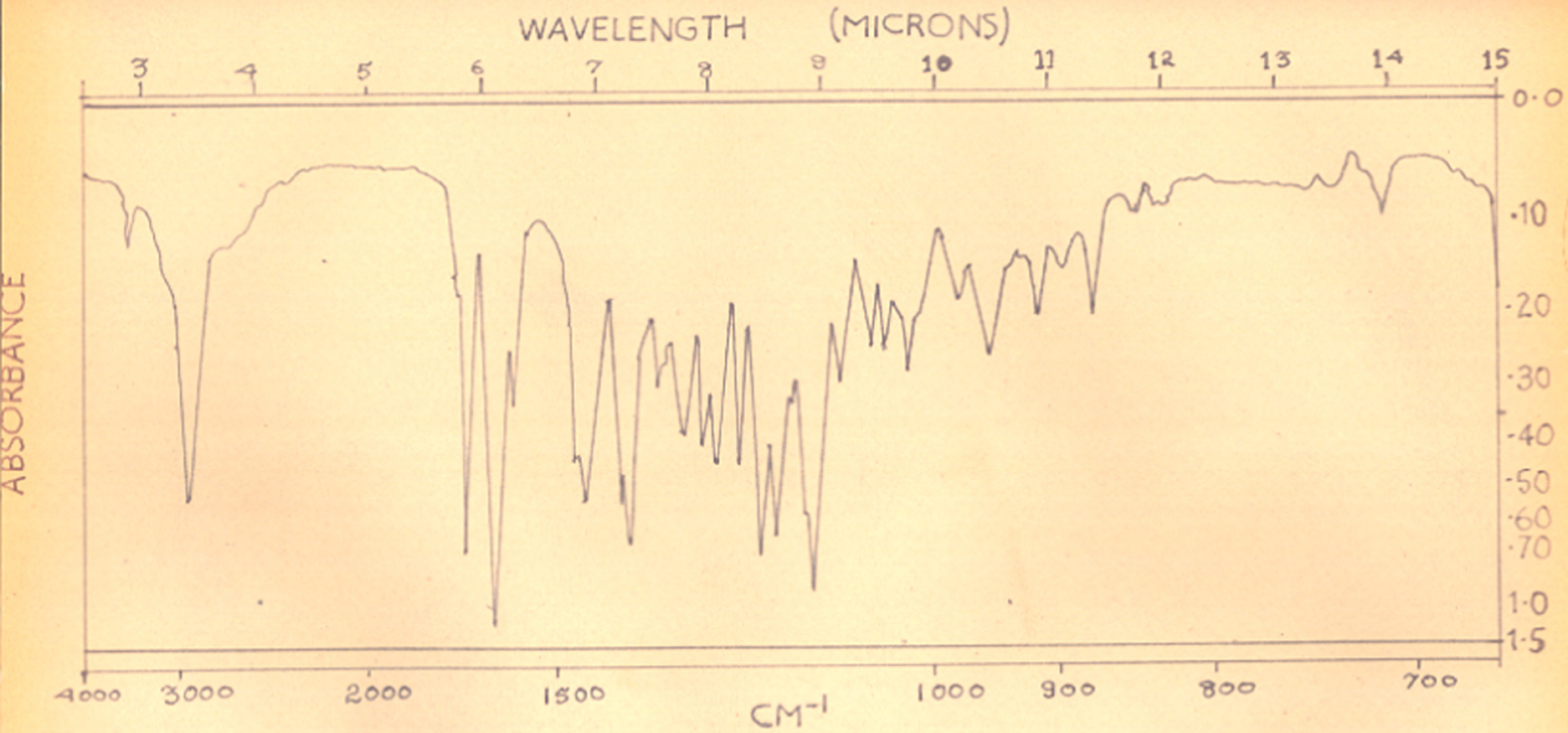
When the dimethyl ether-ester of (A) was subjected to oxidation by chromic acid in glacial acetic acid, the alcoholic group was oxidised to a carbonyl group. The IR spectrum of this oxidised product (Fig. 5) indicated the presence of an additional carbonyl and the absence of an alcoholic group.

The shifts of the carbonyl frequencies in the IR spectra of (A) and its derivatives are quite interesting and hence are tabulated for comparison in Table No. I.

Table No. I

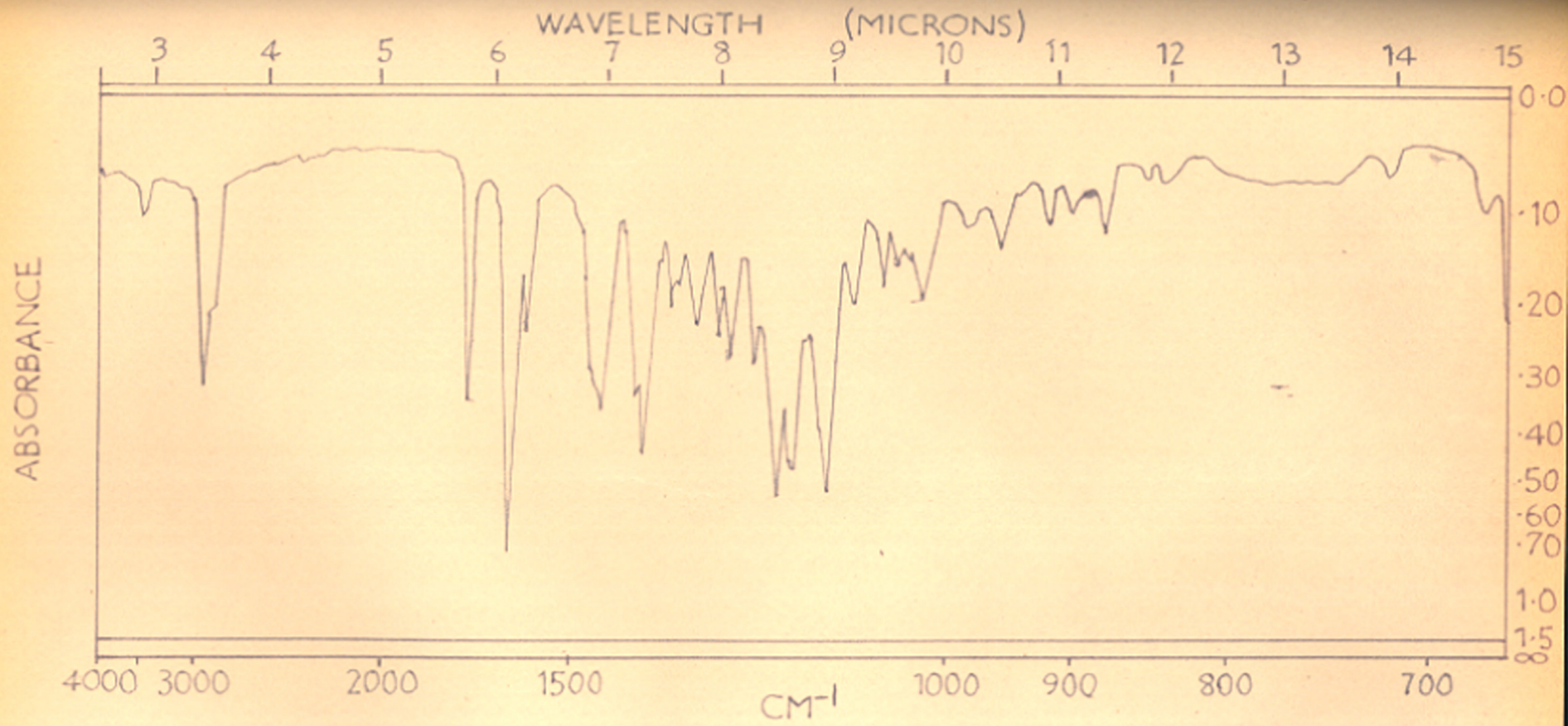
IR Spectra of (OH and CO regions) phenol (A) and its derivatives frequencies cm^{-1}

Compounds	OH	CO		
1. Octahydromorellin	-	1745	1710	1635
2. Phenol (A)	3460	-	1710	1635
3. Acetate		1755, 1730	-	1670
4. Methyl ester	3480	1745	-	1635
5. Dimethyl ether-ester	3500	1750	-	1690
6. Pyrolysis Product	-	1750	-	1635
7. Oxidation Product	-	1750	1710	1690



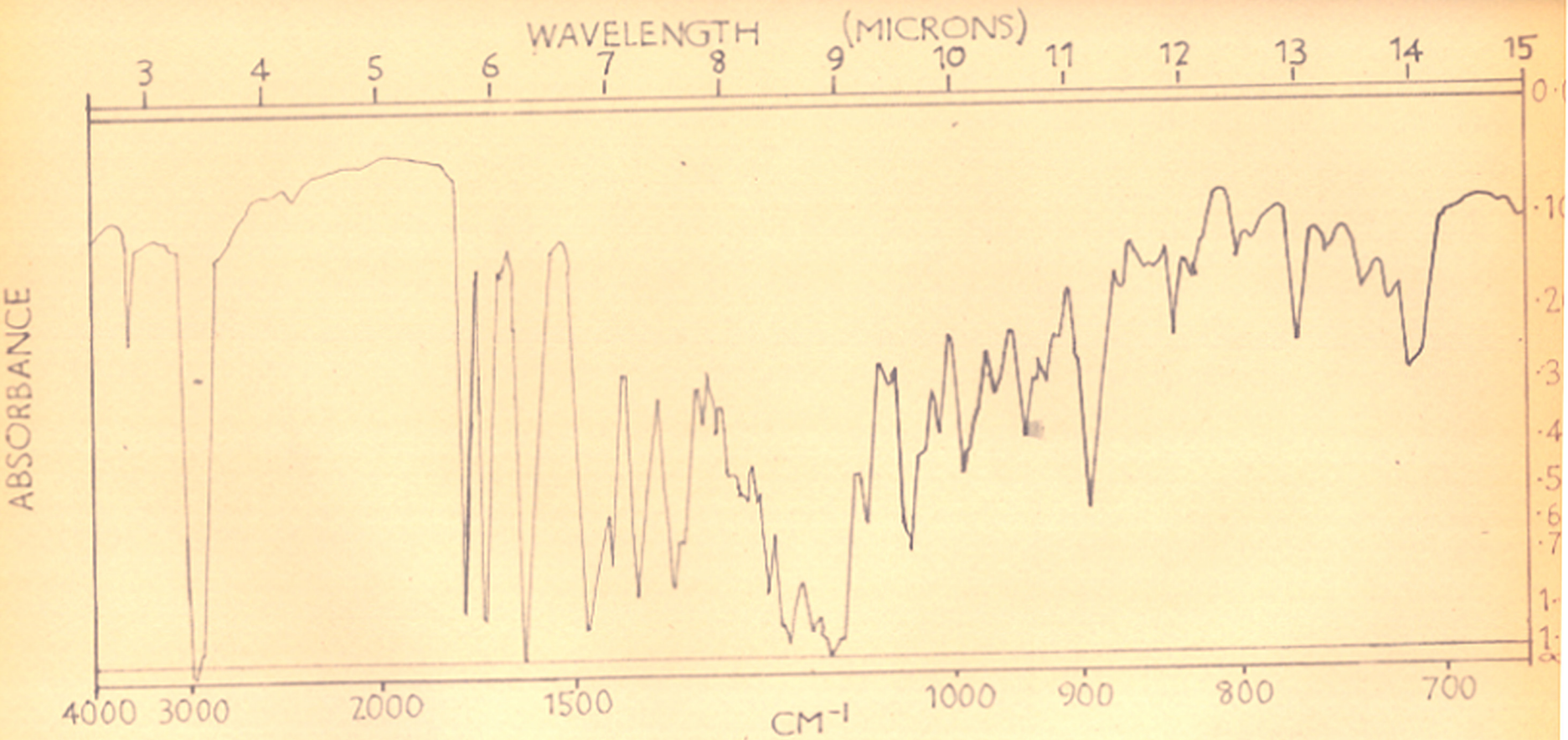
IR spectrum of phenol (A) in CCl_4

FIG 1



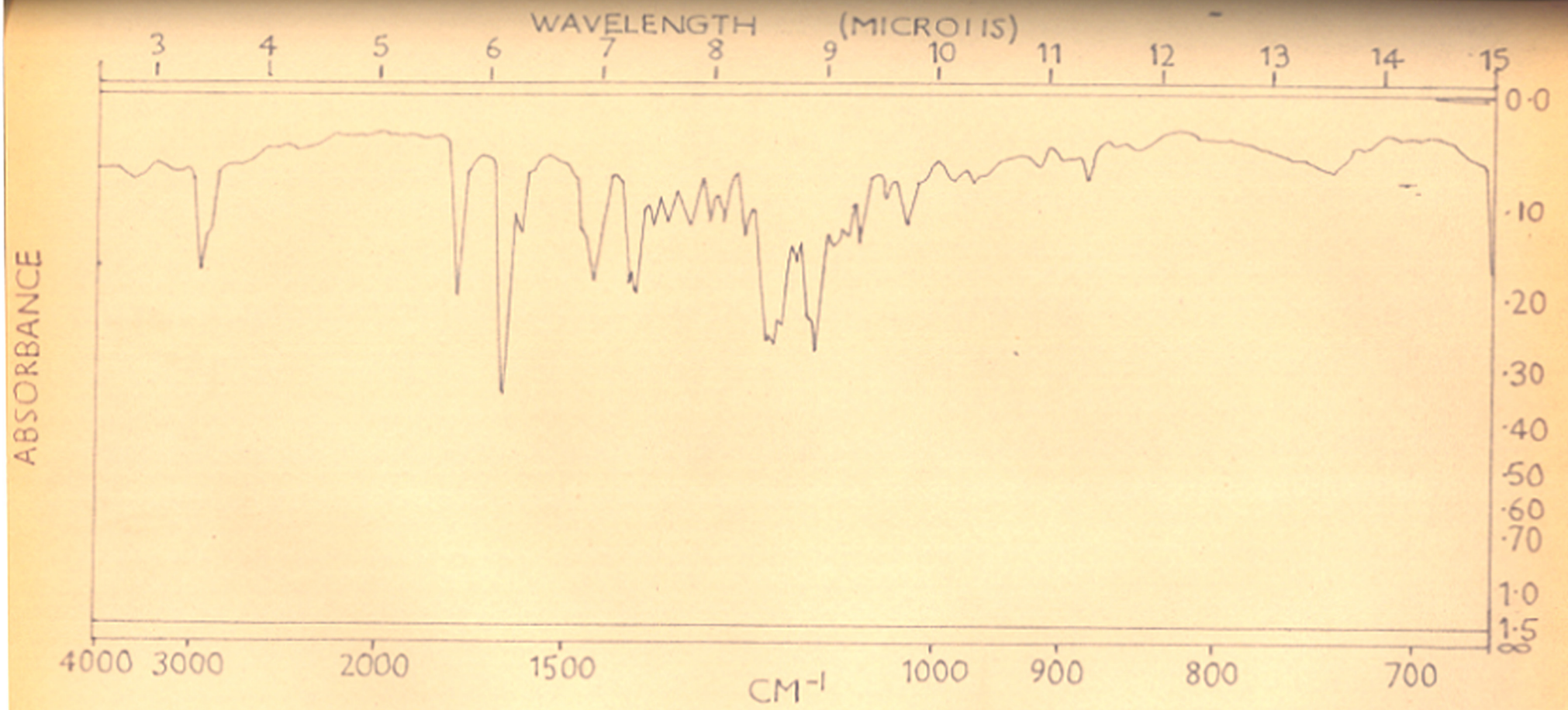
IR spectrum of methyl ester of (A) in CCl_4

FIG 2



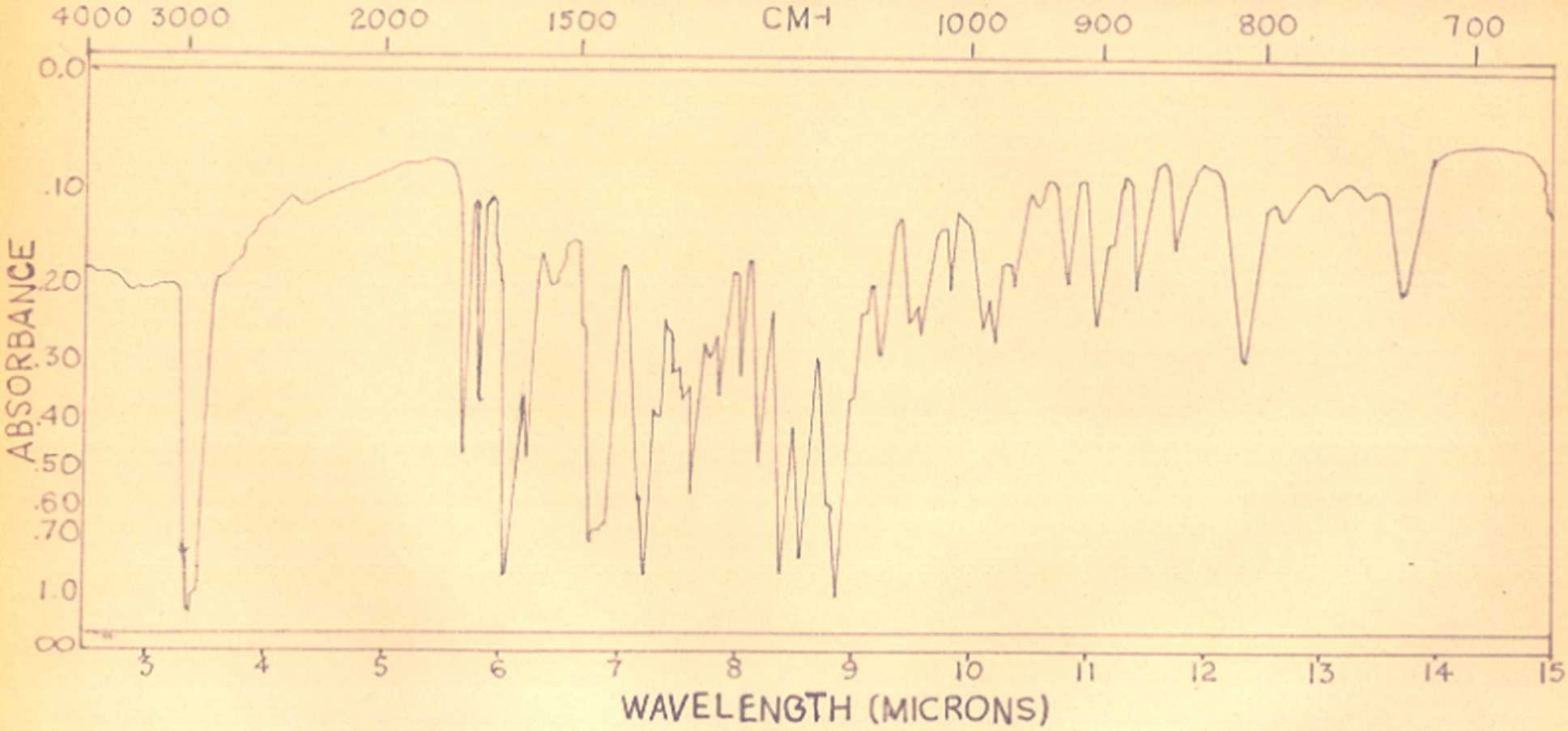
IR spectrum of dimethyl ether-ester of (A) in Nujol

FIG 3



I R spectrum of the dehydrated product of (A) in - CCl_4

FIG 4



IR spectrum of the oxidation product of dimethyl ether-ester of (A) in Nujol

FIG 5

IR spectrum of acetate of phenol (A)

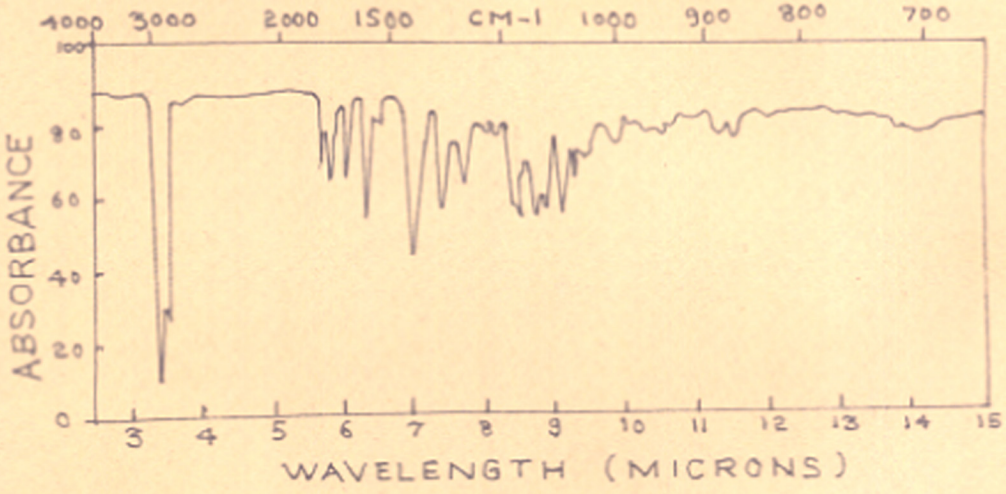


FIG 6 Nujol

IR spectrum of octahydromorellin
C=O region

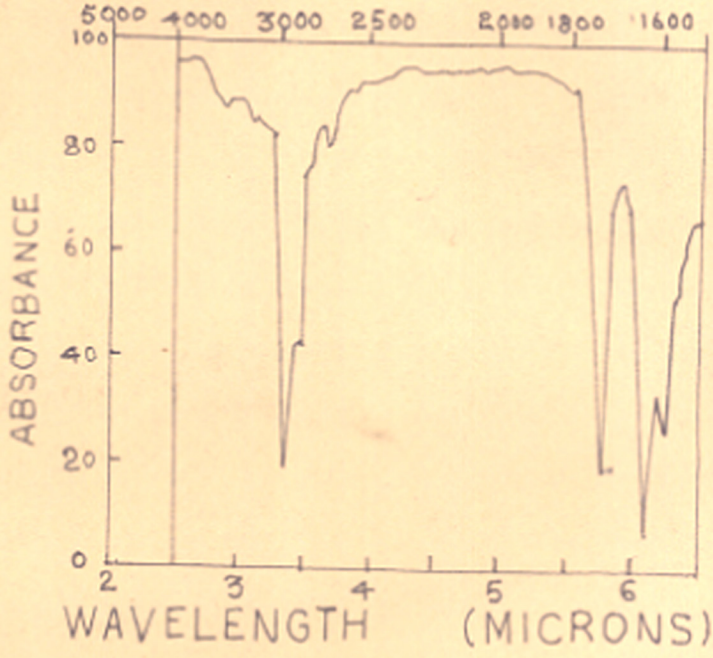


FIG 7

Octahydromorellin (IV) does not show absorption in the hydroxyl region, while in the spectrum of the phenol (A) there is a band at 3460 cm^{-1}. The band at 1745 cm^{-1} in the spectrum of octahydromorellin (Fig. 7) which is due to an aldehyde group is absent in the spectrum of (A). The band at 1710 cm^{-1} in (A) which is due to an unconjugated carbonyl could be assigned to the free carboxyl group formed during the alkaline hydrolysis by cleavage of bicyclooctanone ring of octahydromorellin. The band at 1635 cm^{-1} in the spectrum could be assigned to the chelated carbonyl. The peak due to the hydroxyl at 3460 cm^{-1} in the IR spectrum of (A) (Fig. 1) shows very negligible shift in the spectra of its ester and dimethyl ether-ester. The carbonyl peak at 1710 cm^{-1} in (A) is shifted to 1745 cm^{-1} in the methyl ester and the same is observed at 1750 cm^{-1} in its dimethyl ether-ester and the lactone. While the peak due to the chelated carbonyl at 1635 cm^{-1} in (A) does not show any shift in the spectra of methyl ester and the lactone. Only in the IR spectra of dimethyl ether-ester (Fig. 3) and the acetate (IX) (Fig. 6)the band at 1635 cm^{-1} is shifted to 1690 cm^{-1} and 1670 cm^{-1} respectively.

The 3460 cm^{-1} band of (A) is apparently to be assigned to the hydroxyl group generated in the hydrolysis of octahydromorellin. Lactonization of (A)

brought about by phosphorus oxychloride or under conditions of acetylation involves this hydroxyl group.

The Nuclear Magnetic Resonance (NMR) spectrum of (A) (Fig. 8) in deuterated chloroform ($CDCl_3$) showed a total proton count of 46, using the single proton signal at 6.45* as standard. A signal at - 2.0 indicated a chelated hydroxyl and it could be easily exchanged with D_2O. The signal corresponding to the aldehyde group of morellin and its hydrogenated derivatives was absent in the spectrum of (A). It seems very definite from this, that the chemical changes occurring during the alkaline hydrolysis also involve the aldehyde group of octahydromorellin, probably in an aldol type of condensation with one of the carbons adjacent to a carbonyl. The hydroxyl of the -COOH and the alcoholic -OH group could not be seen. It was further observed that all the seven methyl groups present in octahydromorellin are intact in (A). The integration of the methylene region indicated approximately 21 protons. There was a broad signal at 6.45 which integrated for a single proton and could be assigned to a hydrogen α to a carbon carrying a hydroxyl group. There were no signals in the vinyl region.

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*Chemical shifts are cited on τ scale.

In the NMR spectrum of the methyl ester of (A) (Fig. 9) the signal due to the chelated hydroxyl group was observed at - 1.91. The $-OCH_3$ signal appeared at 6.4. The broad signal of the ∝-hydrogen attached to carbon bearing a hydroxyl was seen at 6.45. Integration of the methyl region indicated the presence of seven Ω-methyls.

In the NMR spectrum of dimethyl ether-ester (Fig. 10) the signal due to the chelated hydroxyl was not observed. The two $-OCH_3$ signals appeared at 6.2 and 6.4, while the signal due to the ∝-hydrogen on a carbon bearing hydroxyl remained unaffected at 6.45.

The signal due to the chelated hydroxyl was observed at - 1.8 in the NMR spectrum of dehydrated product of (A) (Fig. 11). The signal due to the ∝-hydrogen on a carbon bearing an oxygen at 6.45 showed a downfield shift of 0.63 p.p.m. It is thus clear that the hydroxyl involved in the lactone formation is attached to this carbon. The dehydration product of (A) was subjected to hydrogenation in presence of 10 per cent Pd/C, but it did not absorb any hydrogen, and hence the possibility of the formation of a double bond during the dehydration was ruled out. The integration of the methyl region showed seven Ω-methyl groups.

The downfield shift of the ∝-hydrogen attached to

the carbon bearing hydroxyl, was also observed in the NMR spectrum of the acetate of (A) (Fig. 12). This time the signal was observed at 5.6. It is obvious that (A) undergoes a similar type of change as observed in the dehydration experiment during its acetylation. The signal due to the acetoxyl group appeared at 7.59.

From the mass-spectral molecular weight (570), the chemical and the spectral data and in the light of the structure of morellin, it appeared that during the alkaline hydrolysis octahydromorellin underwent two reactions, one in which the aldehyde group condensed with the carbon adjacent to the chelated carbonyl, an aldol type of condensation in which a secondary hydroxyl group was formed. In the other reaction the bicyclo-octanone ring cleaved to form a carboxylic acid. The former type of reaction had not been observed with octahydrodesoxymorellin (XII) which can be understood in terms of the absence of an aldehyde group. The latter type of change has been suggested by Auterhoff[5] in gambogic acid and hence similar reaction could be expected in octahydromorellin having the same basic skeleton.

In the light of the data presented above and since the C_{16} phenol has been obtained by the alkali

fusion of (A), structure (V) is suggested for (A).

The product obtained in alkaline hydrolysis of octahydrodesoxymorellin (XIII) and the product (XI) obtained by the oxidation of the alcoholic group of the dimethyl ether-ester of (A), provide additional support to the structure (V) suggested for phenol (A).

NMR data on phenol (A) (Fig. 8)

Solvent $CDCl_3$

Chemical shift	Multiplicity	No. of H's	Assignment
9.09	d (J = 6 cps)	6	Me_2 of the isopropyl group
8.77	s	3	Me group of the newly formed seven membered ring
8.72	s	3	4 Me on the tertiary carbon atoms
8.67	s	9	
7.0 - 8.5		21	All other CH and $-CH_2-$
6.50	bs	1	Hα to oxygen
- 2.0	s	1	chelated -OH

s = singlet d = doublet

m = multiplet bs = broad signal

NMR spectrum of phenol (A)

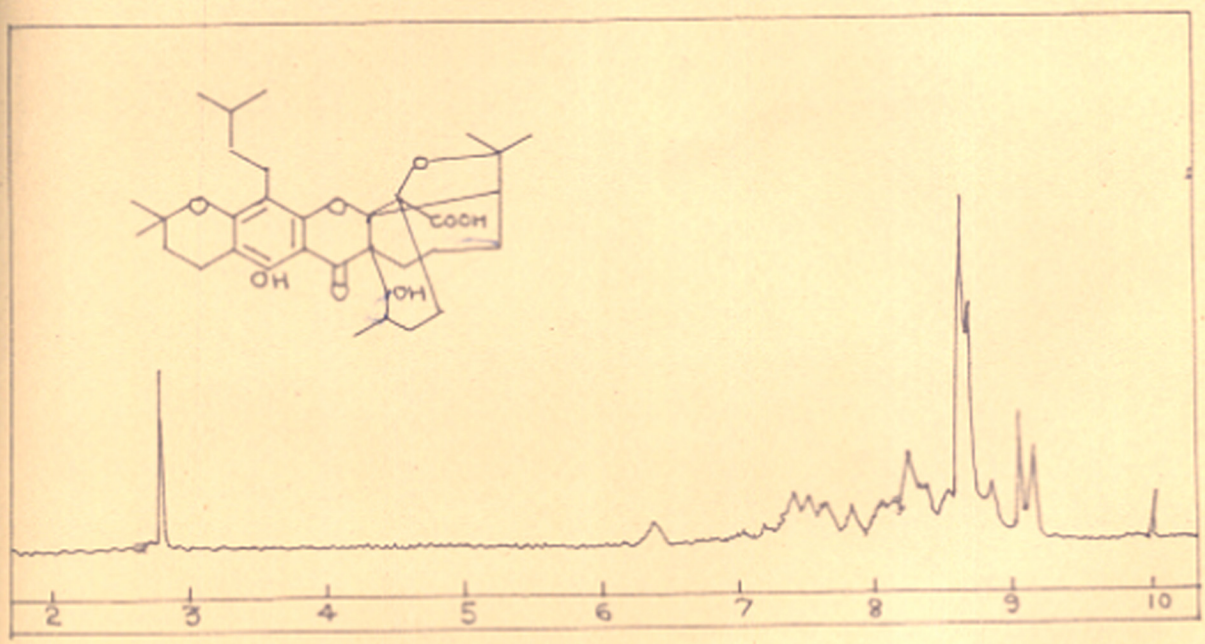

FIG 8 ($CDCl_3$)

NMR spectrum of methyl ester of (A)

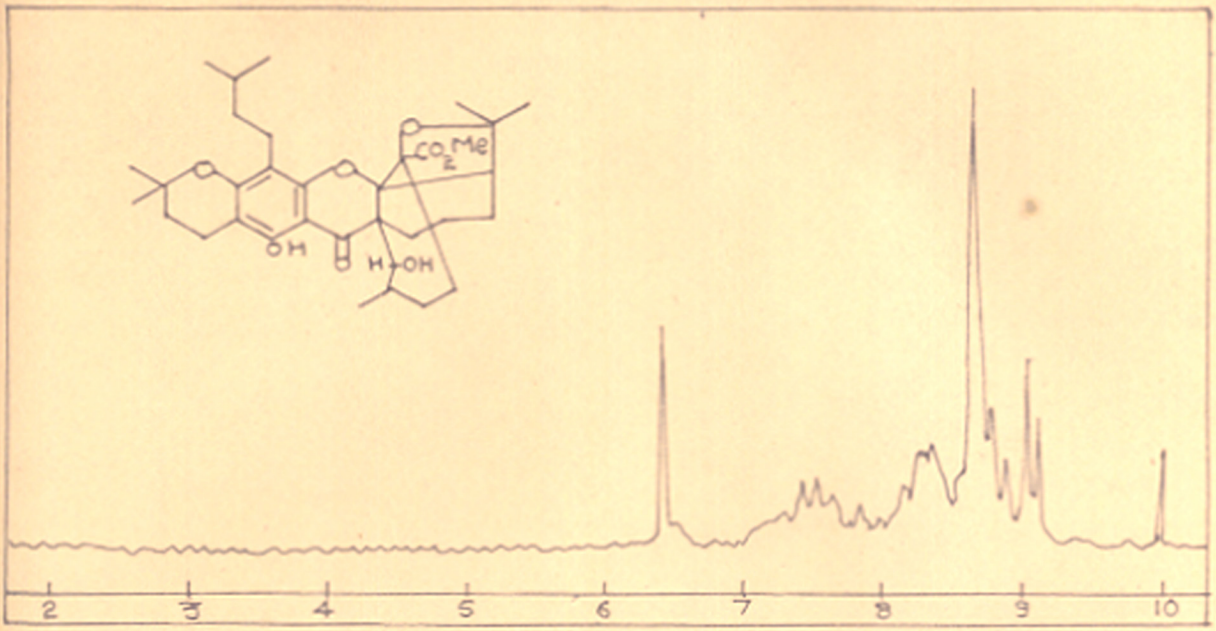

FIG 9 (CCl_4)

NMR spectrum of
dimethyl ether — ester of (A)

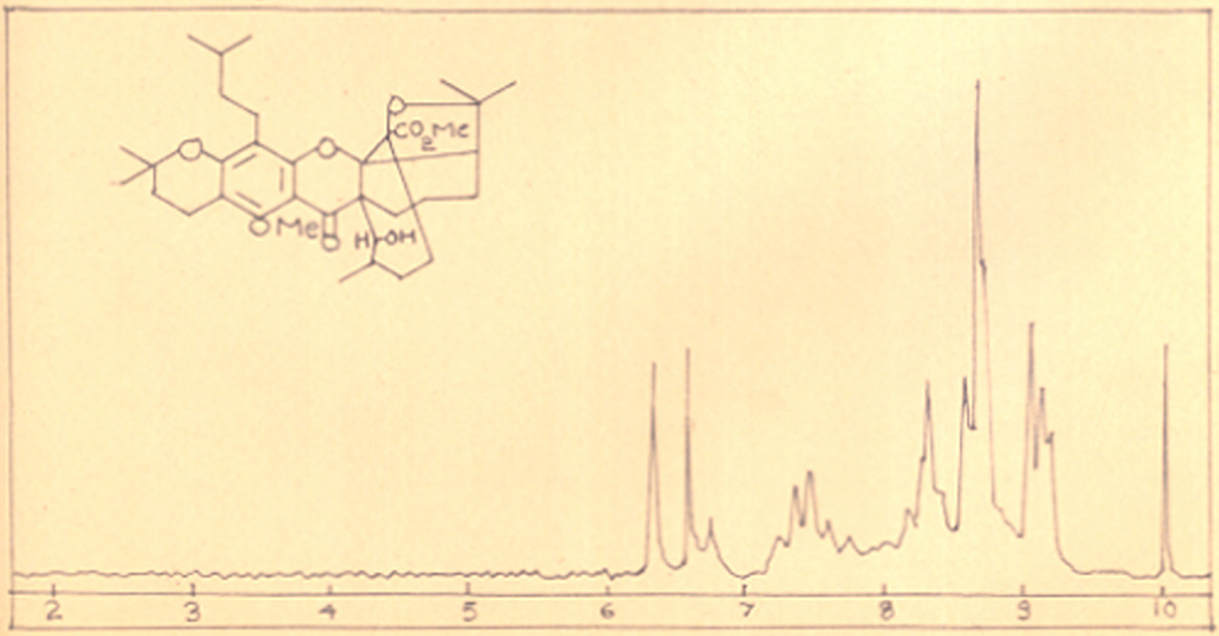

FIG 10 (CCl_4)

NMR data on methyl ester of (A) (Fig.9)

Solvent CCl_4

Chemical shift	Multiplicity	No. of H's	Assignment
9.09	d (J = 6 cps)	6	Me_2 of the isopropyl group
8.83	s	3	Me of the new seven membered ring
8.67	s	12	4 Me on tertiary carbon atoms
7.0 - 8.5	-	21	All other CH and CH_2
6.59	bs	1	Hα to oxygen
6.4	s	3	ester - OCH_3
-1.91	s	1	chelated -OH

NMR data on dimethyl ether-ester of (A) (Fig. 10)

Solvent CCl_4

9.16	d (J = 6 cps)	6	Me_2 of the isopropyl group
9.1	s	3	Me of the new seven membered ring
8.75	s	3	4 Me on tertiary carbon atoms
8.67	s	6	
8.6	s	3	
6.7 - 8.5	-	21	All other CH and CH_2
6.5	s	1	Hα to oxygen
6.4	s	3	-OCH_3 of the ester
6.2	s	3	-OCH_3 of the ether

NMR spectrum of
dehydrated product of(A)

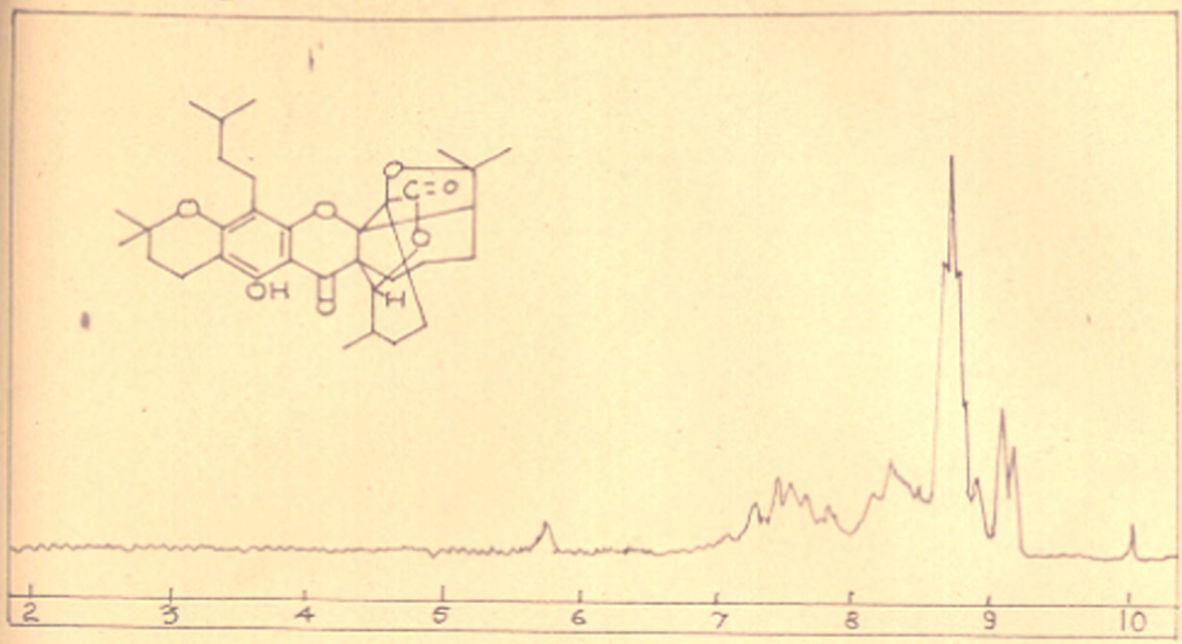

FIG 11 (CCl_4)

NMR spectrum of acetate of (A)

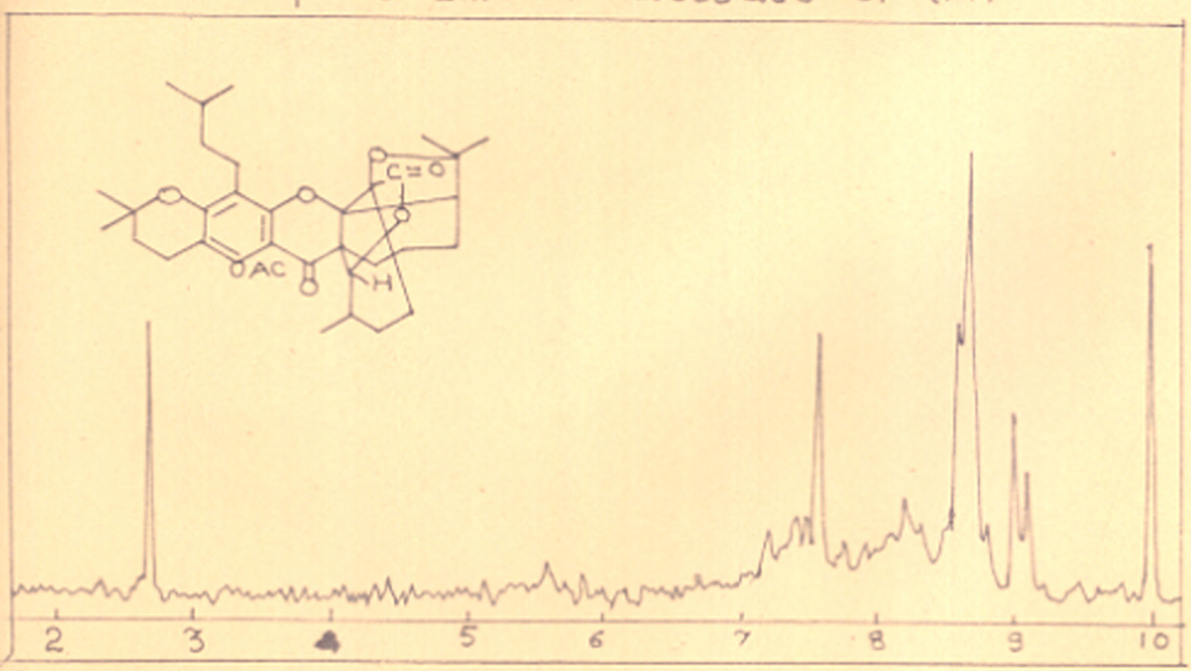

FIG 12 ($CDCl_3$)

NMR data on pyrolysis product of(A) (Fig. 11)

Solvent CCl_4

Chemical shift	Multiplicity	No. of H's	Assignment
9.14	d (J = 6 cps)	6	Me_2 of the isopropyl group
9.02	s	3	Me of the new seven membered ring
8.72	s	3	4 Me on tertiary carbon atoms
8.68	s	6	
8.64	s	3	
6.5 - 8.5	-	20	All other CH and CH_2
5.85	bs	1	Tertiary hydrogen on a carbon linked to the oxygen of the lactone ring
-1.66	s	1	Chelated -OH

NMR data on the acetate of (A) (Fig. 12)

Solvent $CDCl_3$

9.09	d (J = 6 cps)	6	Me_2 of the isopropyl group
8.8	s	3	Me of the new seven membered ring
8.71	s	12	4 Me on tertiary carbon atom
8.6	s	-	
6.5 - 8.5	-	21	All other CH and CH_2 excluding acetoxyl group
7.59	s	3	Acetoxyl group
5.6	bs	1	Tertiary -H on a carbon linked to oxygen of the lactone ring

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

Hydrolysis of octahydromorellin with 15% ethanolic potassium hydroxide

Octahydromorellin (15 g) was dissolved in ethanol (250 ml), a solution of potassium hydroxide (75 g) in water (250 ml) was added and refluxed for 4 hrs. The ethanolic solution of octahydromorellin which was initially yellow, turned red with the addition of alkali. Ethanol was distilled off and the distillate absorbed in Brady's reagent. No dinitrophenyl hydrazone derivative was obtained. The alkaline solution after the distillation of ethanol was cooled with ice and acidified by slow addition of 50% sulphuric acid. A brown sticky mass separated out in the acidified mixture which did not have a fatty acid odour. The acidified solution was extracted with ether. The ether extract was separated as sodium bicarbonate, carbonate and sodium hydroxide soluble fractions.

Sodium bicarbonate soluble fraction

The bicarbonate extract was acidified with 50% sulphuric acid. The yellow solid obtained was crystallized from methanol in creamish yellow needles (6 g), m.p. 132°. The mixed m.p. with phenol A was undepressed. After filtering the crystalline phenol A, the filtrate did not yield any other compound.

Sodium carbonate soluble fraction

The carbonate soluble fraction on acidification with 50% sulphuric acid gave a yellowish brown sticky mass. This was extracted with ether, washed with water, dried over sodium sulphate, filtered and ether distilled off. The yellowish residue gave fine creamish yellow needles (7.5 g), m.p. 130° when crystallized from dilute methanol. Recrystallization from methanol raised the m.p. to 132°. (Found: C, 70.1; 70.4; H, 8.5, 8.4% active hydrogen value 3. Mass spectral molecular weight 570. $C_{33}H_{46}O_8$ requires: C, 70.3; H, 8.7%). Though the compound was slightly soluble in bicarbonate and completely soluble in 10% aqueous carbonate, it did not give a positive iodide-iodate and hydroxamic test for the presence of a carboxyl group. It gave an olive-green ferric colour.

Sodium hydroxide soluble fraction

Acidification of the alkali soluble fraction gave a yellowish brown oily matter which was extracted with ether. The ether extract was washed with water, dried and ether distilled off (4 g). The residue could not be crystallized. It gave a green ferric colour like octahydromorellin.

Neutral fraction

On distillation of the ether extract a yellowish brown oily matter was obtained. It gave a green ferric

colour. The residue could not be crystallized.

Methylation of phenol A by diazomethane

Phenol A (500 mg) was dissolved in dry ether (30 ml). To this excess of diazomethane in ether was added. This was kept at 20° overnight. Excess of diazomethane was destroyed by a few drops of acetic acid. Ether was distilled off and the residue crystallized from methanol. On slow evaporation shinning creamish yellow needles (450 mg) separated out m.p. 92°. Recrystallization from the same solvent raised the m.p. of the compound to 94°. (Found: C, 70.8, 70.9; H, 8.8, 8.9% - OCH_3 6.1% showed it to be a monomethyl derivative. $C_{34}H_{48}O_8$ requires: C, 70.7; H, 8.8%). The compound gave an olive-green ferric colour indicating the presence of chelated hydroxyl group. It did not give a positive hydroxamic test for the ester group.

Remethylation of the ester of phenol (A)

The ester of phenol A (200 mg) was dissolved in dry methanol (10 ml). Excess of diazomethane in ether was added and kept over-night at 20°. Excess of diazomethane was destroyed by a few drops of acetic acid, this was washed with water and ether distilled off. The residue was crystallized from methanol in fine yellowish needles m.p. 94°. It gave an olive green ferric colour.

Methylation of phenol A with dimethyl sulphate, potassium carbonate in acetone

A mixture of phenol A (200 mg), potassium carbonate (5 g), dimethyl sulphate (3 ml) and acetone (40 ml) was refluxed for 8 hrs, when a test portion was free from ferric colour. After distilling acetone, water was added and kept at R.T. overnight and extracted with ether. The ether extract was washed with water. Removal of ether gave a pale yellow residue which did not give ferric colour and had no tendency to crystallize.

Methylation of phenol A by methyl iodide, silver oxide in dimethyl formamide

A mixture of phenol A (500 mg) silver oxide (3 g) and methyl iodide (3 ml) in dimethyl formamide (40 ml) was shaken for 24 hr. This was filtered and dimethyl formamide distilled off over a water-bath under reduced pressure. The residue was extracted with benzene. This was passed through an alumina column and eluted with the same solvent. The eluate was almost colourless, which on removal of benzene gave a residue. The residue was crystallized from methanol in colourless cubes (350 mg) m.p. 90° (Found: C, 71.2; H, 8.4% - OMe 11.7% indicated two methoxyls. $C_{35}H_{50}O_8$ requires: C, 71.0; H, 8.1% OMe 12.2%).

Acetylation of phenol A with acetic anhydride and fused sodium acetate

A mixture of phenol A (200 mg), acetic anhydride (10 ml) and fused sodium acetate (1 g) was refluxed for 4 hr and poured over ice, left overnight in contact with water and extracted with ether. The ether extract was washed with water and ether distilled off. The pale yellow residue was crystallized from methanol (130 mg), m.p. 230°. The m.p. was the same as that of the kostinecki product obtained by Raghavan[1] (Found: C, 70.1; H, 7.7% acetyl value showed it to be a monoacetate. $C_{35}H_{46}O_8$ requires: C, 70.06; H, 7.9). It did not give ferric colour.

Pyrolysis of phenol A

Phenol A (1 g) was dissolved in diphenyloxide (30 ml) and refluxed for one hour. Then most of the diphenyloxide was distilled off. The residue was subjected to steam distillation. The residue was extracted with ether. The ether extract was separated into carbonate soluble and neutral fractions. The carbonate soluble fraction on acidification gave phenol A (500 mg).

The neutral fraction on removal of ether yielded a pale yellow residue (350 mg), which crystallized from methanol m.p. 192°. It gave a green ferric colour (Found: C, 71.8; H, 7.8% active hydrogen

value = 1, $C_{33}H_{44}O_7$ requires: C, 71.5; H, 8.1%).

Dehydration of phenol A with phosphorous oxychloride

Phenol A (100 mg) was dissolved in phosphorous oxychloride (10 ml). The flask was stoppered and the reaction mixture was heated on a water-bath for an hour, cooled and poured over crushed ice. After two hours it was extracted with ether, the unreacted phenol A was removed by giving a carbonate wash. The ether extract was washed with water, ether distilled off and the residue crystallized from methanol (40 mg), m.p. 192°. It showed a green ferric colour (Found: C, 71.8; H, 7.8%. $C_{33}H_{44}O_7$ requires: C, 71.5; H, 8.1%). The mixed m.p. with the pyrolysis product of phenol A was undepressed.

Hydrogenation of pyrolysis product

An attempt at the hydrogenation of the pyrolysis product of phenol A using 10% Pd/C was unsuccessful as it did not absorb hydrogen.

Decarboxylation of phenol A

To a solution of phenol A (100 mg) in diphenyl oxide (20 ml), copper bronze and a few drops of quinoline were added and refluxed for 4 hr. After filtration diphenyloxide was distilled off and traces of it were removed by steam distillation. The residue was taken in ether and washed with dil. hydrochloric

to remove quinoline. Unreacted phenol A was removed by washing the ether extract with 10% aqueous sodium carbonate. The ether extract was washed with water, removal of ether gave a residue which crystallized from methanol (30 mg) m.p. 192°. The mixed m.p. with the pyrolysis product was undepressed.

Hydrolysis of the pyrolysis product

The pyrolysis product (100 mg) was refluxed with 5% ethanolic potassium hydroxide (30 ml) for one hour. The reaction mixture was cooled, acidified with dilute hydrochloric acid and taken in ether. The ether extract was washed with 10% aqueous sodium carbonate. It appeared that most of the hydrolysed compound was soluble in carbonate, as the ether extract did not leave any residue after removal of ether.

The carbonate extract was acidified with dil. hydrochloric acid, taken in ether, washed with water and ether removed. The residue readily crystallized from methanol (80 mg) m.p. 132°, mixed m.p. with phenol A was undepressed.

Alkaline hydrolysis of the methyl ester of phenol A

The methyl ester of phenol A (100 mg) was refluxed for one hour with 5% ethanolic potassium hydroxide (30 ml), it was cooled, acidified and then taken in ether. The ether extract was washed with 10% aqueous sodium carbonate. The carbonate extract

on acidification and subsequent extraction with ether and removal of ether yielded a residue (90 mg) which could be readily crystallized from methanol (80 mg) m.p. 132°, mixed m.p. with phenol A was undepressed.

Mild alkaline hydrolysis of the acetate of phenol A

The acetate of phenol A (100 mg) was refluxed for one hour with 10% ethanolic potassium hydroxide (30 ml). It was cooled, acidified with dilute hydrochloric acid and extracted with ether. The ether extract was washed with 10% aqueous sodium carbonate and then with water. The carbonate extract and the aqueous washings were again acidified with dilute hydrochloric acid and taken in ether. The ether extract was washed with water and subsequently ether was distilled off. The residue readily crystallized from methanol (60 mg) m.p. 132°, mixed m.p. with phenol A was undepressed.

Hydrogenation of desoxymorellin

Desoxymorellin (1 g) was dissolved in methanol (50 ml), and hydrogenated over 10% Pd/C (300 mg) to absorb four mols (190 cc) of hydrogen. Filtration and distillation of ethanol gave a residue which after passing through a silica-gel column and elution with benzene gave a colourless crystalline product which

was recrystallized from methanol (800 mg) m.p. 110°.

Alkaline hydrolysis of octahydrodesoxymorellin

Octahydrodesoxymorellin (500 mg) was dissolved in 15% ethanolic potassium hydroxide (40 ml) and refluxed for eight hours over a water-bath. It was heated further to remove ethanol, cooled, acidified with dil. hydrochloric acid and extracted with ether. The ether extract was washed with 10% aqueous sodium carbonate. The carbonate extract was acidified with dil. hydrochloric acid and taken in ether. This was washed with water and ether removed. The amorphous residue (120 mg) had a m.p. 118°. As mentioned it was carbonate soluble.

Oxidation of dimethyl ether-ester of phenol A

To a solution of dimethyl ether-ester of phenol A (100 mg) in glacial acetic acid (20 ml) was added chromic acid (60 mg) and heated over a water-bath for one hour. This was diluted with water (20 ml) and then extracted with ether. The ether extract washed with water and ether distilled off. The residue was amorphous m.p. 98°.

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PART III

MOZINGO REDUCTION PRODUCTS OF MORELLIN

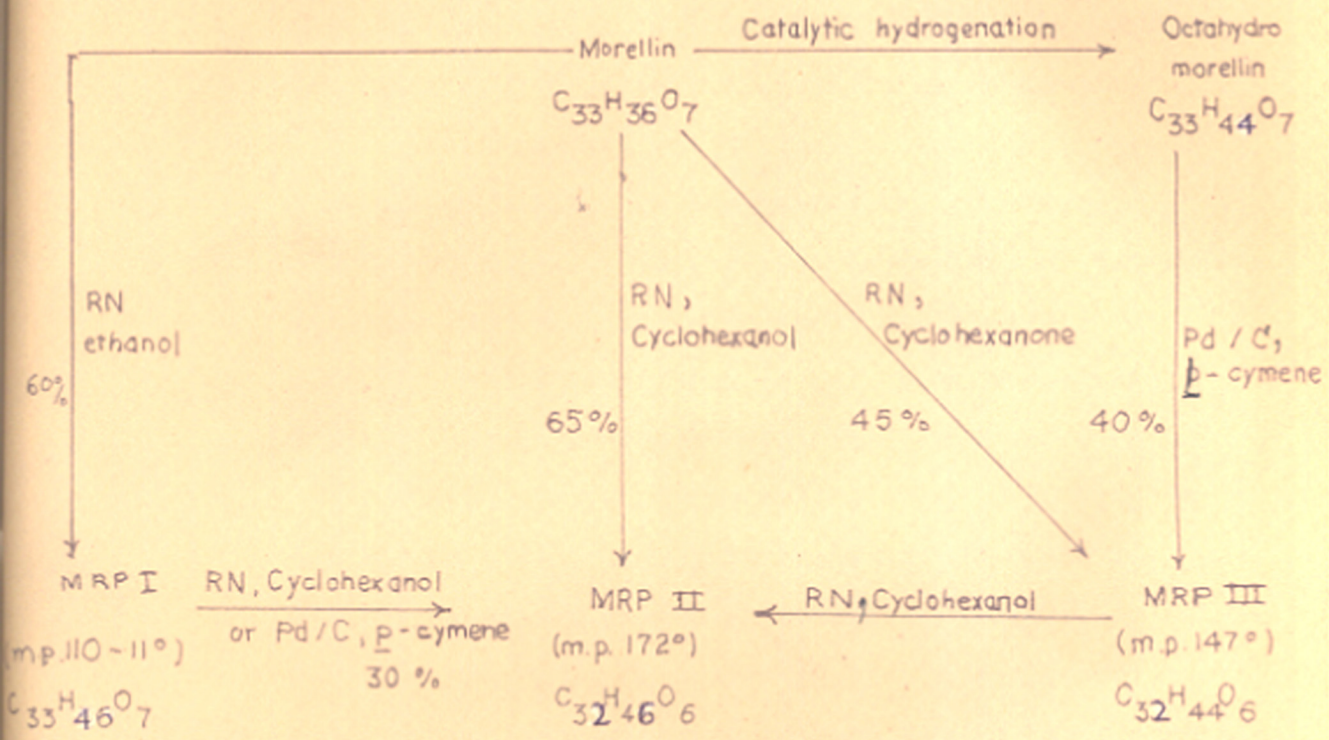
INTRODUCTION

It was observed that stable derivatives of morellin suitable for degradative work could be prepared by Raney-nickel reduction under the conditions first described by Mozingo[1], in which a massive proportion of the nickel catalyst to the substance (eg. 10:1) is employed. On subjecting morellin to this reaction in boiling ethanol, a colourless crystalline compound (MRP I $C_{33}H_{50}O_7$ m.p. 110°)[2] was obtained. The products obtained by using cyclohexanol and cyclohexanone as solvents are referred to as MRP II and MRP III respectively[3]. The basis for the use of cyclohexanol and cyclohexanone is the work of Kleiderer and Kornfield[4], who had carried out Raney-nickel reductions (e.g. Benzoin to dibenzyl) with cyclohexanol as hydrogen donor, as well as oxidations (cholestanol to cholestanone) with cyclohexanone as hydrogen acceptor. Chart I summarizes the results obtained by Raney-nickel reductions; the solvents mentioned were used at their boiling points.

On attempted dehydrogenation with 12 per cent palladium-charcoal in boiling p-cymene for 48 hours MRP I gave a crystalline compound (MRP II 172°) analysing for $C_{33}H_{48}O_6$, and therefore indicating that

Chart I

RN = Raney nickel



: Reduction product A.

: Reduction product B.

: Reduction product C.

I

dehydration rather than dehydrogenation had taken place. MRP II could also be prepared by a one-step reduction of morellin with Raney-nickel in boiling cyclohexanol, and also by subjecting MRP I to further treatment with Raney-nickel, but in cyclohexanol.

In sharp contrast with morellin, MRP II was a stable compound. It was unaffected by ethanolic sodium hydroxide at reflux under a variety of conditions and when much was not known about the chemistry of morellin, MRP II was found to be a most convenient starting point for further degradative work.

On the basis of the molecular weights by Rast method and their elementary analysis MRP I, II and III were assigned molecular formulae $C_{33}H_{50}O_7$, $C_{33}H_{48}O_6$ and $C_{33}H_{44}O_6$ respectively. A positive ferric colour and a deep wine-red colouration in the magnesium-hydrochloric acid test indicated a chromanone nucleus. Hence it was of interest to compare the ultra-violet spectra of reduction products I, II and III with the spectrum of 5,7-dihydroxy-2,2-dimethylchromanone. The chromanone had a maximum at 293 mμ and a broad weak band at about 345 mμ. The general similarity in the shape of the absorption curves showed the chomanone nature of MRP I, II and III, although the intensity of the peak at 300 mμ for MRP III is only about half that of the corresponding peak for MRP I.

The frequencies in the double bond region of the IR spectra of these reduction products showed a common band at 1640 cm^{-1} which was assigned to a chelated carbonyl as in 5-hydroxy chromanone. It is obvious that such a chelated carbonyl is unaffected by treatment with Raney-nickel under the conditions used by Mozingo. The other band is at 1745 cm^{-1} for MRP I and III, while for MRP II there is only a very weak absorption in this range. The usual assignments for a band at 1740 cm^{-1} are a 5-membered ring ketone and a lactone of a six-membered ring. Since these products were obtained after a vigorous Raney-nickel reduction, a ketone carbonyl was ruled out. The reduction products did not answer the hydroxamic acid test and hence the possibility of a lactone was ruled out. It was further observed from the IR spectra that only in MRP I and II there was absorption in the hydroxyl region (3460 cm^{-1}). There was no peak in that region of the IR spectrum of MRP III.

Stepwise degradations of MRP II were undertaken, one of which was an alkaline permanganate oxidation of MRP II yielding n-valeric acid. This showed the presence of a normal C_5 side-chain, but no other fragment of the molecule was isolated. Other oxidation experiments did not throw much light on the constitution of MRP II.

MRP II was fused with five times its weight of potassium hydroxide at 290° and the reaction products were given a functional separation. The bicarbonate soluble portion gave a mixture of steam-volatile fatty acids and a dicarboxylic acid $C_{12}H_{12}O_4$ m.p. 281° (decomp). A phenol $C_{16}H_{24}O_4$, m.p. 164° was obtained from the carbonate as well as sodium hydroxide fraction. The acid was characterized as napthalene 1-3 dicarboxylic acid which was later confirmed by its synthesis[3].

Further work on the C_{16} phenol obtained during the alkali fusion of MRP II showed that it had the structure (I)[5]. The characterization of C_{16} phenol did not throw much light on the constitution of MRP II. Both morellin and isomorellin gave the same crystalline reduction product. (MRP I) m.p. 110° on treatment with Raney-nickel in boiling ethanol and the elementary analysis indicated that it was a decahydro derivative of morellin.

The molecular weights for MRP I, II and III determined by Rast method were not reliable as shown by the mass spectra of these compounds which were made available later. Considering the complexity of the morellin molecule it was not possible for the earlier workers to assign structures to these reduction products in the light of the physical and chemical data available to them.

PRESENT WORK

After the elucidation of the structure of morellin and with the accumulation of chemical and spectral data, constitutional work on the various degradation products of morellin was undertaken.

On the basis of the mass spectral molecular weights for MRP I and II (554 and 526), chemical analysis and other evidence to follow, molecular formulae earlier assigned to these reduction products I, II and III had to be changed to $C_{33}H_{46}O_7$, $C_{32}H_{46}O_6$ and $C_{32}H_{44}O_6$ respectively.

Taking into consideration the molecular formula $C_{33}H_{36}O_7$ of morellin, it appeared that the basic skeleton was unaffected in all the reduction products. The four double bonds in the morellin molecule got saturated during the process of reduction. It could further be noted that the chelated carbonyl was unaffected in all the three cases as indicated by a green ferric colour. As described earlier, MRP II gave C_{16} phenol when it was subjected to drastic alkalifusion showing the presence of the 8-isoamyl-5-7-dihydroxy-2,2-dimethyl chroman moiety.

A comparison of the IR spectra of these compounds showed that only in the case of MRP I (Fig. 1) and II (Fig. 2) there was absorption in the hydroxyl

region while there is no such band in the spectrum of MRP III (Fig. 3). The alcoholic nature of one of the hydroxyl groups in MRP I and II was evident as the spectra of their methyl ethers still showed a band in the hydroxyl region.

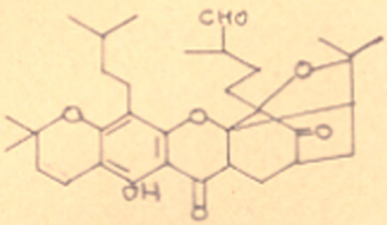
In the carbonyl region it was observed that there was a band at 1640 cm^{-1} common in all the three spectra, while the band at 1740 cm^{-1} which was probably due to the cyclic ketone was observed only in MRP I and III. It could therefore be concluded that only in the case of MRP II, it had undergone reduction to liberate a secondary hydroxyl group. The hydroxyl group in MRP I was apparently formed from the aldehyde group of morellin, as other carbonyl absorptions in its spectrum are unaffected. This was later confirmed from the NMR spectrum.

The NMR spectra of MRP I (Fig. 4), II (Fig. 5) and III (Fig. 6) show a total absence of the vinyl protons, and the characteristic aldehyde signal which are observed in the morellin spectrum. The signal due to chelated hydroxyl appeared around - 1.9 τ. Integration of the methyl region indicated 21 hydrogens i.e. seven methyl groups in all the three reduction products, while that of the methylene region showed 22, 20 and 20 protons for MRP I, II and III respectively.

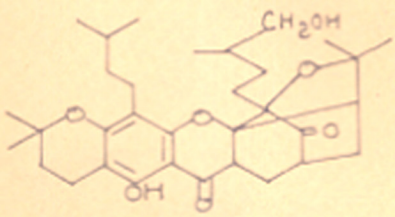
It could be easily observed from the IR and NMR

spectra that these reduction products are very closely related to each other and have the same basic skeleton as that of morellin. A consideration of the molecular formulae of $C_{33}H_{46}O_7$ of MRP I and octahydromorellin reveals that the former has a molecule of hydrogen more than the latter which has the structure (II). The NMR spectrum of MRP I (Fig 4) does not show an aldehyde signal but instead a signal appeared at 6.45τ integrating for two protons As mentioned earlier the chelated and the cyclic carbonyls were unaffected. Keeping in view these observations it could reasonably be expected that the aldehyde group gets reduced to a primary alcoholic group during the Raney-nickel reduction. These data in addition to the mass-spectral molecular weight (554) leave no doubt about the structure (III) assigned to MRP I. The molecular formula $C_{32}H_{46}O_6$ of MRP II, assigned on the basis of elementary analysis and mass spectral molecular weight (526) show that it has one carbon atom and one oxygen atom less than MRP I.

Preparation of MRP II from MRP I under dehydrogenating condition and its preparation by Raney-nickel reduction of morellin in cyclohexanol for 24 hr suggested the elimination of aldehyde group in the form of carbon monoxide might be taking place[6]. The other change taking place is the reduction of the cyclic ketone.

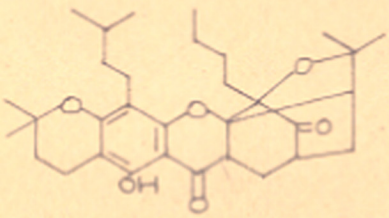


II

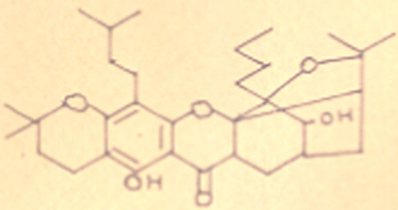


III

(A)



(C) V



IV

(B)

Considering the absence of an aldehyde group the absence of the cyclic carbonyl and the presence of an alcoholic hydroxyl.

In the light of the data cited so far the structure (IV) has been assigned for MRP II.

Similarly the molecular formula $C_{32}H_{44}O_6$ assigned to MRP III shows two hydrogen atoms less than for MRP II. The presence of the cyclic ketone as indicated from the IR spectra and the absence of the alcoholic hydroxyl in MRP III suggest that the carbonyl of the bicyclooctanone did not get reduced in presence of cyclohexanone (which is a hydrogen acceptor) and Raney-nickel. On the basis of these data MRP III has been assigned the structure (V).

IR spectra of MRP I, II and III

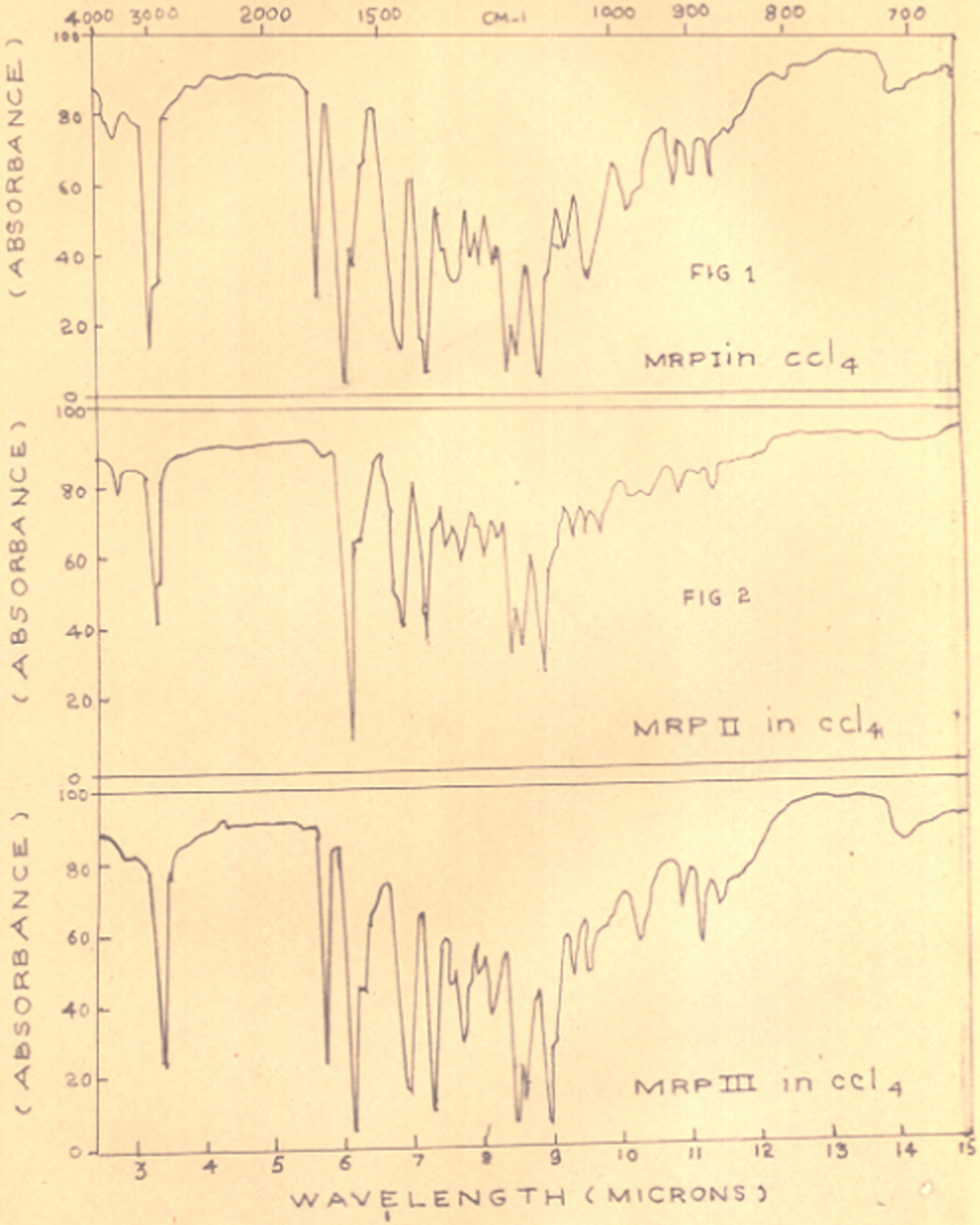


FIG 3

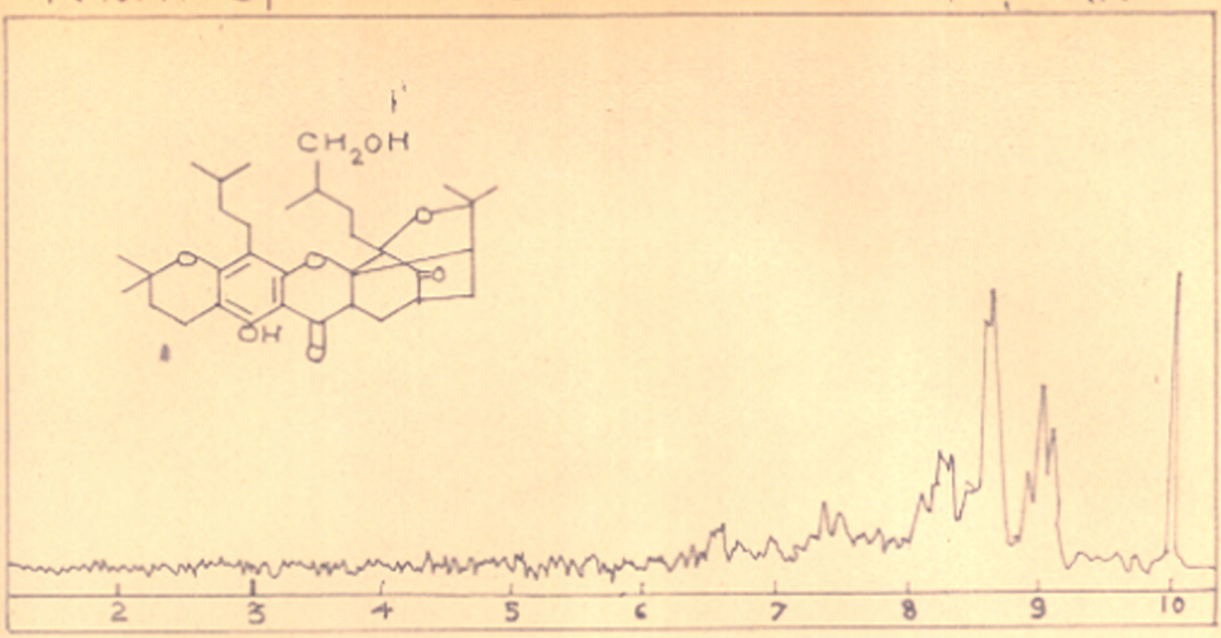


FIG 4 (CCl_4)

NMR Spectrum of MRP II methyl ether

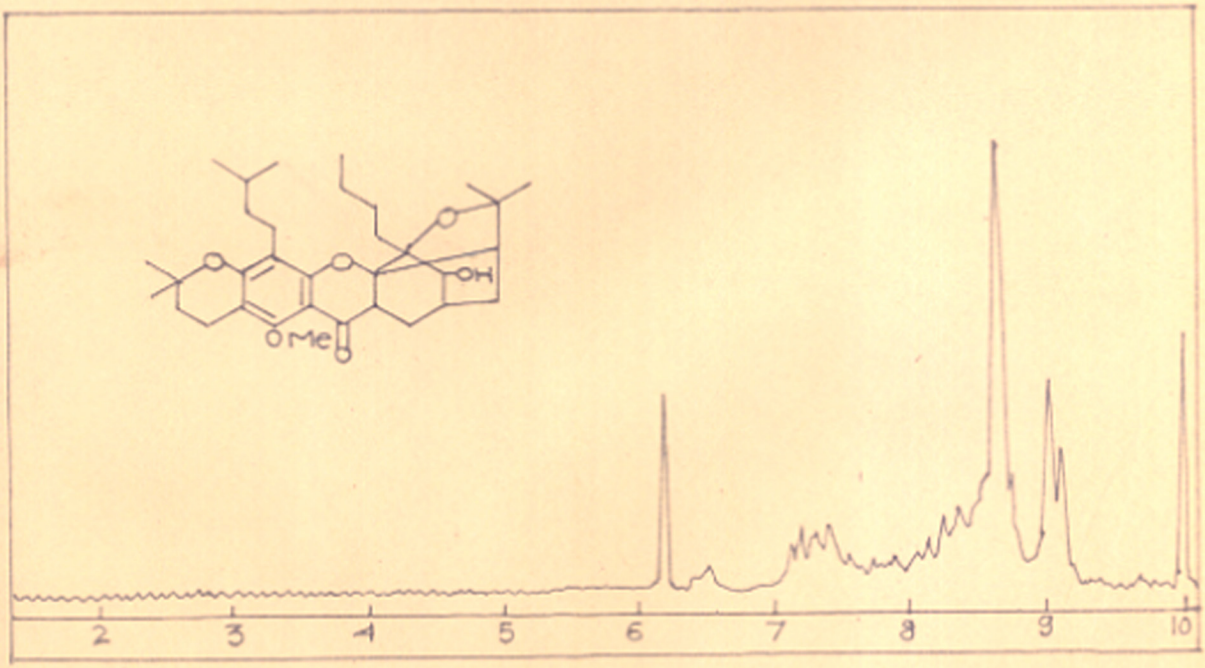


FIG 5 (CCl_4)

NMR spectrum of MRP III

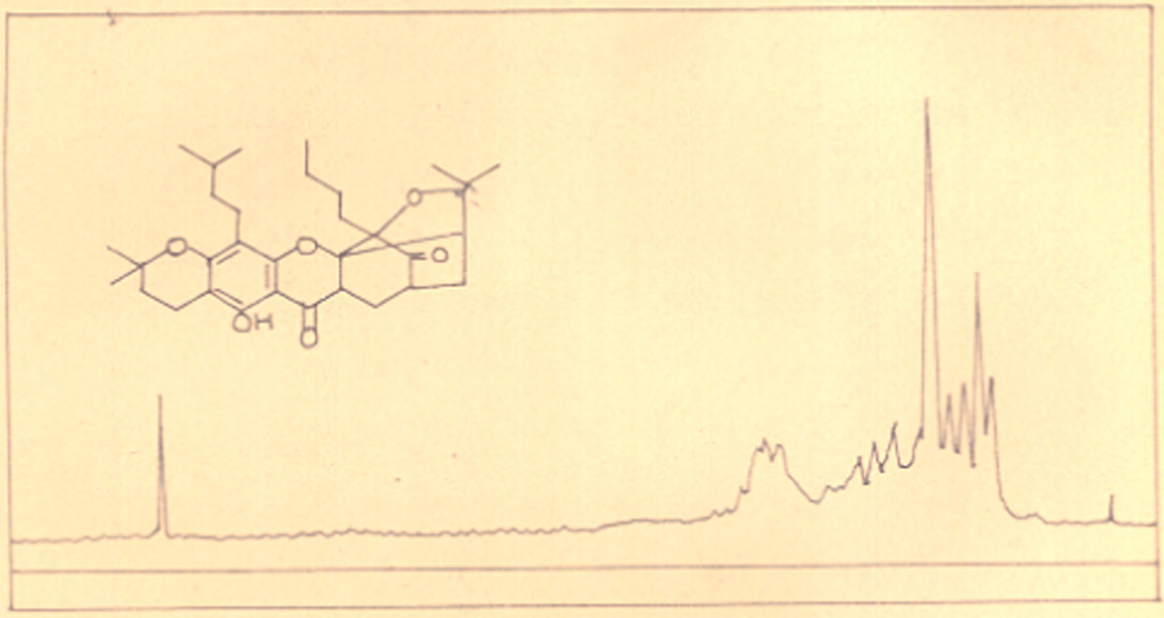


FIG 6 ($CDCl_3$)

EXPERIMENTAL

Methylation of MRP I

A mixture of MRP I (200 mg), dimethyl sulphate (1 ml), potassium carbonate (5 g) and acetone (30 ml) was refluxed for 5 hr, when a test portion of the reaction mixture did not show ferric colour. Acetone was distilled off from the reaction mixture. The residue (170 mg) after cooling, was treated with water and kept at RT for 24 hr. It was extracted with ether, the ether extract washed with water and ether distilled off. The residue had no tendency to crystallize.

Methylation of MRP II

MRP II was methylated by employing the same procedure as used for methylating MRP I. Methyl ether of MRP II was a colourless crystalline compound m.p. 152° (Found: C, 73.1; H, 8.1%. $C_{33}H_{48}O_6$ requires: C, 73.3; H, 8.5).

Methylation of MRP III

MRP III was also methylated, following the procedure described above. Its crystalline methyl ether had a mp. 122° (Found: C, 73.4; H, 8.3%. $C_{33}H_{46}O_6$ requires: C, 73.4; H, 8.4%).

Conversion of MRP III to MRP II

A mixture of MRP III (500 mg), Raney nickel (5 g) and cyclohexanol (10 ml) was refluxed on a hot plate

for 12 hr, filtered and cyclohexanone removed by steam distillation. The residue so obtained (450 mg) on chromatography over silic gel column using acetone-benzene (1:9) as eluent, gave a residue on removal of the solvent. The residue readily crystallized from methanol (210 mg) m.p. 172°. Mixed m.p. with MRP II was undepressed.

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PART IV

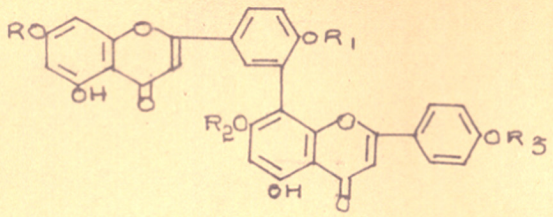
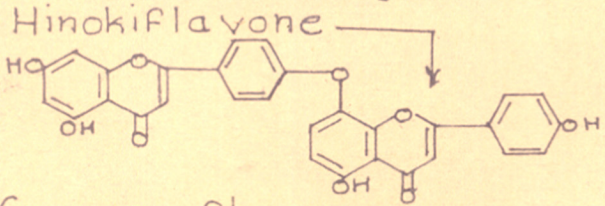
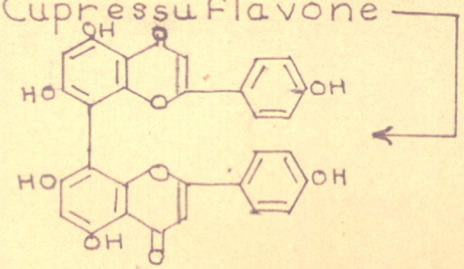
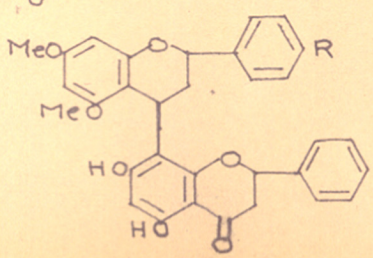
MORELLOFLAVONE: A NEW BIFLAVONOID FROM GARCINIA MORELLA

INTRODUCTION

The real beginning of the entry of the biflavonyl pigments as a class among the increasing number of natural products can be traced to the researches of Nakazawa[1] on ginkgetin (1941-55), of Kariyone and Kawano[2] on sciadopitysin and of Baker and Ollis[3] (1958) on ginkgetin, isoginkgetin and sciadopitysin, although mention must be made of the initial work of Furkawa[4] on ginkgetin which did not result in any concrete or correct structure proposal linking two C_{15} units. Table I lists the biflavonyl pigments isolated so far ~~are~~ of which are all apigenin derivatives. It however does not include those which are made up of two C_{15} units, but differ in the state of oxidation of the C ring and are called by different names such as biflavans and proanthocyanidins.

Some of the reactions which lead to the determination of the structure are hydrolysis with caustic potash or barium hydroxide and oxidation with potassium permanganate or alkaline hydrogen peroxide to yield the various smaller fragments. The clue to the nature of the linkage between the two C_{15} units lies in the isolation of the diphenyl or the diphenyl ether derivative depending on whether the link is Ar-Ar or Ar-o-Ar. So far the -C-C- link has been found

TABLE I

No.	Name and structure	occurrence	reference	
			Isolation structure	synthesis
1	Ginkgetin R=H R1=Me R2=H R3=	Ginkgo biloba	4	15
2	Isoginkgetin R=H R1=Me R2=H R3=Me	Ginkgo biloba	3	
3	Sciadopitysin R=Me R1=Me R2=H R3=Me	Umbrella pine (coniferales)	2,3	
4	kayaflavone R=H R1=Me R2=Me R3=Me	Torreya nucifera	7,8	
5	Sotetsuflavone R=H R1=H R2=Me R3=H	cycas revoluta (sotetsu)	8,9	
6	Amentoflavone R=H R1=H R2=H R3=H		10	
7	Bilobetin R=H R1=H R2=H R3=Me	Ginkgo biloba	11	
8	Hinokiflavone 	chamaecyparis obtusa	12,13	
9	Cupressuflavone 	cupressus torulosa	14	16
	 I = R = H Ia = R = OH			

to be between 3', 8; in certain cases the 3', 6 link has not been ruled out, but the fact that the compound undergoes methylation readily has been offered as an explanation in favour of the 3', 8 linkage. The only example of C-O-C link in biflavonoids is provided by hinokiflavone in which the units are combined through the oxygen at 4'-position and the carbon in the 8-position.

Very important light is shed by the UV spectra which is based on the fact that the chromophores if essentially the same in both units of the biflavonoid it would present a spectrum which would have the same absorption maxima as the parent flavonoid but will greatly differ in intensity. However deviations from this should be expected if the biflavonoid is built up of two dissimilar units. Also the shifts produced by the addition of alkali and other reagents have been very useful in the location of the hydroxyl groups[5].

Although these biflavonoid pigments would be expected to exhibit optical activity no such example is known so far and a study of similarly substituted diphenyls has shown that the steric disposition of the groups is not sufficient enough to permit the existence of optical isomers.

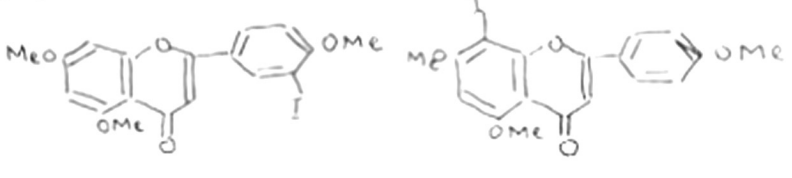
The present day investigations on these pigments have by and large done away with extensive degradative

experiments partly due to lack of availability of the material and partly because of the enormous data made available by physical methods. Examples of structure determination mainly from mass spectral data are the biflavan, xanthorrhone (I) and 4'-hydroxyxanthorrhone I and Ia respectively (I; R = H, Ia; R = OH) from the phenolic resins of Australian xanthorrohoea ("grass-trees")[6].

Later years have seen the isolation of only very few new biflavonyl pigments and a new feature of the recently isolated cupressuflavone is the linkage between the 8-positions of both the apigenin units.

The synthesis of ginkgetin tetramethyl ether by Nakazawa[15] by the Ullmann reaction between Ib and Ic established beyond doubt the 3'-8 linkage and it followed that the assumption of a similar linkage in related compounds is correct. The structure of cupressuflavone has also been confirmed by comparison of its hexamethyl ether with 8-8' biapigeninyl hexamethyl ether synthesized earlier by Nakazawa[16].

Biogenetically biflavonyls have been formulated as arising by oxidative coupling of flavonoid precursors[5].



Ib Ic

PRESENT WORK

Following the elucidation of the structure of morellin[17], a complex xanthone derivative occurring in the pericarp of the seeds of Garcinia morella, several new but closely related compounds have been reported[18] from the same plant; some form the subject matter of the previous parts of this thesis. During the course of the complete screening of the plant Garcinia morella, the heartwood has yielded a new biflavonoid, morelloflavone, the isolation and structure elucidation of which are presented below.

The isolation of morelloflavone (A) was achieved by extraction of the coarsely powdered heartwood with acetone. A preliminary purification of the residue from the acetone extract was effected by repeated chromatography on a silica gel column using acetone-benzene (2:3) as solvent. The resulting product was further purified by preparative layer chromatography on silica gel using the same solvent system; (A) crystallized from methanol in bright yellow cubes, m.p. 298° (decomp.). It gave a green colour with ethanolic ferric chloride and a deep wine-red colour in the Shinoda test.

By methylation with dimethyl sulphate and

potassium carbonate in acetone for two hours (A) formed a heptamethyl ether indicating the presence of seven phenolic hydroxyls. (A) was devoid of methoxyl groups as shown by its NMR spectrum. It failed to undergo hydrogenation under a variety of conditions including the use of Adams catalyst in glacial acetic acid at room temperature, which went to show the absence of any readily reducible double bond. From the elementary analysis of (A) and its methyl ether and the mass spectral M of the latter (654) kindly determined by Dr. R.S. Kapil, the molecular formula $C_{30}H_{20}O_{11}$ was assigned to (A). By hydrolysis with 15 per cent potassium hydroxide for 4 hr., the methyl ether of (A) gave a yellow crystalline compound, m.p. 116°, which did not exhibit the Shinoda test and gave a green ferric colour.

The UV spectrum of (A) (λ_{max}^{EtOH} 345, 288, 275, 258 mμ) was suggestive of a flavonoid skleton with probably a flavone ring containing a 4'-oxygenated B-ring.

UV spectra of a few flavones and flavanones in ethanol

No.	Compound	λ_{max}	λ_{max}	λ_{max}
1	Morelloflavone	345	288 275	255
2	Apigenin (5,7,4'-trihydroxy flavone)	340	269	-
3	Naringenin (5,7,4'-trihydroxy-flavanone)	330*	284	-
4	Luteolin (5,7,3',4'-tetrahydroxy flavone)	350	268*	255
5	Eriodictyol (5,7,3',4'-tetrahydroxy flavanone)	330	289	-

*Inflection

The IR spectrum of (A) (Fig. 1) showed a broad band at around 3200 cm^{-1} and a strong band at 1645 cm^{-1}, the former assignable to OH groups and the latter to the C=O of a 5-hydroxyflavone. The spectrum of the methyl ether (Fig. 2) showed bands at 1670, 1645 and 1600 cm^{-1} and the hydroxyl region was transparent. The shift of one of the C=O bands to higher frequency on methylation is reminiscent of a similar shift in going from a 5-hydroxychromanone or 5-hydroxyflavanone to its methyl ether. The spectrum of the hydrolysis product (Fig. 3) of the methyl ether of (A) in nujol is also devoid of

absorption in the hydroxyl region and a strong band at 1645 cm^{-1} with a shoulder at 1655 cm^{-1} is characteristic of a strongly chelated C=O group.

The NMR data* throw further light on the structural features of morelloflavone. The spectrum in deuterated acetone (Fig. 4) showed signals in the region between 2 and 6 The spectrum in pyridine is transparent, showing the absence of methoxyls and aliphatic protons. A pair of doublets at 4.05 and 4.95 (J = 12 cps), the former partly merged with a signal at 3.9, do not represent vinyl protons since the compound failed to undergo hydrogenation. The proton appearing at 4.05 is probably α to oxygen and adjacent to a phenyl ring as in a flavanone; and since a methylene group is absent and the other proton appears at 4.95 as a doublet, it is probable that 3-position of the flavanone is substituted. The high field signals at and around 3.9 representing aromatic protons are ascribable to those of a phloroglucinol nucleus. The other signals appear as clusters between 3.2 and 3.64 and 2.3 and 3.0. A sharp singlet at 2.75 is due to the 3-proton of a flavone, and a broad hump at 5.5 represents phenolic hydroxyls.

The NMR spectrum of the methyl ether of (A) (Fig. 5) shows seven methoxyls between 6.0 and 6.4

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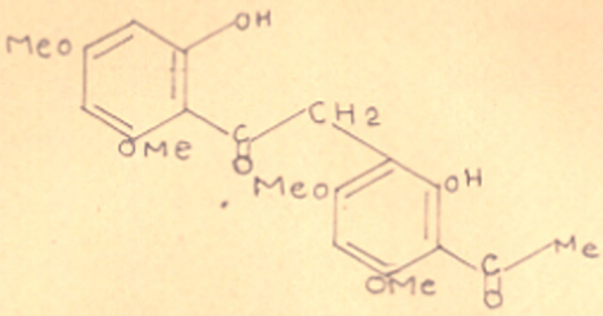
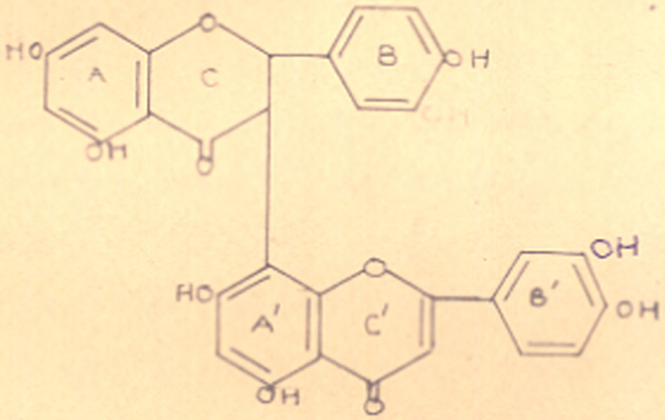
*Chemical shifts are cited on the τ scale.

and a pair of doublets at 4.14 and 5.09. The other signals appear between 2.5 and 3.9. The spectrum of the hydrolysis product of the methyl ether of (A) (Fig. 6) is very revealing, and shows the presence of an acetyl group as a sharp singlet at 7.40. Three sharp singlets at 6.18, 6.10 and 5.75 are seen; the first two correspond to two methoxyls each and the last probably represents an uncoupled methylene group. A broad multiplet at 3.9 integrating for 3 protons is undoubtedly due to aromatic protons flanked on either side by methoxyls and/or hydroxyls because of its high-field line position. In the low field region two singlets at -3.8 and -3.9 are obviously due to chelated hydroxyls. The mass spectral molecular weight of the hydrolysis product (390), kindly determined by Dr. Hanus, together with the above data lead to the structure (II).

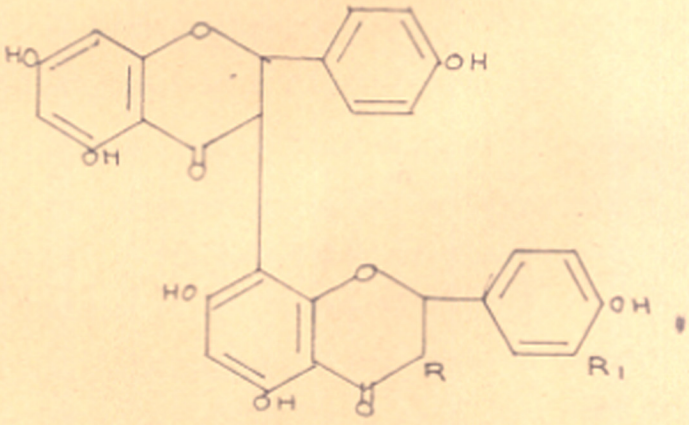
Since an acetyl group has been liberated by alkaline hydrolysis, the presence of a flavone nucleus is indicated; and as seven aromatic protons and three methoxyls have been lost, the cleavage must involve the loss of two aromatic rings. The hydrolysis product being a deoxybenzoin, one of the aromatic rings lost by hydrolysis must be the B-ring of flavanone. Morello-flavone has the structure (III), in which the seventh



II



III



IV R = OH R' = H

V R = H R' = H

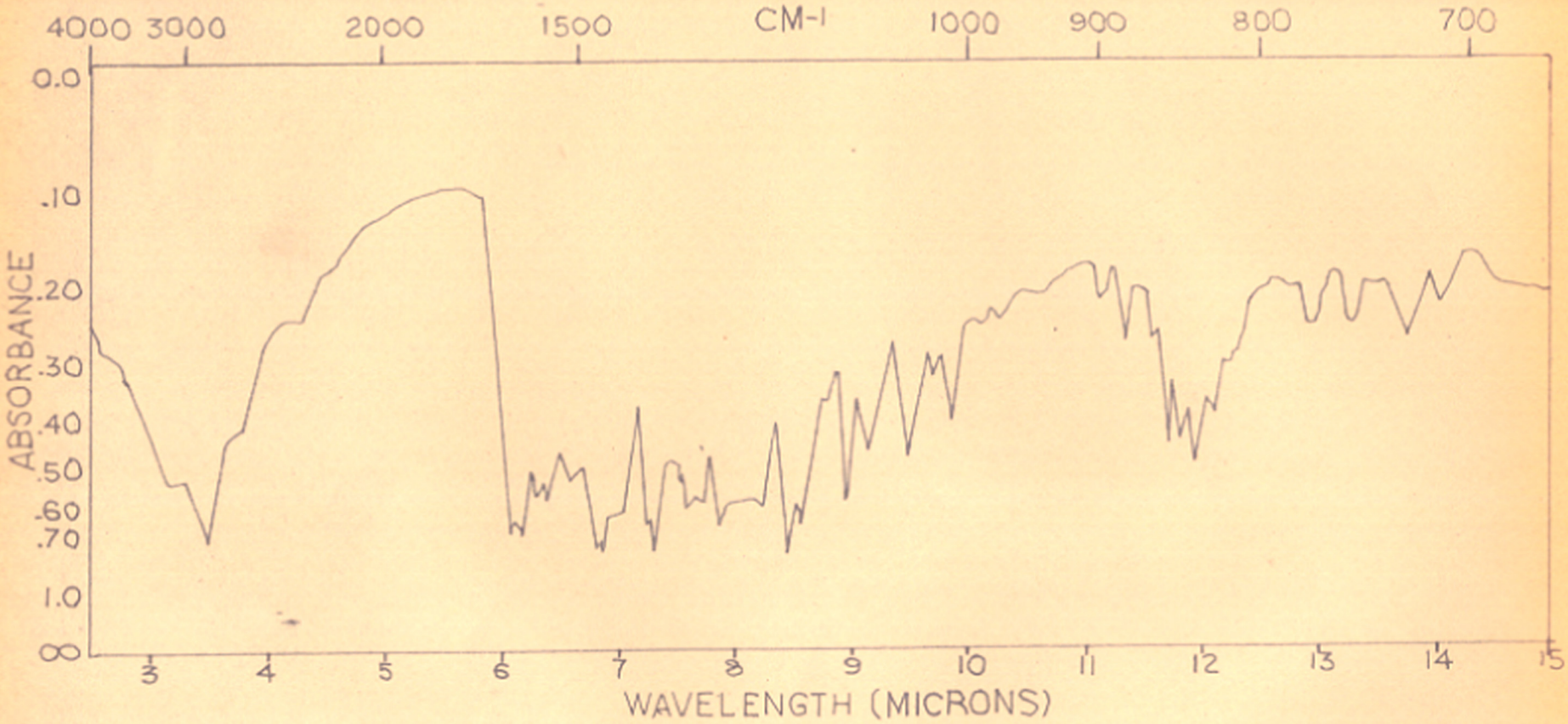
VI R = OH R' = OH

hydroxyl group may be in either of the two B-rings.

In addition to (II), the isolation of acetoveratrone and veratric acid by the mild alkaline hydrolysis of heptamethyl ether of (III), establishes the 3',4'-hydroxylation pattern in the B-ring of the flavone moiety which is also consistent with the NMR data. Since in the spectrum of the methyl ether of (III) the lowest signal in the aromatic region is a quartet.

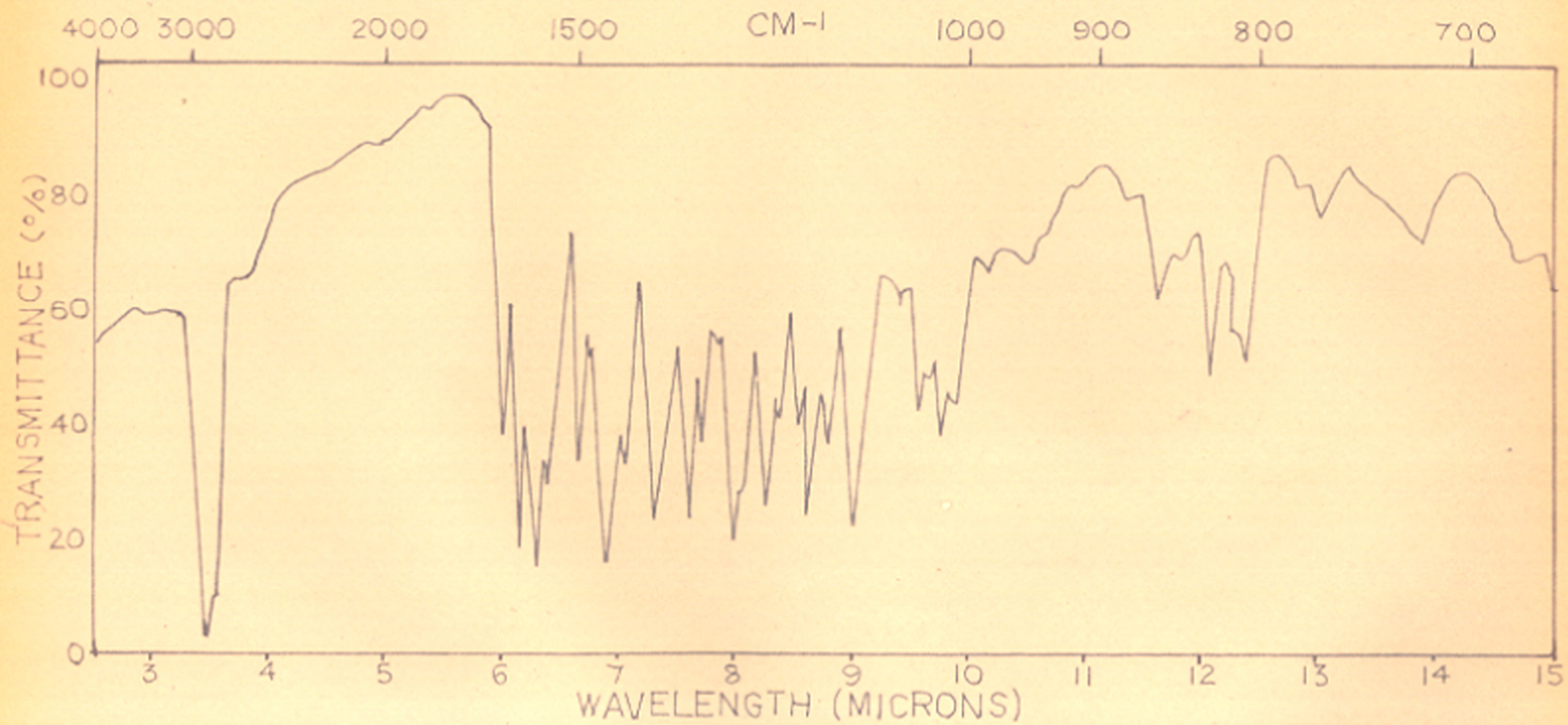
The appearance of a fragment at m/e 547 in the mass spectrum of the methyl ether of (III) confirms that the B-ring of the flavanone has only one methoxyl group[19-20] and it follows from the NMR data that it is at the 4'-position. The mass spectrum shows major peaks at 654, 547, 474, 181, 180, 152, 137 and 121 which are explicable on the basis of the fragments that could be represented by structures as shown in Chart I. (Please refer to page 133)

Morelloflavone is a novel biflavonoid in which a flavanone and a flavone are linked in the 3 and 8 positions. While this part of the thesis was being written a paper appeared in Tetrahedron Letters reporting the isolation of three new closely related biflavonoids IV, V and VI from the heartwood of Garcinia buchananii, and their structure elucidation on the basis of NMR and mass spectral data[18]. These three flavonoids along with morelloflavone represent the class of biflavonoids in which the two flavonoid units have a 3-8 linkage.



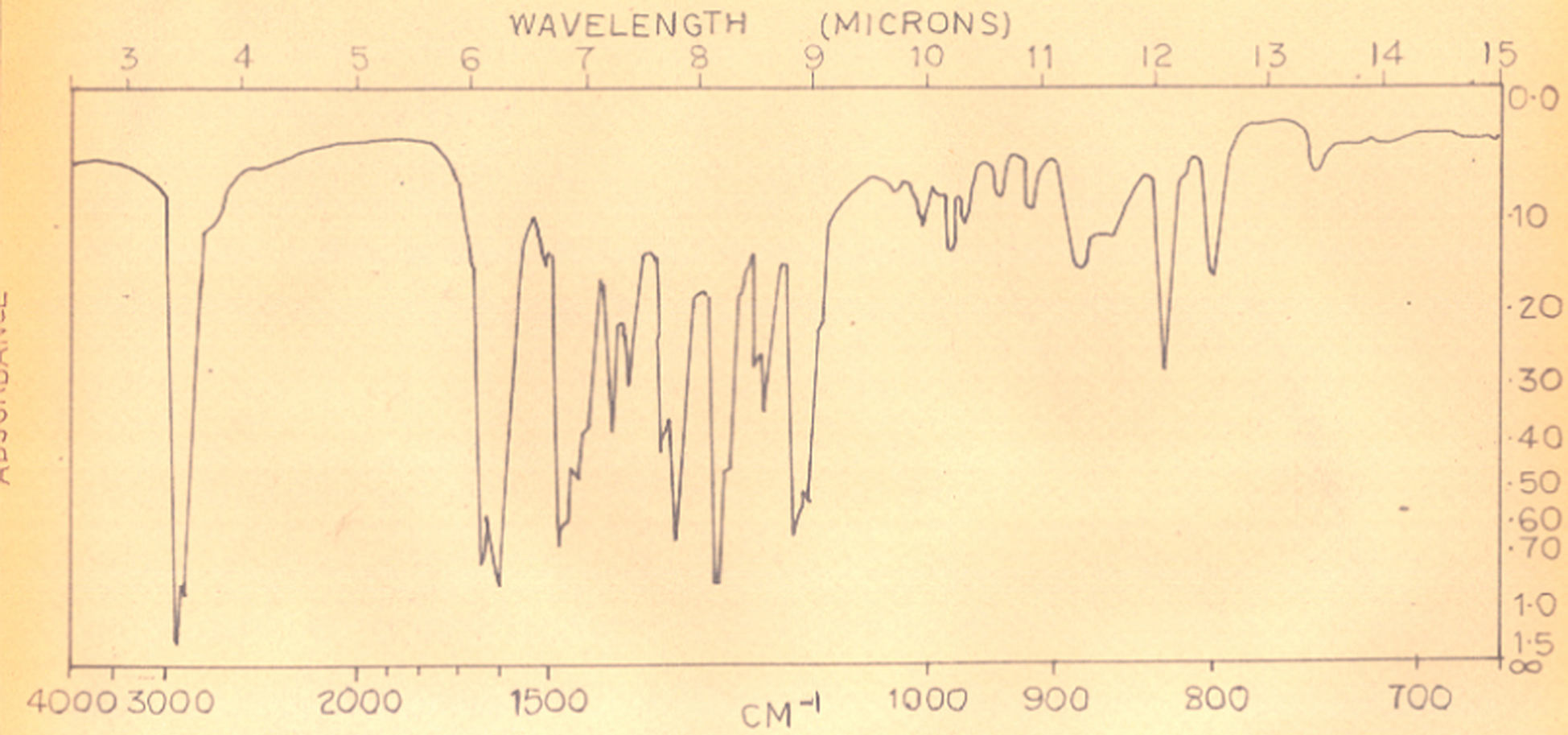
IR spectrum of morelloflavone in Nujol

FIG 1



I R spectrum of heptamethylether of morelloflavone in Nujol

FIG 2



IR spectrum of hydrolysis product of heptamethylether of morelloflavone

FIG 3

NMR spectrum of
methyl ether of morelloflavone

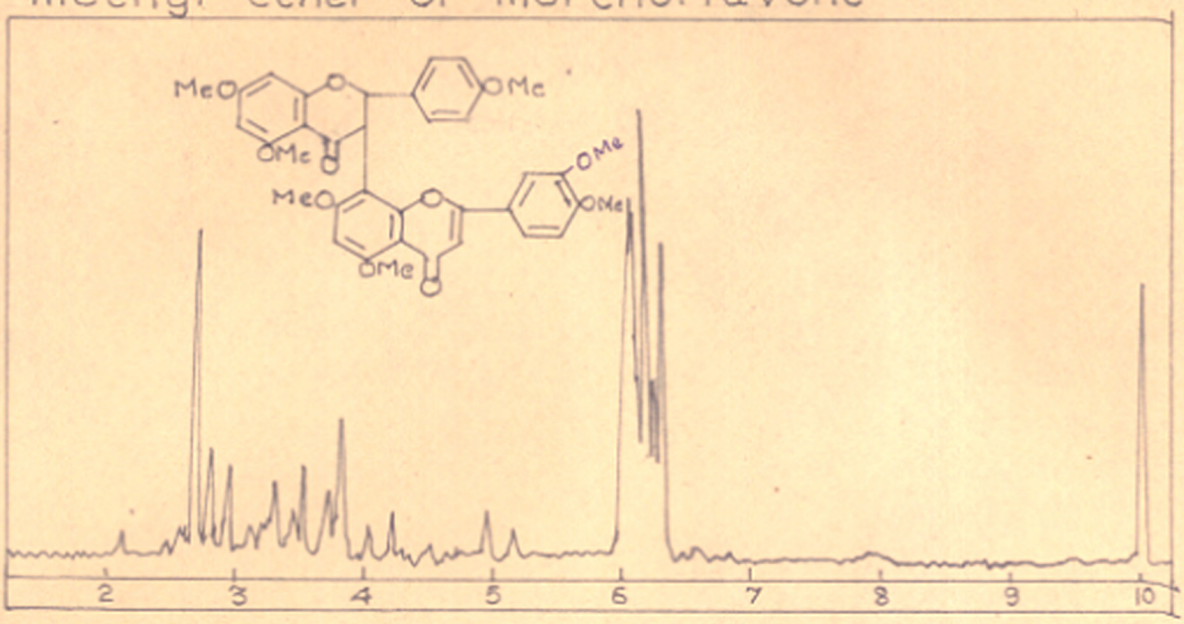


FIG 5 ($CDCl_3$)

NMR spectrum of
morelloflavone

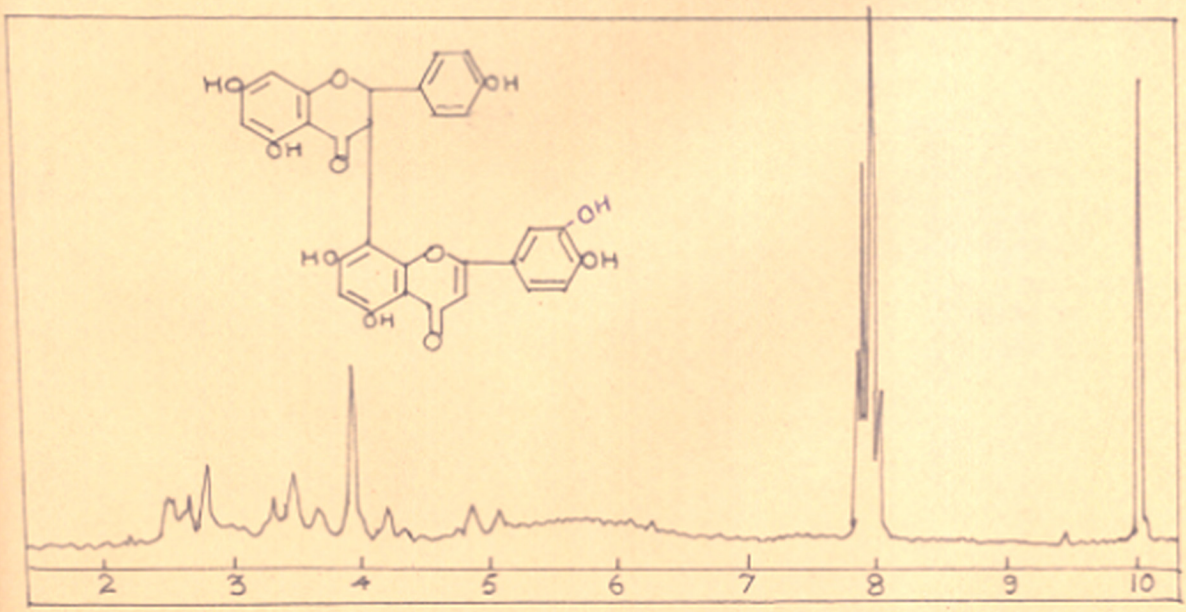


FIG 4 (Deuterated acetone)

NMR data on heptamethyl ether of morelloflavone

Solvent = $CDCl_3$

Chemical shift	Multiplicity	No of H	Assignment
6.0 - 6.4	-	21	7 - OMe groups
5.09	d (J = 12 cps)	1	3-proton of flavanone
4.14	d (J = 12 cps)	1	2-proton of flavanone
3.9	s	2	6- and 8- protons of the A-ring of flavanone
3.8	s	1	6-proton of the A-ring of flavone
3.65	d (J = 9.5 cps)	2	3',6'-protons of B-ring of flavanone
3.3	d (J = 8 cps)	1	5'-proton of B-ring of flavanone
3.1	d (J = 2.5 cps)	1	2-proton of flavanone
2.9	s	1	3-proton of flavone
2.75	d (J = 9.5 cps)	1	5'-proton of B-ring of flavone
2.67	q	1	6'-proton of B-ring of flavone

NMR data on morelloflavone

Solvent = (Deuterated acetone)

5.5	bs	5	5-phenolic -OH's
4.95	d (J = 12 cps)	1	3-proton of the flavanone
4.05	d (J = 12 cps)	1	2-proton of the flavanone
3.9	s	2	2 aromatic protons of A-ring and one
3.85	s	1	one aromatic proton of A-ring
3.20-3.64	-	4	4 aromatic protons adjacent to hydroxyls
2.30-3.0	-	3	Aromatic hydrogens 3
2.75	s	1	3-proton of the flavone

Two chelated -OH were observed at -2.6 and -3.50 when the spectrum was recorded in deuterated (DMSO)

NMR spectrum of hydrolysis product of heptamethyl ether of morelloflavone

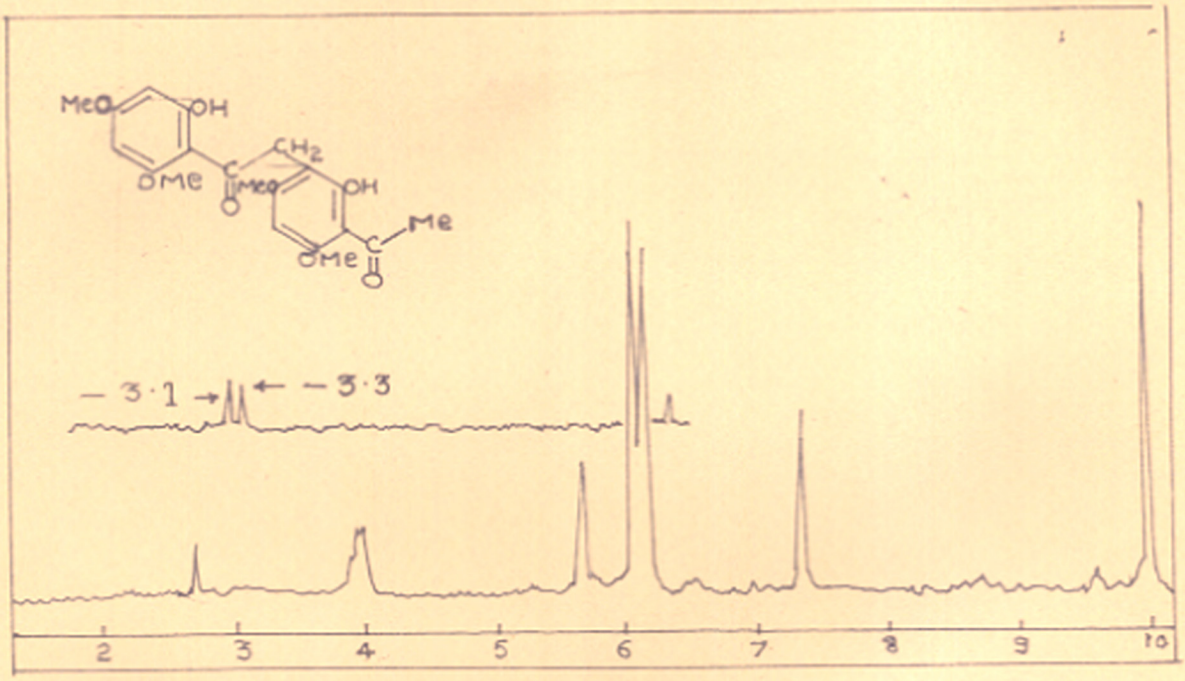


FIG 6 ($CDCl_3$).

NMR data on the hydrolysis product of heptamethyl ether of morelloflavone Fig.6

Solvent = $CDCl_3$

Chemical shift	Multiplicity	No.of H	Assignment
7.4	s	3	-COMe
6.1 and 6.18	s	6	4 -OMe
5.75	s	2	$-CH_2-$ flanked between a carbonyl and -Ar-group
3.95	bm	3	3 aromatic H of a phloroglucinol nucleus
-3.1 and -3.3	s	2	2 chelated -OH group

EXPERIMENTAL

Isolation of morelloflavone (A)

The coarsely powdered heartwood of *Garcinia morella* (1.2 kg) was extracted with acetone (3 L), filtered and acetone distilled off. The residue was extracted with a mixture of acetone-benzene (4:6). A preliminary purification of this solution was effected by repeatedly passing through a silica gel column using the same solvent for elution. Further purification was achieved by preparative layer chromatography on the same adsorbent using the same solvent system. The purified product was crystallized from methanol (2.5 g) m.p. 298° (decomp.). (Found: C, 65.3; H, 3.6%. $C_{30}H_{20}O_{11}$ requires: C, 65.7; H, 4.0%). It showed a green ferric colour and gave a wine-red colour in the Shinoda test.

Methylation of (A)

A mixture of (A) (1 g), acetone (80 ml) potassium carbonate (15 g) and dimethyl sulphate (6 ml) was refluxed for 2 hr, when a test portion of the reaction mixture did not show ferric colour. Acetone was distilled off from the reaction mixture and the residue treated with water, kept at R.T. for 24 hr, filtered and washed with water. The crude product was purified

by passing it through an alumina column using acetone-benzene (3:7) as solvent. The solvent was distilled off and the residue crystallized from methanol in pale yellow cubes (750 mg) m.p. 198°. (Found:C, 68.2; H, 5.5; -OMe, 31%. $C_{37}H_{34}O_{11}$ requires: C, 67.9; H, 5.2. $-OCH_3$, 32%). The compound did not show ferric colour but gave a wine red colour in the Shinoda test.

(a) Hydrolysis of the methyl ether of (A)

Methyl ether of (A) (500 mg) was dissolved in 15 per cent ethanolic potassium hydroxide (15 ml) and refluxed for 4 hr, cooled, diluted with water (10 ml) and acidified with dilute hydrochloric acid. The acidified reaction mixture was taken up in chloroform, washed with water and chloroform distilled off. The major component was separated by preparative layer chromatography using silica gel as adsorbent and acetone-benzene (1:9) as the solvent system. The bright yellow band (Rf 0.7) was removed from the plates, extracted with methanol and crystallized from the same solvent (70 mg). Recrystallization gave bright yellow cubes (40 mg) m.p. 116°. It gave a green ferric colour and was soluble in aqueous sodium carbonate.

(b) Hydrolysis of the heptamethyl ether of (A):

A mild hydrolysis of heptamethyl ether of (A)

(500 mg) by 7% ethanolic potassium hydroxide (30 ml) for 6 hr, on cooling followed by acidification with dilute hydrochloric acid gave an amorphous solid. This was extracted with chloroform . The chloroform extract was washed with 10% aqueous sodium carbonate. The carbonate extract on acidification with dil. hydrochloric acid gave an amorphous solid which was again extracted with chloroform and the solvent distilled off. The residue on chromatography over a silica-gel column using acetone-benzene (1:19) as eluent, gave a compound which readily crystallized from benzene-hexane (40 mg) m.p. of the crude crystalline compound was 182°. The compound neither showed ferric colour nor did it give a positive Shinoda test. The compound ~~is probably an acid which is being further investigated~~ was characterised as veratric acid.

Hydrogenation of (A)

(A) (100 mg) was dissolved in glacial acetic acid (10 ml). This was added to a suspension of Adams' catalyst (30 mg) in glacial acetic acid (10 ml), which was saturated with hydrogen, and stirred for 6 hr.(A) did not absorb any hydrogen. After filtration and removal of solvent (A) was recovered unaffected.

Acetylation of (A)

(A) (100 mg) was dissolved in acetic anhydride (5 ml) and a few drops of pyridine were added. This

was kept at R.T. for 24 hr, poured over crushed ice, kept for 24 hr, filtered and the residue washed with water and dried. The residue was amorphous and had no tendency to crystallize, m.p. above 300°. However the compound did not show ferric colour.

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PART V

PIGMENTS OF *GARCINIA XANTHOCHYMUS*

XANTHOCHYMOL (A)

PRESENT WORK

The peels of the fruits of Garcinia xanthochymus were kept soaked in benzene for 3 days and the solution that developed an orange colour was filtered. Removal of benzene gave a dark brown viscous oil. A benzene solution of this was chromatographed on a silica gel column using acetone-benzene (1:9) as eluent. The yellow eluate on removal of the solvent gave an oil which crystallized from hexane or carbon tetrachloride in lemon yellow needles, m.p. 135°.

From the colour reactions given below (A) is not indicated to be a flavone or a xanthone.

Colour reactions of (A)

Shinoda test	yellow
Asahina test	red and yellow after the addition of conc. hydrochloric acid
Alcoholic ferric chloride	green
Conc. sulphuric acid	orange yellow
Gibbs test	negative
Gossypetone test	negative
o-Dinitrobenzene in aq. sodium hydroxide for o and p-dihydroxy compounds	negative

However, the colour produced in the Asahina test indicated that xanthochymol probably had a skeleton similar to that

of morellin (A) was soluble in aqueous sodium hydroxide and totally insoluble in aqueous sodium carbonate and suggested the absence of a carboxyl group.

The mass spectral molecular weight (602), kindly determined by Prof. Shannon of CSIRO (Australia), and the elementary analysis agree with the molecular formula $C_{38}H_{50}O_6$. The presence of two active hydrogens is indicated by the shift of the molecular ion by two when exchanged with D_2O.

The UV spectrum of (A) in ethanol showed absorptions at 270 mμ and 224-230 mμ. A comparison of the UV spectra of (A) and morellin (λ_{max}^{EtoH} 236, 288 and 360 mμ) revealed the absence of a chromophore conjugated with the chromene system which is characterised by a band at 350 mμ.

Hydrogenation of (A) over Pd/C catalyst indicated the presence of five double bonds. Methylation of (A) and its decahydroderivative by dimethyl sulphate and potassium carbonate in acetone did not give crystalline methyl ethers. (A) did not give a crystalline acetate when acetylated with acetic anhydride and pyridine. However these experiments showed that xanthochymol had two phenolic hydroxyl groups. Ozonolysis of (A) gave formaldehyde and acetone, which were characterised as their dinitrophenylhydrazone derivatives. The residue obtained in the ozonolysis experiment is being examined.

The IR spectrum of (A) in chloroform showed bands at 3546, 3333, 1724, 1658 and 1639 cm^{-1}. The bands at 1658 and 1639 cm^{-1} could be assigned to a conjugated carbonyl group and C=C vibrations respectively. The absorption at 1724 cm^{-1} could be assigned provisionally to a six-membered ring carbonyl. The absorption in the hydroxyl region was due to hydroxyl groups.

The IR spectrum of decahydroxanthochymol in carbon tetrachloride showed bands at 3250, 1745, 1690 and 1625 cm^{-1}. The new band at 1690 cm^{-1} suggested the presence of an α,β-unsaturated carbonyl group in xanthochymol.

The NMR spectrum of (A) in deuterated DMSO indicated a large number of protons in different environments. The signals in the region between 2.7 and 3.4 integrating for three protons appeared to represent aromatic or vinyl protons. A signal at 2.82 can probably ascribed to lone aromatic proton since it did not disappear on hydrogenation. A broad signal at 5.09, from its nature, appeared to represent vinyl protons of three prenyl side-chains. The signals at 5.50 and 5.35 are attributable to a terminal methylene group. The remaining protons absorbed between 7.20 and 9.20. These represented methylenes and methyls contained in prenyl side-chains or in similar

surroundings and on saturated carbon atoms.

In the NMR spectrum of the hydrogenated product the region between 3.0 and 6.0 was transparent and indicated that the signals at 5.50, 5.35, 5.09 and 3.17 in the parent compound represented vinyl protons. A singlet at 2.82 showed the presence of a lone aromatic proton.

The IR spectrum of the methyl ether of xanthochymol did not show absorption in the hydroxyl region and displayed bands at 1724, 1666, 1639 and 1590 cm^{-1}.

The IR spectrum of the methyl ether of decahydroxanthochymol showed bands at 1740, 1690, 1655 and 1600 cm^{-1}. In view of the absence of absorption in the hydroxyl region, the absence of an alcoholic hydroxyl group could be inferred with certainty. Further work is in progress.

Table I

IR data cm^{-1}

Xanthochymol	Methyl ether of xantho-chymol	H_{10} xantho-chymol	Methyl ether H_{10}-xantho chymol
3333, 3546	-	3280, 3490	-
1725	1724	1740	1740
1658	1666	1695	1690
1639	1639	1625	1655
894	885	-	1600

Mass spectral data

The molecular ion at m/e 602 confirms the molecular formula. Deuteration *in situ* shifted the molecular ion by two mass units showing the presence of two exchangable hydrogen atoms. Peaks corresponding to the loss of a molecule of water, carbon monoxide and C_5H_9 ion are significant. Several metastable peaks are present to support the fragmentation modes.

The direct loss of a fragment with m/e 137 as charged and neutral species from the molecular ion is observed. A fragment with mass 136 is also lost from the molecular ion (m/e 466). This rearrangement involves the transfer of a proton from the $C_{10}H_{17}$ side-chain (m/e 137) to the rest of the molecule (carbonyl α to

the side-chain)

m/e 602 ——— (A) ——— m/e 466 + 136 (neutral fragment)

m/e 602 ——— (B) ——— m/e 465 + m/e 137 (both as charged and neutral species)

The peaks at m/e 466 and m/e 465 have relatively the same intensity.

In the mass spectrum of the hydrogenated product (heated inlet) the molecular ion was not observed. However, a peak is observed at m/e 474 (466 + 8 = 474) which corresponds to the rearranged fragment observed in the spectrum of xanthochymol (in the loss of 136). No straight cleavage product corresponding to the loss of C_{10} side-chain is obtained. The fragment at m/e 136 appears to have the composition $C_{10}H_{16}$ and is probably devoid of oxygen. Obviously it contains 3 double bonds. One double bond is formed during rearrangement. The $C_{10}H_{17}$ fragment can be constituted as containing either one double bond and a ring or two double bonds. Since other evidences show the presence of five double bonds the $C_{10}H_{17}$ fragment is probably monocyclic with one double bond. The remaining centres of unsaturation are in the rest of the molecule.

Peaks corresponding to the direct loss of 151 and 123 mass units from the parent ion are observed in

the spectrum of xanthochymol. The fragments (A) and (B) appear to lose 123 and 124 mass units in one step to give peaks at m/e 343 and 341 respectively. The loss of 123 is a result of straight cleavage and 124 is formed by a rearrangement.

$$\text{m/e } 466 \xrightarrow[\text{Calculated } 252.4]{\text{Found } 252.5} \text{m/e } 343 + 123$$

(Both as neutral and charged species)

$$\text{m/e } 465 \xrightarrow[\text{Calculated } 250.0]{\text{Found } 250.0} \text{m/e } 341 + 124$$

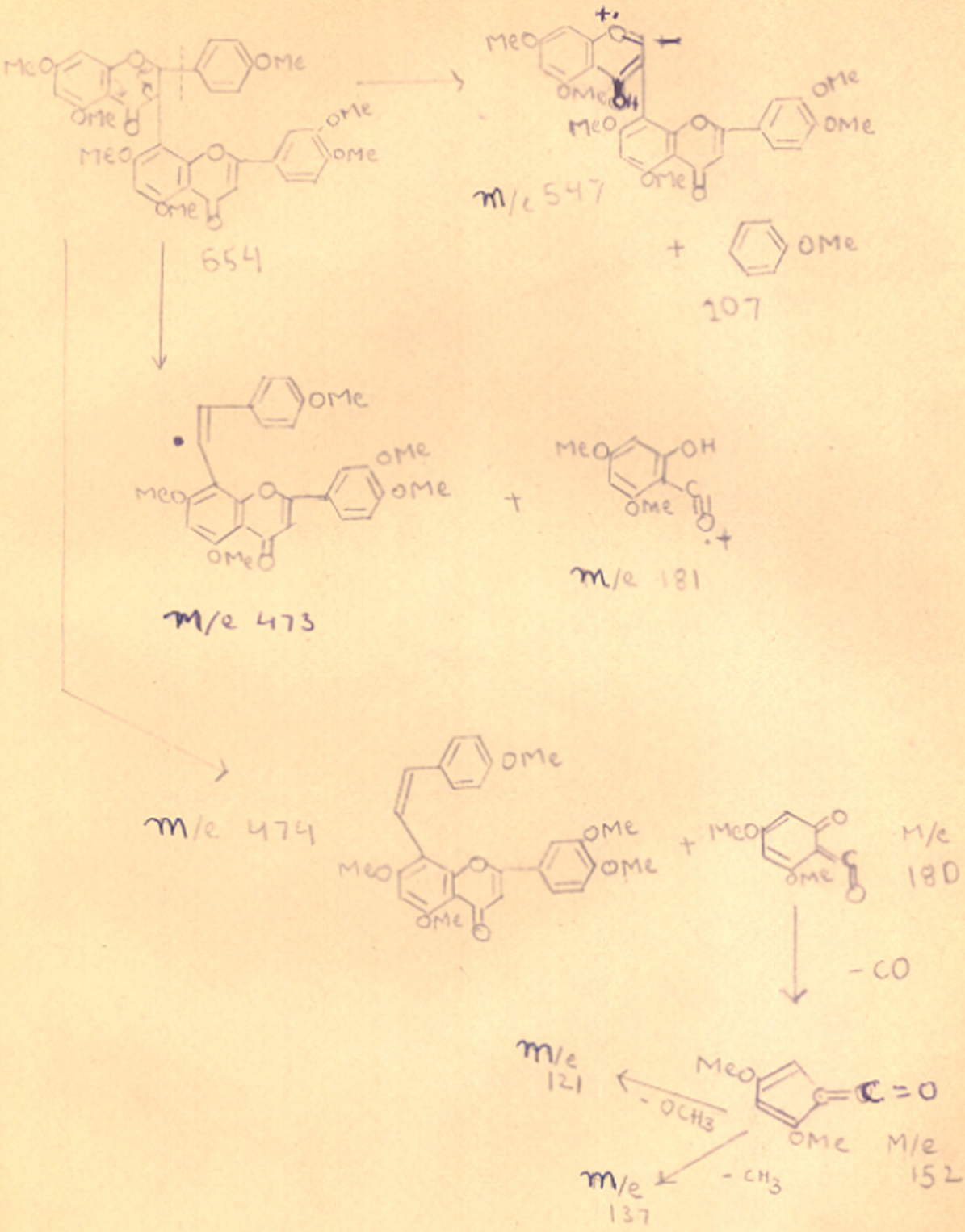
neutral fragment

A comparison of the spectra of xanthochymol and decahydroxanthochymol shows that the 123 fragment (C_9H_{15}) contains two double bonds. It is also concluded that the C_9H_{15} fragment is attached through a carbonyl to the main skeleton since after the cleavage of 123 from the m/e 466 is followed by a loss of 28 mass units corresponding to the loss of -CO-.

Further work is in progress.

CHART 1

133


MeO
OMe
OMe
OMe
OMe
OMe
654
m/e 547
+
OMe
107
m/e 473
+
MeO
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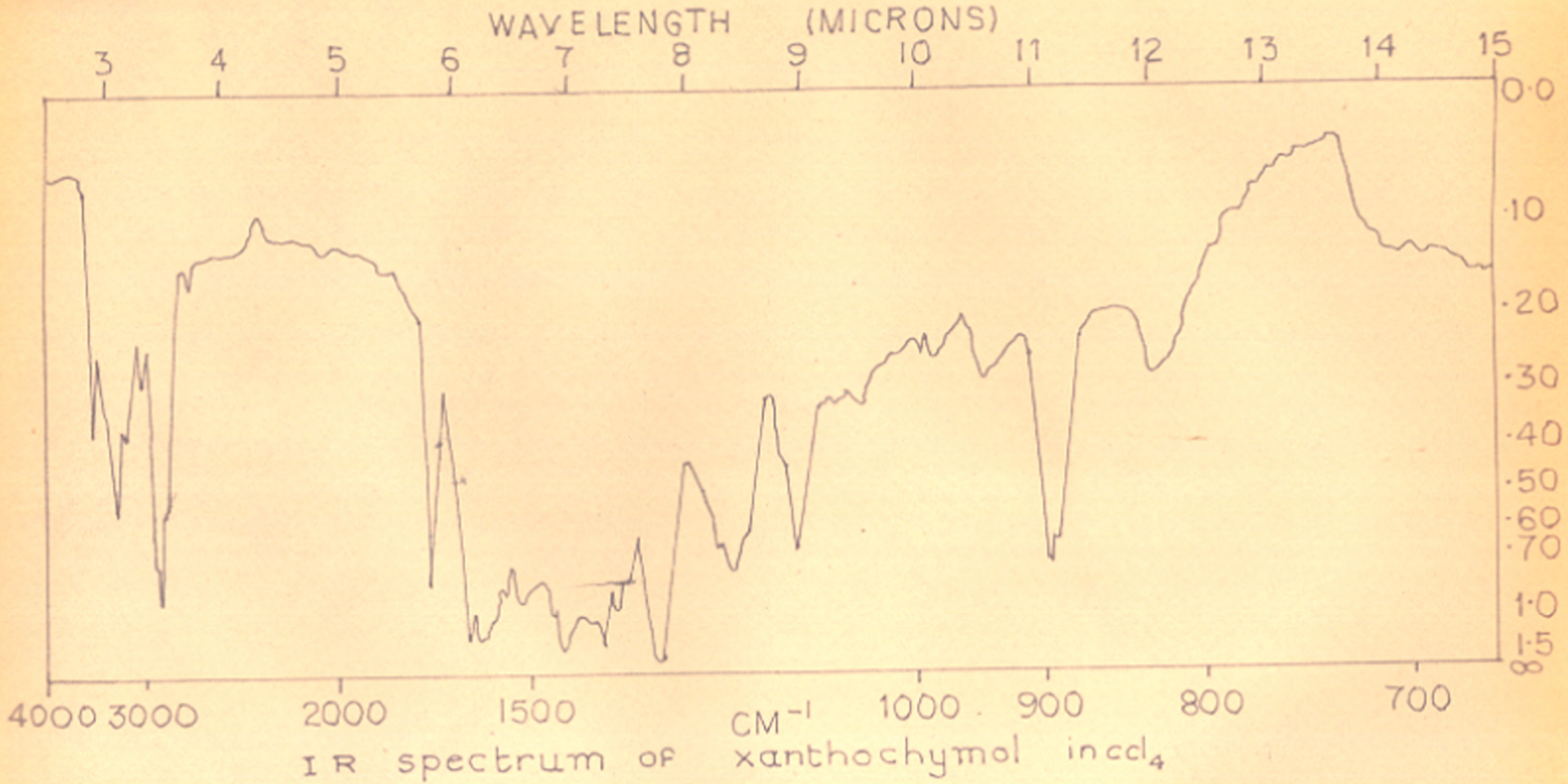
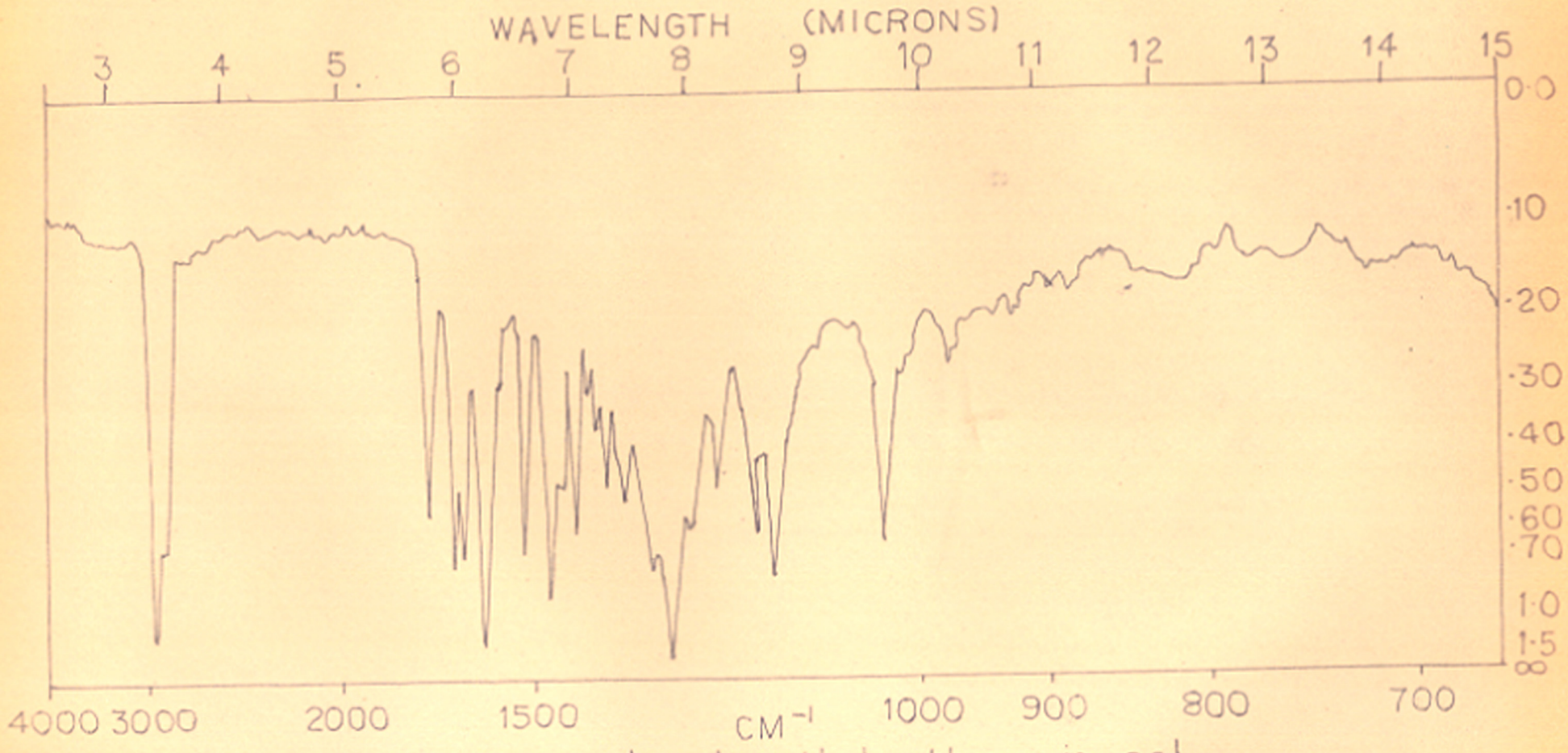
- OCH3
- CH3
m/e 137
m/e 152



IR spectrum of xanthochymol in CCl_4

FIG 1



IR spectrum of H 10 – xanthochymol methyl ether in ccl_4

FIG 2

NMR spectrum of
methyl ether of xanthochymol

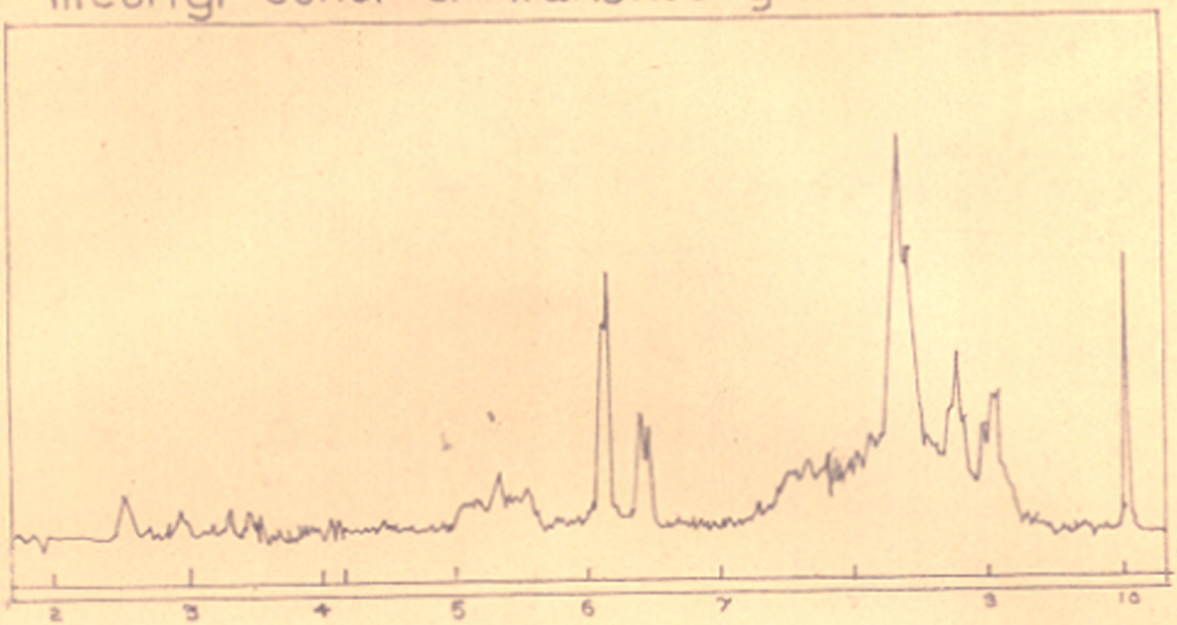


FIG 4 (CCl_4)

NMR spectrum of
xanthochymol

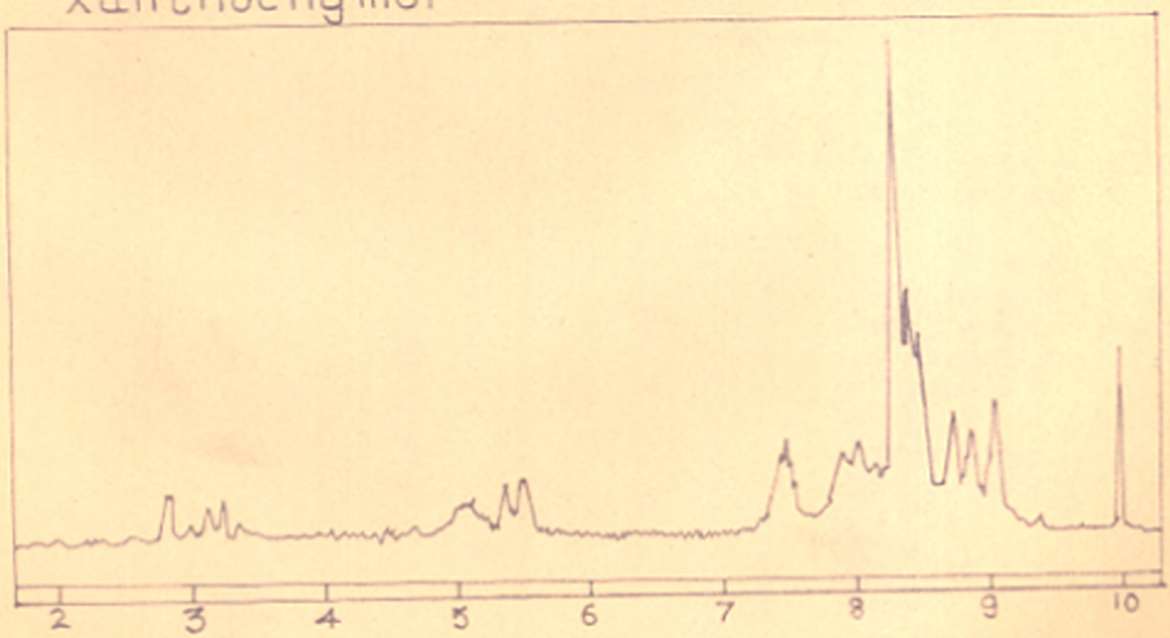


FIG 3 (Deuterated DMSO)

NMR data on the methyl ether of xantho-chymol (Fig. 3)

Solvent = CCl_4

Chemical shift (δ)	Multiplicity	No. of H	Assignment
9.04	-	9	Methyls on saturated carbon atom
8.75	-		
8.40	-	12	Methyls on double bonds and/or attached to carbon bearing oxygen
8.34	-	12	
6.60	-	-	Not assigned
6.44	-	-	
5.0 - 5.6	-	5	Vinyl H
3.32	-		
2.90	-	2	Vinyl H
2.50	s	1	Aromatic H

NMR data on xanthochymol (Fig. 4)

Solvent = Deuterated DMSO

9.05	s	3	Methyls on saturated carbon atoms
8.89	s	3	
8.75	s	3	
8.50	s	6	Methyls on double bonds and/or attached to carbon bearing oxygen
8.44	s	6	
8.35	s	12	
8.04	-	-	$-CH_2-$
7.49	-	-	Methylenes adjacent to C=C or -C=O
5.50	s	5	Vinyl H
5.35	s		
5.09	b		
3.17	q	2	
2.82	s	1	Aromatic H

EXPERIMENTAL

Isolation

The dried peels of the fruits of *Garcinia xanthochymus* (2 kg) were kept soaked in benzene (5 L) for 3 days. The solution developed an orange colour, this was filtered and removal of benzene gave a dark brown viscous oil. The benzene solution of this was chromatographed on a silica gel column using acetone-benzene (1:9) as eluent. The yellow eluate on removal of the solvent gave an oil which crystallized from hexane or carbon tetrachloride in lemon yellow needles. Recrystallization from hexane gave bright lemon yellow needles m.p. 135° (9.0 g) (Found: C, 75.4, 75.6; H, 8.0, 8.1%. $C_{38}H_{50}O_6$ requires: C, 75.7; H, 8.3% Mol. wt. 602 (mass spec.) It gave a green ferric colour but did not give wine red colour in the Shinoda test.

Hydrogenation of xanthocymol

The catalyst (10% Pd/C 30 mg) was suspended in methanol (15 ml) and was saturated with hydrogen, to this xanthochymol (100 mg) was added and stirred. It absorbed five mols of hydrogen. The solution was filtered to remove the catalyst, and the solvent was distilled off. The residue had no tendency to

crystallize.

Methylation of xanthochymol

A mixture of xanthochymol (300 mg) potassium carbonate (5 g), acetone (50 ml) and dimethyl sulphate (1.5 ml) was refluxed for 6 hr, when a test portion of the reaction mixture did not show any ferric colour. After removal of acetone it was cooled, treated with water and kept at R.T. for 24 hr. It was extracted with ether, the ether extract washed with water and ether distilled off. The residue (300 mg), even after a chromatographic separation over alumina, had no tendency to crystallize.

Acetylation of xanthochymol

A mixture of xanthochymol (100 mg), acetic anhydride (5 ml) and a few drops of pyridine was refluxed for 4 hr, cooled, poured over crushed ice and kept at R.T. for 24 hr. The amorphous solid so obtained was filtered, washed with water and dried (80 mg).

Ozonolysis of xanthochymol

A calculated amount of ozone gas (5 mols) was passed through a solution of xanthochymol (700 mg) in ethyl acetate (25 ml). The outgoing gases were first scrubbed through ethyl acetate and then through cold water. No more ozone was passed through the solution when the outgoing gases gave a test for excess of ozone.

The solution of the ozonized xanthochymol was added to a suspension of Adams catalyst (80 mg) in ethyl acetate (10 ml) which was earlier saturated with hydrogen. Ozonized xanthochymol absorbed 4.5 moles of hydrogen. This was filtered to remove catalyst, washed with fresh ethyl acetate. The combined filtrates were distilled and the distillate collected in Brady's reagent, which immediately showed the formation of precipitate. This was filtered and washed with distilled water. The precipitate was crystallized from methanol m.p.125°. Mixed m.p. with acetone D.N.P. was undepressed. The contents of the scrubber, in which the outgoing gases were passed through cold water, were treated with Brady's reagent, it gave a bright orange yellow precipitate, which was filtered and washed with distilled water. The residue was crystallized from pure methanol (50 mg) m.p. 141° mixed m.p. with formaldehyde D.N.P. was

undepressed.

The residue obtained in the ozonolysis experiment is being further investigated.

SUMMARY

PART I

(A) Constitution of morellic and isomorellic acids

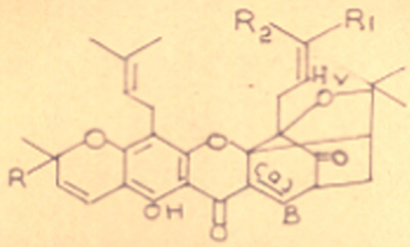
Morellin is a pigment of the pericarp of Garcinia morella, and its structure has recently been established as (I) on the basis of chemical, IR, UV, NMR and X-ray crystallographic data. From the latex of G. morella obtained from Mangalore in South India, two new pigments, morellic and isomorellic acids closely related to morellin, have been isolated. Their structures have been deduced as (II) and (III), mainly from the NMR data.

Morellic and isomorellic acids were isolated from the carbonate soluble fraction of the latex. They were both amorphous, but on heating with acetic anhydride and sodium acetate gave acetyl derivatives which crystallised from methanol in yellow cubes m.p. 240° and 174° respectively. Elemental analysis and mass spectral molecular weights led to the same molecular formula, $C_{35}H_{38}O_9$, for both the acetates, and the acetyl values showed that they were monoacetates. With diazomethane the two acids gave monomethyl esters. Morellic acid was converted to isomorellic acid by running its solution in acetone-benzene (1:9), through a column of

silica gel or fullers earth or by treatment at room temperature with hydrochloric acid in acetone, showing that morellic and isomorellic acids were probably related to each other in the same way as morellin and isomorellin. The NMR spectra of the acetates of morellic and isomorellic acids in comparison with those of morellin and isomorellin readily showed that morellic and isomorellic acids are carboxylic acids (II) and (III) corresponding to morellin and isomorellin. In the NMR spectra of the acetates of the two acids the aldehyde signals of morellin and isomorellin were replaced by broad hydroxyl signals at 1.35 and 1.45. Seven C-methyl groups, a 2,2-dimethylchromene system, a prenyl side-chain attached to an aromatic ring, and the bicyclooctenone part of the morellin skeleton were all readily recognizable. The vinyl proton (H_γ) of the α,β-unsaturated carboxylic acid side-chain of morellic acid acetate appeared at 3.85 and the corresponding absorption of isomorellic acid acetate was at 3.25, the downfield shift indicating the isomerisation of a carboxyl group from a trans to a cis configuration in relation to H_γ proton.

(B) Configuration of the carboxyl group in gambogic acid

Ollis et al. have stated that"the stereochemistry of the side-chain carrying the carboxyl group in gambogic acid is probably as shown in (IV), but the NMR results



I = R = CH_3 , R_1 = CH_3 , R_2 = CHO

II = R = CH_3, R_1 = CH_3 , R_2 = COOH

III = R = CH_3 , R_1 = COOH , R_2 = CH_3

IV = R = $CH_2CH_2CH{=}CMe_2$, R_1 = CH_3 , R_2 = COOH

V = R = $CH_2CH_2CH{=}CMe_2$, R_1 = COOH , R_2 = CH_3

VI = R = CH_3 , R_1 = COOH , R_2 = CH_3

single bond at a and - OC_2H_5 at B

are not conclusive". On the basis of the NMR data the stereochemistry of the side-chain carrying the carboxyl group in gambogic acid, has now been deduced as shown in (IV), in which the methylene and the carboxyl groups have a cis-relationship. The trans configuration of the carboxyl and the methylene groups of side-chain has also been observed in isogambogic (V) and isogamboginic acid derivatives.

(C) Pigments from the pericarp of G. morella

The examination of the carbonate soluble fraction of the pericarp pigments of G. morella has yielded isomorellic acid and a compound believed to be an artefact formed by the addition of ethanol to the double bond of the bicyclooctenone ring in isomorellic acid. The fact that (VI) is an ethanol adduct of isomorellic acid has been confirmed by refluxing the dimethyl ether-ester of (III) with dry ethanol in presence of piperidine, when the dimethyl ether-ester of (VI) was obtained. It is probable that these are transformation products of the corresponding cis-derivatives (relation of the carboxyl to the methylene in the side-chain) under the isolation procedure employed in the present work.

PART II

Constitution of the phenol (A): an alkaline hydrolysis product of octahydromorellin

Phenol (A), a product of mild alkaline hydrolysis of octahydromorellin, was isolated by Raghavan, who suggested the molecular formula, ($C_{26}H_{36}O_6$) and a tentative structure with the help of the available chemical and spectral data. In the light of the knowledge of the chemistry of morellin, and of additional chemical and NMR data on its derivatives and degradation products, Raghavan's structure for phenol (A) has now been shown to be untenable and a new structure is proposed.

On treatment with diazomethane phenol (A) gave a crystalline monomethyl ester. However, on methylation with methyl iodide and silver oxide in dimethyl formamide, it gave a dimethyl ether-ester, the IR spectrum of which indicated the presence of an alcoholic hydroxyl group. It formed a lactone when heated in diphenyl ether, which on hydrolysis with 5% ethanolic potassium hydroxide gave back phenol (A).

The NMR spectra of phenol (A) and its derivatives throw light on its constitution and indicate the presence of all the seven methyl groups of octahydromorellin, and

the absence of an aldehyde group.

From the mass spectral molecular weight (570), the chemical and spectral data and in the light of the structure of morellin, it appears that during the alkaline hydrolysis of octahydromorellin no carbon atom is lost and two reactions take place; (a) the aldehyde group undergoes an aldol type of condensation with the hydrogen adjacent to the chromanone carbonyl and (b) the bicyclooctanone ring cleaves to form a carboxylic acid. The former reaction is not observed with octahydrodesoxymorellin because of the absence of the aldehyde group, but the cleavage of the bicyclo-octanone ring takes place to form the corresponding carboxylic acid. The latter type of change has been observed by Auterhoff in gambogic acid, and hence such a reaction can be expected in octahydromorellin having the same basic skeleton. In the light of the data presented above structure (VII) has been suggested for phenol (A).

X

XI

VIII

VII

PART III

Constitution of the Mozingo reduction products of morellin

Morellin gave stable derivatives suitable for degradative work when subjected to Raney nickel reduction under the conditions first used by Mozingo. Raghavan obtained three reduction products of morellin designated as MRP I, II and III using ethanol, cyclohexanol and cyclohexanone as solvents.

With the knowledge of the structure of morellin, these reduction products were further investigated. MRP I, II and III showed a green ferric colour indicating the presence of a chelated hydroxyl, and they gave monomethyl ethers by methylation with dimethyl sulphate and anhydrous potassium carbonate in dry acetone.

The IR spectra of MRP I and II showed absorption in the hydroxyl region, but in MRP III there was no band in the hydroxyl region. In the carbonyl region the band at 1735 cm^{-1} is absent only in MRP II, indicating the reduction of the carbonyl of the bicyclooctanone ring.

The NMR spectra of these reduction products showed a total proton count of 46, 46 and 44 respectively. Further the spectra of all the three products showed the absence of aromatic and vinyl protons.

From the chemical, IR, NMR and mass spectral data, it is evident that in MRP I the aldehyde group of morellin has been reduced to a primary alcoholic group, in addition to the complete saturation of the four olefinic bonds. In MRP II and III the aldehyde group has been replaced by hydrogen. Only in MRP II the cyclic ketone has been reduced to a secondary alcohol as indicated by the molecular weight 526 (mass spectrum). All these data lead to structures (VIII), (IX) and (X) for MRP I, MRP II and MRP III respectively.

PART IV

Morelloflavone, a new biflavonoid from the heartwood of Garcinia morella

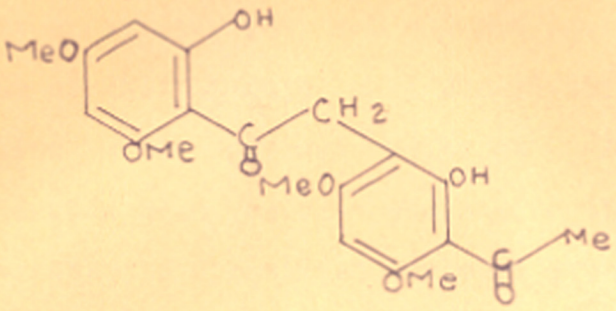
From the heartwood of Garcinia morella a new biflavonoid, morelloflavone, has been isolated and its structure elucidated as (XI) on the basis of UV, NMR, mass spectral and degradative evidence.

Morelloflavone (XI) gave a green colour with ethanolic ferric chloride, indicating the presence of a chelated hydroxyl and a wine-red colour in the Shinoda test which is characteristic of some flavonoids and xanthones.

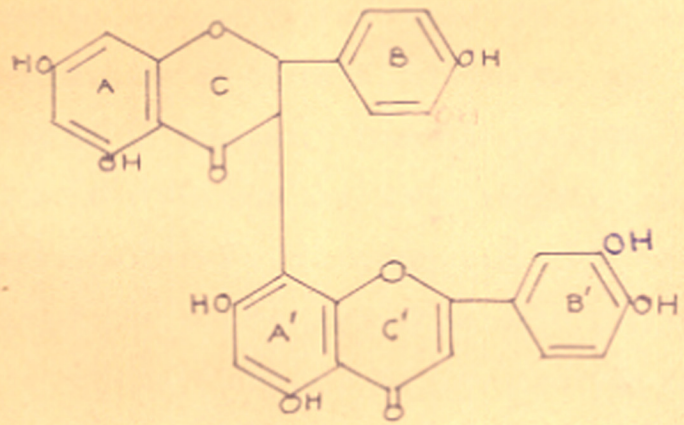
The elemental analysis of (XI) and its methyl ether and the mass spectral molecular weight of the latter (654) agree with the molecular formula $C_{30}H_{20}O_{11}$ for (XI).

By methylation with dimethylsulphate and potassium carbonate in acetone (XI) gave a crystalline heptamethyl ether free from ferric colour. Attempts at hydrogenation of (XI) over palladized carbon or Adams catalyst were unsuccessful. Alkaline hydrolysis of the methyl ether of (XI) with 15% ethanolic potassium hydroxide gave a yellow crystalline compound (B) showing green ferric colour.

The UV spectrum of (XI) in ethanol showed



XII



XI

absorptions at 345, 288, 275 and 255 mμ. The IR spectrum of (XI) in nujol mull showed strong absorption in the hydroxyl region at 3400 cm^{-1}. In the double bond region a broad band at 1645 cm^{-1} is assignable to a chelated carbonyl group, in the absence of evidence for unsaturation arising from C=C.

The NMR spectrum of (XI) in deuterated acetone showed a pair of doublets at 4.05 and 4.95 (J = 12 cps) integrating for one proton each. In the lower region a complex pattern of signals between 2.4 and 4.0 represented 11 protons. These include the 3'-proton (2.73) of the flavone and three aromatic protons of phloroglucinol ring, the others represent the aromatic protons of the two B-rings of morelloflavone. There are signals at - 2.66 and - 3.50 representing two chelated hydroxyl groups. A very broad signal around 5.5 arises from free phenolic hydroxyl groups. The spectrum in pyridine is completely transparent. The spectrum of the methyl ether in $CDCl_3$ indicated the presence of 7 methoxyls appearing in the region 6.0 - 6.3. The spectrum of (B) in the aliphatic region showed a sharp three proton singlet at 7.37 assignable to $COCH_3$ attached to an aromatic ring. Two six-proton absorptions at 6.1 and 6.18 are assigned to four methoxyls. There is a sharp two-proton singlet at 5.75 which can be ascribed to a methylene

flanked between a carbonyl and an aromatic ring. Three aromatic protons are indicated by a multiplet at 3.9. The aromatic protons represented by signals between 2.4 - 3.6 are not observed in the spectrum of (B), amounting to loss of seven aromatic protons. The presence of two chelated hydroxyls is shown by absorptions at - 3.3 and - 3.4, which disappear on exchange with D_2O. Together with the mass spectral molecular weight (390) for (B) these data lead to structure (XII) for (B). Isolation of veratric acid and acetoveratrone from the products of mild alkaline hydrolysis of the heptamethyl ether of morelloflavone, confirms the 3'-4' hydroxylation pattern in the B-ring of the flavanone.

On the basis of the structure assigned for (B) and in the light of the data discussed, morelloflavone has been constituted as (XI).

PART V

Xanthochymol

From the fruits of Garcinia xanthochymus a new pigment, xanthochymol has been isolated. On the basis of the elemental analysis and molecular weight (602; mass spectrum), it has been assigned the molecular formula $C_{38}H_{50}O_6$.

Xanthochymol (C) exhibits a green colour with ethanolic ferric chloride. It is sparingly soluble in aqueous sodium hydroxide and totally insoluble in aqueous sodium carbonate, indicating the absence of carboxyl group.

Xanthochymol underwent hydrogenation over palladized carbon in ethanol giving a decahydro-derivative. By methylation with dimethyl sulphate and potassium carbonate in acetone, it gave a dimethyl ether.

The UV spectrum of (C) in ethanol showed absorptions at 270 and 224-230 mμ. The IR spectrum of (C) in CCl_4 showed bands at 3333, 1724, 1658 and 1639 cm^{-1}. The bands at 1658 and 1639 cm^{-1} are attributable to a conjugated carbonyl and C=C stretching vibrations respectively. The band at 1724 cm^{-1} has been assigned to a six-membered ring carbonyl. The IR spectrum of the decahydroxanthochymol in CCl_4 in the double bond region showed bands at 1740, 1695 and 1625 cm^{-1}. The appearance of a band at 1695 cm^{-1} points

to the presnece of a α-β unsaturated carbonyl in (C).

The NMR spectrum of (C) in deuterated DMSO showed an approximate proton count of fifty. The region between 2.7 and 3.4 integrated for three protons. A signal appearing as a singlet at 2.82 represented a lone aromatic proton. A quartet at 3.17 is assigned to vinyl protons. A broad signal at 5.0 for 3 protons is characteristic of the vinyl proton on three γ,γ-dimethyl allyl side-chains; one terminal methylene is indicated by broad signals at 5.50 and 5.35. The rest of the signals appear between 7.20 and 9.20, and these arise from methyls and methylene in different environments. In the spectrum of decahydroxanthochymol the region between 3.0 and 6.0 is transparent, indicating that the number of vinyl protons is seven. A singlet at 3.0 is attributable to a lone aromatic proton. Further work is in progress.

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

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