CONSTITUTION OF MORELLIN

A

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INTRODUCTION

The presence of an orange colouring matter in the seeds of Garcinia morella was first noticed by M. G. Rao.[1] Extraction with boiling alcohol gave a crystalline orange colouring matter, which he named morellin. Since then it has been of considerable interest and special reference may be made to the work of B. S. Rao[2] and P. L. N. Rao.[3] B. S. Rao observed that morellin, m.p. 154°, was optically active and was a somewhat unstable substance, being resinified by prolonged boiling with alcohol or heating at 100° for a few hours. He assigned the molecular formula $C_{30}H_{34}O_6$ and observed that morellin yielded a tetra-acetate, a dimethyl ether, and a trimethyl ether which could be prepared through the sodio derivative. Two ethylenic bonds were present, morellin yielding a dihydrochloride and a tetrabromide. The presence of two carbonyl groups was indicated by the formation of an amorphous dioxime; the presence of one was confirmed by the formation of a crystalline mononitroguanylhydrazone. When morellin in ether was shaken with aqueous potassium hydroxide, or when morellin was digested with acetyl chloride and potassium carbonate in benzene, it formed an isomer named isomorellin, m.p. 116°. Fusion with caustic potash at 220° gave dl-methylheptenol (I), phloroglucinol and four acids: acetic, isovaleric, methylsuccinic, and homophthalic.

A liquid dienic ditertiary glycol, $C_{16}H_{22}O_2$, with an amyl alcohol odour was also isolated.

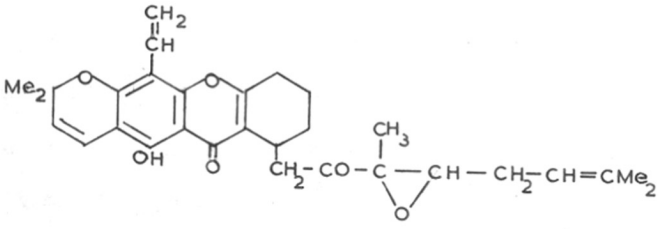
$Me\text{-}CHOH\text{-}CH_2\text{-}CH_2\text{-}CH{=}CMe_2$

(6-Methyl-2-heptenol)

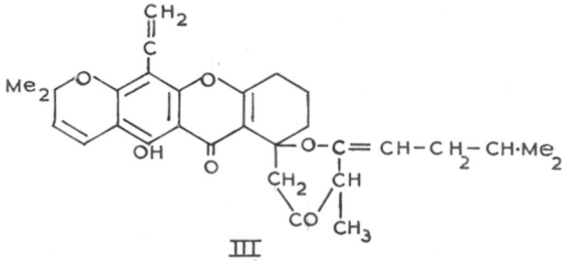
(I)

P. L. N. Rao and his collaborators have published a series of notes on morellin, but unfortunately no experimental details have been disclosed, and work carried out in this laboratory has failed to confirm most of their results and interpretations. They found that B. S. Rao's morellin was not homogeneous since it separated into three fractions, morellin T (m.p. 80°), M (m.p. 156°) and L (m.p. 60°) by chromatography of a benzene solution on silica gel. The analysis indicated hydration of morellin on chromatography. Isomorellin, m.p. 120-121°, was obtained in 50 per cent yield by treatment with boiling pyridine for six hours and they attributed the isomerization to the epimerization of groups round an asymmetric centre or the rearrangement of double bonds. Morellin and its derivatives readily absorbed four moles of hydrogen in the presence of Adam's platinum oxide catalyst; the octahydro derivatives (amorphous, m.p. 65°) of morellin and isomorellin were

identical. Octahydromorellin formed a tetra-acetate. Octahydro-O-dimethylmorellin on treatment with cold alcoholic caustic potash gave a phenol which did not contain a carboxyl group and a lactone structure was therefore excluded. P. L. N. Rao et al. obtained "the known dimethyl ether" by further methylation of monomethyl morellin, m.p. 156°. By treatment with boiling aqueous sodium hydroxide they obtained acetaldehyde and a mixture of volatile ketones, one of which was acetone. By oxidation with 2 per cent aqueous potassium permanganate they obtained oxalic acid (5 moles) and α-hydroxyisobutyric acid (0.6 mole), together with acetic, benzoic and other acids. They concluded that "the formation of both α-hydroxyisobutyric acid and acetone is thus indicative of a chromene ring." On the basis of the above evidence Rao et al. suggested two structures (II) and (III), both of which were modifications of a structure proposed earlier for morellin, based on the $C_{30}H_{40}O_6$ formula.[4]



II



III

Both the above structures are based on the $C_{30}H_{34}O_6$ formula. Although they found a higher molecular weight for morellin by the X-ray method, they "reserve an explanation for this discrepancy." Several features of structures (II) and (III) are not in conformity with the experimental data, particularly the behaviour of morellin on hydrogenation. The proposed structures contain only one phenolic hydroxyl group, but P. L. N. Rao *et al.* obtained the "known dimethyl ether" by further methylation of morellin monomethyl ether. By oxidation with 2 per cent permanganate they obtained oxalic acid (5 moles) and α-hydroxyisobutyric acid, together with acetic, benzoic and other acids; they conclude that "the formation of both α-hydroxyisobutyric acid and acetone is thus indicative of a chromene ring." But more recent evidence (see present work) does not support this suggestion.

According to R. R. Rao and Natarajan[5] morellin is "highly antibacterial," but it is also "highly toxic and

therefore not likely to assume any importance in chemotherapy." On the other hand P. L. N. Rao and Verma have reported that "2-4 per cent solutions of morellin in olive oil can be administered subcutaneously to mice in doses up to 450 mg. per kg. body weight without causing any mortality or necrosis."

However, Bringi et al.[5] observed that morellin, isomorellin and desoxymorellin showed no activity against any of eleven organisms including Streptococcus haemolyticus and Staphylococcus aureus, using the ditch plate technique and blood-agar.

A re-investigation of the chemistry of morellin was undertaken in this laboratory, first by Bringi et al.,[6] and continued in the present work. Bringi studied the chromatographic behaviour of B. S. Rao's morellin, the molecular formula of morellin, and the preparation of crystalline derivatives, followed by degradative experiments which led to tentative suggestions regarding the constitution of morellin.

When a solution of B. S. Rao's morellin in hexane or benzene was chromatographed on various adsorbents such as Florex XXX or magnesium carbonate, four orange zones were formed; the colourless percolate gave a fat, and elution of the bottom zone gave rise to a new pigment

(m.p. 126°) to which the name desoxymorellin was assigned. The next band gave isomorellin, m.p. 120-121, in a yield of 85 per cent, which showed that B. S. Rao's morellin which contains essentially morellin isomerized to isomorellin by the action of the adsorbent. It was therefore desirable to employ only methods of crystallization to purify morellin. Crystallization of B. S. Rao's morellin from hexane or extraction of the crude morellin obtained by the initial alcohol extraction with hexane in a Soxhlet removed the wax as well as desoxymorellin, and repeated crystallization from ethanol or methanol yielded morellin as glistening prismatic needles, m.p. 158-160°.

B. S. Rao's procedures for the morellin-isomorellin transformation have been mentioned earlier. Bringi observed that the isomerization can also be effected by adding aqueous sodium hydroxide to an acetone solution of morellin and acidifying the red solution immediately; but chromatography of a benzene solution of morellin through Florex appeared to be the most suitable method for obtaining pure isomorellin.

Repeated elementary analysis (C, H and O) of morellin and isomorellin and molecular weight determinations carried out by the X-ray method on morellin and isomorellin monomethyl ether led Bringi to suggest a

revised formula, $C_{33}H_{38}O_7$, for morellin.

A dark brownish green ferric colour and a positive Dimroth test (red colour with boric acid in acetic anhydride) showed the presence of a chelated hydroxyl group. Anomalous results were obtained in trying to decide the number of hydroxyl groups in morellin. Contrary to B. S. Rao and P. L. N. Rao, Bringi was unable to prepare a dimethyl or trimethyl ether or a tetra-acetate. By methylation by the usual acetone-potassium carbonate method both morellin and isomorellin gave crystalline monomethyl ethers (m.p. 156-158° and 141-143°); isomorellin monomethyl ether was also obtained by treatment of morellin monomethyl ether in acetone with hydrochloric acid. The presence of one hydroxyl group was also indicated by the formation of monobenzenesulphonyl and monotoluenesulphonyl esters. However, acetylation by means of acetic anhydride and pyridine or sodium acetate, or by isopropenyl acetate and p-toluenesulphonic acid gave the same diacetate (m.p. 178°), the formation of which was explained by one phenolic and one enolic hydroxyl group as present in a hydroxychromanone such as toxicarol (IV). The possibility of an enolizable $CO\text{-}CH_2\text{-}CO$- group was ruled out as morellin did not give a copper salt under normal conditions.

Morellin and its diacetate were susceptible to reductive acetylation by zinc, acetic acid and anhydride, but only one additional acetoxy group was introduced and not two as required for a quinone. Besides, other reactions for a quinone were negative, although the orange-yellow colour of morellin in aqueous dioxane could be decolourized by means of sodium hydrosulphite.

Under a variety of conditions catalytic hydrogenations of morellin were carried out. With Raney nickel or palladium-charcoal morellin absorbed four moles of hydrogen to yield amorphous products, but selective reduction with palladium on calcium carbonate or the Lindlar catalyst gave crystalline dihydro and tetrahydro derivatives (m.p. 125-126° and 167-169°). Tetrahydromorellin had a green ferric colour, very different from the brownish green ferric colour shown by morellin. Another striking difference was the wine-red colour exhibited by tetrahydromorellin by reduction with sodium amalgam and subsequent acidification. Tetrahydromorellin gave a crystalline monomethyl ether, m.p. 174-175°. Tetrahydromorellin reacted with hydroxylamine hydrochloride and pyridine in alcohol at 10° to give a crystalline product, m.p. 192-193°, the analysis of which agreed with the anhydride of a monoxime rather than a monoxime.

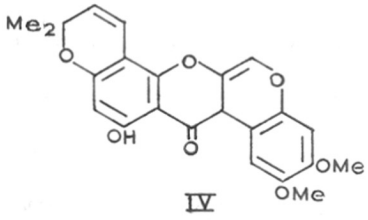
Morellin monomethyl ether gave a crystalline tetrahydro derivative, m.p. 137-138°, which was different from the product, m.p. 174-175°, obtained by the methylation of tetrahydromorellin. Isomorellin gave a tetrahydro derivative, m.p. 159-160°, the monomethyl ether of which was identical with the monomethyl ether of tetrahydromorellin.

Both morellin and isomorellin gave the same crystalline reduction product, m.p. 110-111°, on treatment with Raney nickel in boiling alcohol, and the elementary analysis indicated that it was a decahydro or dodecahydro derivative of morellin; but this simple view was not supported by the properties of the compound and the known behaviour of ketones in the Mozingo reduction. We will revert to this problem later in connection with two other Raney nickel reduction products prepared in the course of the present work.

Alkali fusion of morellin with five times its weight of caustic potash at 220° gave acetic, isovaleric, methyl succinic and homophthalic acids, methyl heptenol and phloroglucinol, fully confirming B. S. Rao's results. Hydrolysis of morellin with 7.5 per cent ethanolic sodium hydroxide gave acetone, propionaldehyde and traces of acetaldehyde. Tetrahydromorellin on the other hand did not yield acetone, but an aldehyde, the

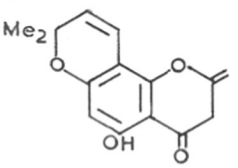
dinitrophenylhydrazone of which (m.p. 141-142°) analyzed for the dinitrophenylhydrazone of α-hydroxyisobutyraldehyde.

On oxidation with alkaline permanganate morellin gave acetone and α-hydroxyisobutyric acid. The formation of acetone by alkaline hydrolysis and of α-hydroxyisobutyric acid by oxidation indicated the presence of a 2,2-dimethylchromene nucleus as in toxicarol (IV) and several other natural products.

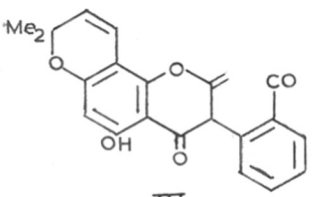


IV

The formation of a monomethyl ether but a diacetate by morellin further strengthened the analogy with toxicarol, from which Clark prepared a monomethyl ether, but a diacetate due to the enolization of the chromanone carbonyl group. In the light of these observations the part structure (V) was advanced for morellin. The double bond in the pyrone ring was so situated in order to account for the fact that a chromanone colour reaction developed only at the tetrahydro stage. Since homophthalic acid was formed on alkali fusion of morellin, the partial structure was expanded to (VI).



V

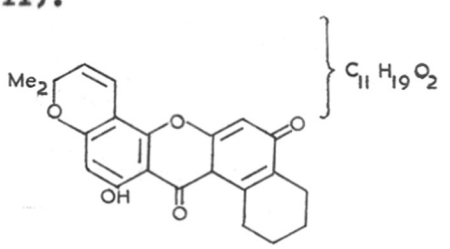


VI

The possibility of homophthalic acid arising from an isocoumarin or indan-1,2-dione nucleus was excluded by infra-red studies. Further, unlike isocoumarin morellin did not yield a hydroxamic acid with hydroxylanine; and unlike indan-1,2-dione morellin did not form a quinoxaline with o-phenylenediamine.

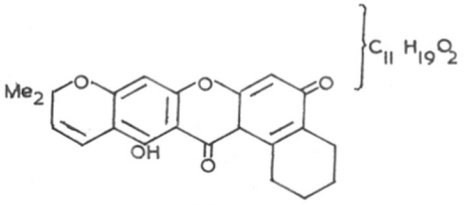
Isovaleric acid, methylsuccinic acid and methylheptenol were the other products in the alkali fusion of morellin. Assuming that isovaleric acid came from the isoprenoid residue in the dimethylchromene nucleus, the other two products accounted for the remaining 12 carbon atoms in morellin.

The ultra-violet and infra-red spectra of morellin and the products of hydrogenation then led to the partial structure (VII).



VII

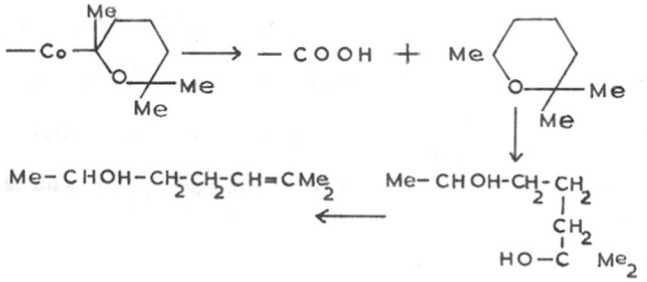
By taking recourse to the analogy with toxicarol, the morellin-isomorellin transformation was explained by assuming the opening of the γ-pyrone ring and cyclization in the opposite direction to give the linear isomer (VIII).



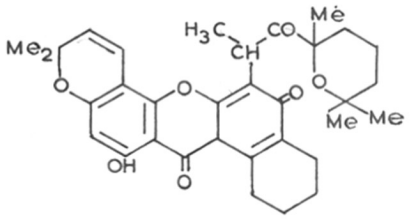
VIII

This view was apparently strengthened by the fact that isomorellin gave a positive Gibbs test (2,6-dichloroquinone-chloroimide colouration) which showed a vacant position <u>para</u> to a phenolic hydroxyl group. However, the isomerization of morellin monomethyl ether to isomorellin monomethyl ether was not in conformity with the above suggestion.

The $C_{11}H_{19}O_2$ residue and its mode of attachment to the main part of the molecule were not established. Assuming that one of the oxygen atoms in the C_{11} residue was a carbonyl group and the other an ether group, the formation of methylheptenol was explained by the following scheme.



Structure (IX) for morellin was then tentatively suggested as the basis for further work, although it was realized that the available experimental evidence was inadequate.



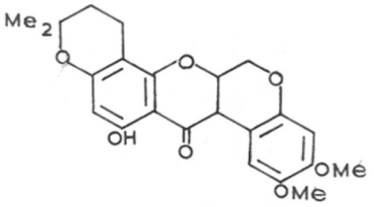
IX

The validity of the structure (IX) proposed for morellin was discussed in the light of infra-red and ultra-violet data.[8] Morellin did not show any free hydroxyl band in the infra-red. Tetrahydromorellin and octahydromorellin exhibited weak broad bands at 3280 and 3550 cm^{-1}. Hexahydromorellin and the Raney nickel reduction product had prominent bands at 3690 and 3550 cm^{-1}. In the double bond region morellin had four bands at 1730, 1668, 1628 and 1585 cm^{-1}. In dihydromorellin the 1628 cm^{-1} band had broadened with maxima at 1625 and 1642 cm^{-1}. The 1585 cm^{-1} band disappeared and this was attributed to an ethylenic bond capable of extremely rapid reduction, as for instance a double bond conjugated with a benzene ring. There was no further change in the double bond region in tetrahydromorellin. In hexahydromorellin the band corresponding to the 1668 cm^{-1} band of morellin was suppressed; the band at 1630 cm^{-1} became

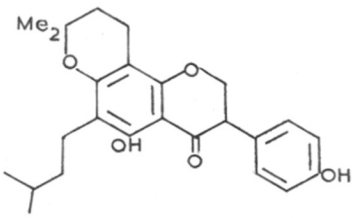
sharp with a single peak at 1640 cm^{-1}. The 1668 cm^{-1} band in morellin was assigned to an $\alpha\beta$-$\alpha'\beta'$ unsaturated carbonyl group, in which one alkene bond was part of a cyclohexene ring. In the entire double bond region the Raney nickel reduction product showed only one band at 1642 cm^{-1} which was assigned to a chelated carbonyl group as in a 5-hydroxychromanone. The characteristic bands at 875 cm^{-1} and in the 1030-1124 cm^{-1} region in the morellin spectrum probably indicated a tetrahydropyran ring.

The ultraviolet spectrum of morellin had three maxima at 236, 288.5 and 360 mμ. Dihydromorellin had a more complex spectrum with two additional maxima in the 260-280 mμ region. Tetrahydromorellin and further reduction products, including the Raney nickel reduction product, had similar absorption spectra having a peak in the 300-306 mμ region in addition to a weak broad band near 345 mμ. Such a type of ultra-violet absorption is characteristic of C-methylphloroacetophenone and 5,7-dihydroxychromanone. Comparison of the spectra of the reduction products with those of dihydro-toxicarol (X) and hexahydro-osajin (XI) showed that the latter compounds had higher intensity absorption in the 300 mμ region, which was attributed to the substituted phenyl groups in the 3-position of the chromanone nucleus. The low intensity of the peak in the

300 mμ region in the spectra of the reduction products of morellin indicated the absence of such a group, thus supporting the tentative structure proposed for morellin (IX).



X



XI

References

1. M. G. Rao, Abstracts of the Chemistry Section, 13th Indian Science Congress, 1915.
2. B. S. Rao, J. Chem. Soc. 1937, 853.
3. P. L. N. Rao and S. C. L. Verma, J. Sci. Industr. Res. 1951, 10B, 184; 1952, 11B, 206; P. L. N. Rao, S. C. L. Verma and D. V. Krishnamurthy, Current Sci. 1951, 20, 234; P. L. N. Rao and D. V. Krishnamurthy, J. Sci. Industr. Res. 1953, 12B, 565.
4. P. L. N. Rao, D. V. Krishnamurthy and S. C. L. Verma, Naturwiss. 1954, 41, 66.
5. R. R. Rao and S. Natarajan, Current Sci. 1950, 19, 59.
6. N. V. Bringi, K. H. Shah and K. Venkataraman, J. Sci. Industr. Res. 1955, 14B, 135-152.
7. E. P. Clark, J. Amer. Chem. Soc. 1931, 53, 2264.
8. N. V. Bringi, M. R. Padhye and K. Venkataraman, J. Sci. Industr. Res. 1956, 15B, 128-138.

PRESENT WORK

PRESENT WORK

The present work starts with the confirmation of the molecular formula of morellin. The molecular formula, $C_{33}H_{38}O_7$, proposed by Bringi *et al.* (see Introduction) has been confirmed by the molecular weight of a few derivatives of morellin determined by the X-ray method.

But mass spectrum : $C_{33}H_{36}O_7$

Derivative of morellin	Molecular weight	
	Found	Required
Monomethyl ether	555	560
Monobenzenesulphonyl ester	682.5	690
p-Bromobenzenesulphonyl ester	765	769

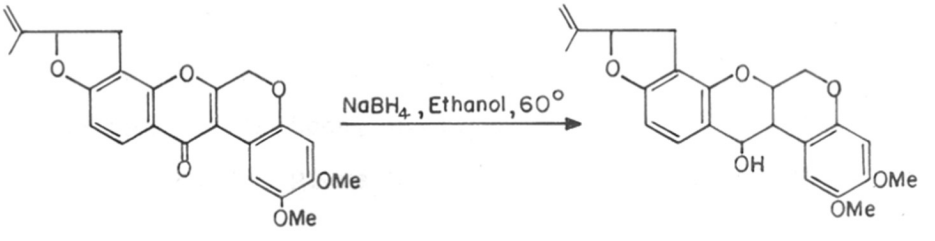
Ethylenic bonds

Although Bringi[1] had observed that morellin absorbed four moles of hydrogen on catalytic hydrogenation, it was not established beyond doubt that no carbonyl group was reduced during the process of hydrogenation. The presence of four ethylenic bonds in morellin has now been confirmed. Morellin absorbs four moles of hydrogen on catalytic hydrogenation, using 12 per cent palladium-charcoal. The first two moles are readily absorbed in 20 minutes, the third mole in an hour, and the fourth mole in three hours

more. Tetrahydromorellin and octahydromorellin had one active hydrogen and the corresponding methyl ethers none, showing that no carbonyl group was reduced during catalytic hydrogenation. Further, the product obtained by the sodium borohydride reduction of tetrahydromorellin in methanol absorbed two moles of hydrogen in presence of palladium-charcoal, which confirmed the presence of four ethylenic bonds in morellin.

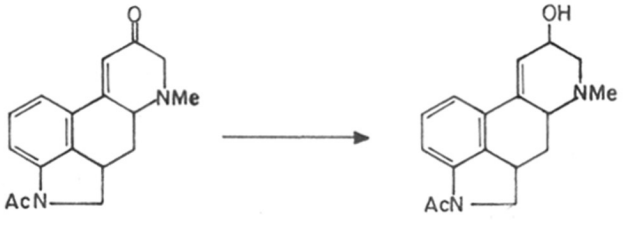
Sodium borohydride reduction

Reduction of morellin with methanolic sodium borohydride gave an amorphous product, which absorbed only three moles of hydrogen by catalytic hydrogenation in presence of palladium-charcoal. It appeared therefore that an ethylenic bond, in addition to one or more carbonyl groups, was reduced by sodium borohydride. Treatment of a dioxane solution of dehydrorotenone (I) with ethanolic sodium borohydride at 60° for ½ hour gave rotenol (II) in nearly 100 per cent yield.[2]



I II

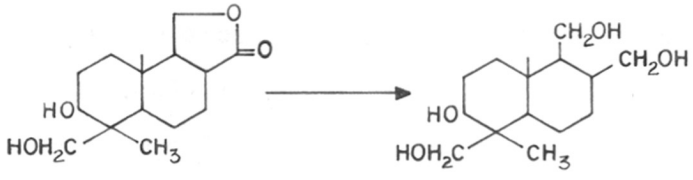
From the last example it may be concluded that in morellin there is a double bond in conjugation with a γ-pyrone carbonyl which gets reduced by sodium borohydride; but the presence of other types of $\alpha\beta$- or $\alpha\beta$-$\alpha'\beta'$-unsaturated carbonyl groups also explain the behaviour towards sodium borohydride. It is clear from the literature that the action of sodium borohydride on $\alpha\beta$-unsaturated ketones can lead to different results. The reduction of Δ^4-3-keto-steroids with sodium borohydride yields a mixture of the corresponding Δ^4-3-hydroxy epimers.[3] One of the reactions in the total synthesis of lysergic acid is the reduction of the $\alpha\beta$-unsaturated ketone (III) to the corresponding alcohol (IV) by sodium borohydride.[4]



III IV

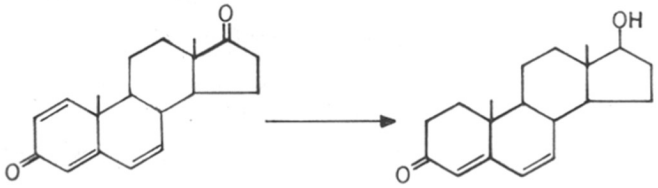
Heymann and Fieser found that the action of sodium borohydride on methyl 3α-hydroxy-12-keto-$\Delta^{9(11)}$-cholenate resulted in attack on the carbomethoxy group rather than the ketone, possibly due to the steric effect of the 18, 20-methyl groups.[5] The literature

contains a few examples of the reduction of a double bond conjugated with a carbonyl group by lithium aluminium hydride or sodium borohydride. Thus iresin (V) underwent reduction to (VI) by lithium aluminium hydride in tetrahydrofuran.[6]



V VI

$\Delta^{1,4,6}$-Androstatriene-3,17-dione (VII), by successive treatment with sodium borohydride and manganese dioxide in chloroform, gave 17β-hydroxy-$\Delta^{4,6}$-androstadien-3-one (VIII).[7] The Δ^{6}-double bond was subsequently found to be unnecessary, and it was also observed that the Δ^{4}-double bond was partly reduced by the action of sodium borohydride on Δ^{4}-androstene-3,17-dione.



VII VIII

Ozonization

In order to study the disposition of the double bonds the action of ozone on morellin and tetrahydromorellin was studied. Ozonolysis of morellin was

carried out in chloroform at -60° for 2 hours. After the decomposition of the ozonide with ice-cold water and distillation of the volatile matter into Brady's reagent, one mole of acetone was readily isolable as its 2,4-dinitrophenylhydrazone. Under similar conditions tetrahydromorellin also gave one mole of acetone. The isolation of acetone from both morellin and tetrahydromorellin showed that an isopropylidene ($Me_2C=$) group did not undergo saturation until a third mole of hydrogen was consumed.

On prolonging the ozonization of morellin for 6 hours and decomposing the ozonide by catalytic hydrogenation with palladium-charcoal, and finally steam distilling the volatile matter, a mixture of aldehydes and ketones was obtained. A benzene solution of the mixed dinitrophenylhydrazones was chromatographed on alumina, when two bands separated, and eluted successively by benzene. The lower band was identified as a mixture of the dinitrophenylhydrazones of formaldehyde, acetaldehyde and acetone by paper chromatography, using a methanol-heptane system. From the upper band pyruvic aldehyde bis-dinitrophenylhydrazone was isolated and identified by mixed m.p. and by comparing its infra-red spectrum with that of authentic pyruvic

aldehyde bis-dinitrophenylhydrazone. The isolated dinitrophenylhydrazone showed bands at 2280, 1589, 1481, 1341, 1319, 1263, 1218, 1148, 1087, 1056, 937, 928, 919, 835 cm^{-1}. The synthetic sample showed bands at 2280, 1587, 1483, 1341, 1313, 1254, 1218, 1148, 1082, 1057, 934, 923, 913 and 831 cm^{-1}. The formation of pyruvic aldehyde by ozonization indicates the presence of a $CH_3COCH=$ group or a $CH_3-\overset{CH_2}{\overset{//}{C}}-CH=$ group; the latter was excluded by a negative test for a terminal methylene group when morellin was treated with periodate and permanganate.[8]

Formation of copper complex

By the usual method of preparing a copper complex in which a blue or bluish green complex separates at the interphase, when an ether solution of a 1,3-diketone is shaken with aqueous copper acetate, morellin gave no copper complex. However it was found that under somewhat special conditions, using a dioxane solution and treating with excess of aqueous copper acetate, morellin gave a red copper complex, which crystallized from chloroform or dimethylformamide-alcohol mixture in blood-red needles analysing for one atom of copper and two molecules of morellin. On hydrolysis with conc hydrochloric acid the copper complex gave isomorellin

(m.p. 120-121°). Catalytic hydrogenation of the copper complex in presence of palladium-charcoal resulted in the uptake of two molecules of hydrogen, and a pale-yellow amorphous compound containing copper was isolated.

Isomorellin also gave a copper complex, while morellin methyl ether, isomorellin methyl ether, tetrahydromorellin and octahydromorellin did not give copper complexes. The formation of the copper complex appeared therefore to involve the phenolic hydroxyl group and a readily reducible ethylenic bond, probably in conjugation with a carbonyl group. Several chalkones, chromones and related compounds were then tested for copper-complexing behaviour.

Table I

Copper complexes of some o-hydroxy carbonyl compounds

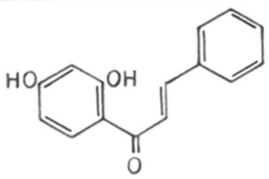
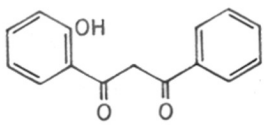
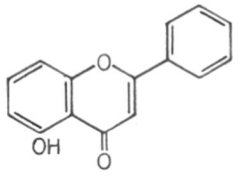
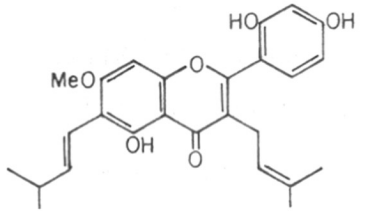
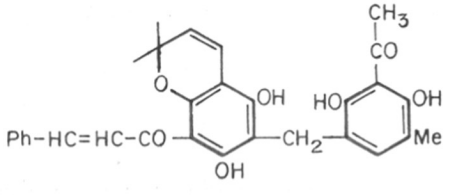
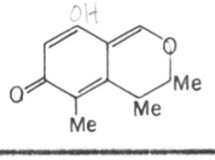
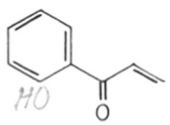
Compound	Formula	Colour of complex
2',4'-Dihydroxychalkone		Green
o-Hydroxydibenzoylmethane		Green

Table I (contd.)

Compound	Formula	Colour of complex
5-Hydroxyflavone		Green
Artocarpin		Green
Rottlerin[9]		Red
Citrinin		Blue-green

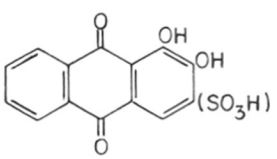
Rottlerin, which has a cinnamoyl group attached to a 5,7-dihydroxychromene nucleus, was the only one of the compounds examined which gave a red copper complex, but this was insoluble in chloroform unlike the complex from morellin. The formation of a red copper complex by

morellin appears to indicate the probable presence of the following group (XI).

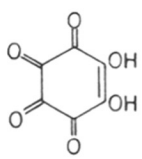


XI

The following compounds are stated in the literature to give red copper complexes: alizarin and its 3-sulphonic acid (IX) and rhodizonic acid (X). Morellin obviously was not an anthraquinone derivative or a rhodizonic type of compound.



IX

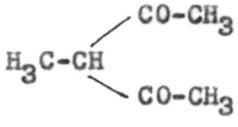


X

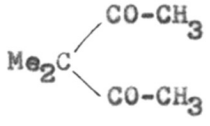
Condensation with o-phenylenediamine

o-Phenylenediamine condensed with morellin, tetrahydromorellin, octahydromorellin and the copper complex of morellin. The products, which were amorphous in all cases, on dissolving in alcohol and treating with a drop of conc hydrochloric acid, gave a deep-red colouration. Such a colouration is characteristic of heptazines which are formed by condensing 1,3-diketones with o-phenylenediamine. According to Viebel[10]

heptazines from compounds of the type $-CO-CH_2-CO$ give a violet colouration with acids. Vaisman *et al.*[11] have studied the condensation of *o*-phenylenediamine with compounds having a $-CO-C(RR')-CO$ or $-CO-CH(R)-CO$ group. They observed that (XII) gave a heptazine forming a red salt with hydrochloric acid, whereas the heptazine from (XIII) was itself coloured.



(XII)



(XIII)

The red colour given by the heptazines from morellin and its derivatives with hydrochloric acid thus shows the presence of a $-CO-CH(R)-CO-$ type of group in morellin.

Raney nickel reductions

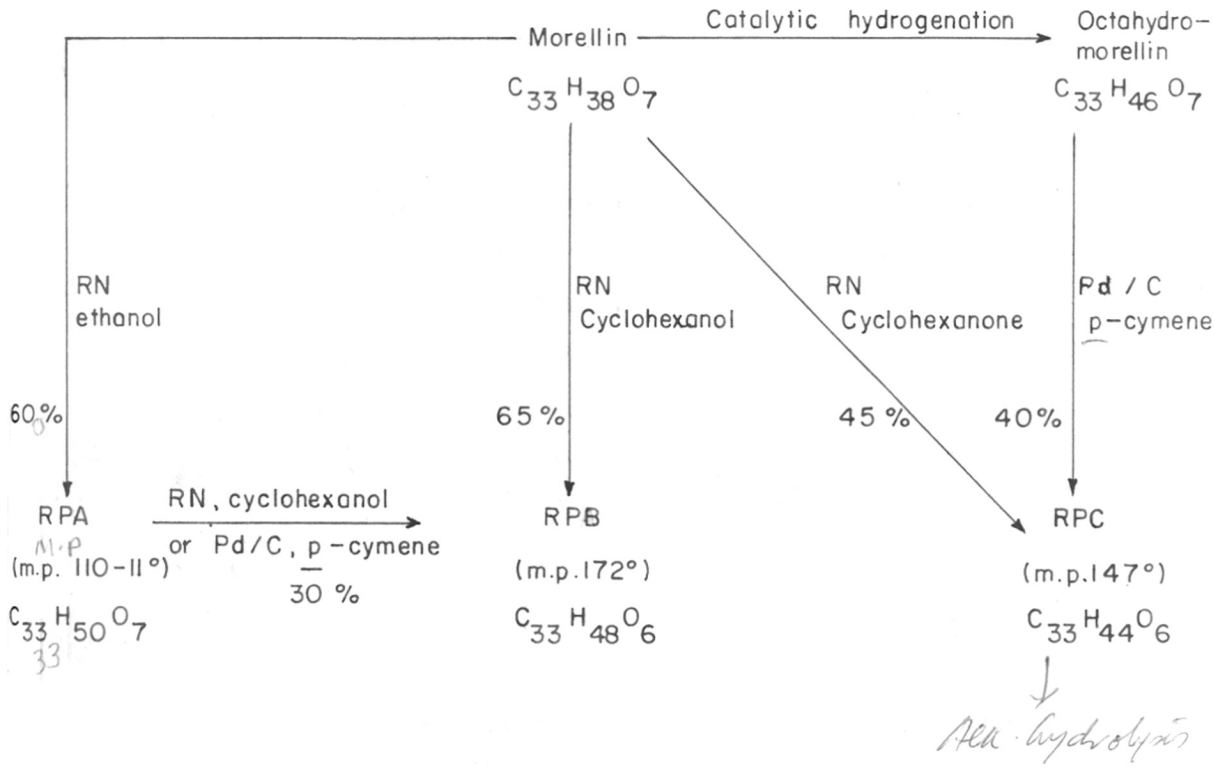
Morellin tends to resinify on treatment with solvents and is alkali-sensitive, but it was observed that stable derivatives suitable for degradative work could be prepared by Raney nickel reduction under the conditions first used by Mozingo,[12] in which a massive proportion of the nickel catalyst to the substance (e.g. 10 to 1) is employed. The reduction products obtained by using ethanol, cyclohexanol and cyclohexanone as solvents are referred to in the sequel as RPA, RPB, and RPC.

The basis for the use of cyclohexanol and cyclohexanone is the work of Kleiderer and Kornfeld,[13] who have carried out Raney nickel reductions (e.g. benzoin to dibenzyl) with cyclohexanol as hydrogen donor, as well as oxidations (e.g. of 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.

Morellin on reduction with Raney nickel in boiling ethanol gave a colourless crystalline compound (RPA, m.p. 110-111°, $C_{33}H_{50}O_7$, described earlier[1]). This compound, on attempted dehydrogenation with 12 per cent palladium-charcoal in boiling p-cymene during 48 hours, gave a crystalline compound (RPB, m.p. 172°), analysing for $C_{33}H_{48}O_6$, and therefore indicating that dehydration rather than dehydrogenation had taken place. RPB could also be prepared by a one-step reduction of morellin with Raney nickel in boiling cyclohexanol and also by subjecting RPA to further treatment with Raney nickel, but in cyclohexanol. Both RPA and RPB analysed for 3 active hydrogen atoms by the lithium aluminium hydride method. Two carbonyl groups have therefore been reduced.

Chart - I

RN = Raney nickel



RPA : Reduction product A

RPB : Reduction product B

RPC : Reduction product C

When the reduction of morellin was carried out in a hydrogen acceptor medium, cyclohexanone, instead of cyclohexanol (a hydrogen donor), the product was RPC, m.p. 147°, which was also obtained by the action of palladium-charcoal on octahydromorellin. It had only one active hydrogen.

Morellin dissolves in ethanolic sodium hydroxide with a deep-red colour from which it cannot be recovered on acidification; the product appeared to resinify readily and no crystalline product of hydrolysis was isolable. But in sharp contrast with morellin, RPB is a stable compound, being unaffected by ethanolic sodium hydroxide at reflux under a variety of conditions. It gave an olive-green ferric colour and a wine-red sodium amalgam colouration after 24 hours. It did not exhibit any change in colour with magnesium and hydrochloric acid. It was insoluble in aqueous sodium carbonate or hydroxide and was recovered unchanged from an attempted hydrolysis with zinc and 4 per cent aqueous potassium hydroxide as in dihydrodehydrotoxicarol or prolonged refluxing with 30 per cent ethanolic sodium hydroxide. RPB was found to be a convenient starting point for further degradative work.

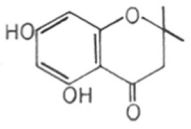
The ultra-violet spectra of morellin and reduction products A, B and C, determined in ethanol in a Beckman DU spectrophotometer, are shown in Fig. 1. Table II records the wave-lengths of the maxima and their intensities.

Table II

U.V. absorption spectra of morellin and Raney nickel reduction products

Compound	λ_{max}, mμ	ε_{max}	λ_{max}, mμ	ε_{max}
Morellin	236	26350	288.5	16600
			360	15130
RPA	300	19010	345	3570
RPB	299	25000	345	4020
RPC	300	10120	345	1890

It is of interest to compare the ultra-violet spectra of reduction products A, B and C with the spectrum of 5,7-dihydroxy-2,2-dimethylchromanone (XIV).



XIV

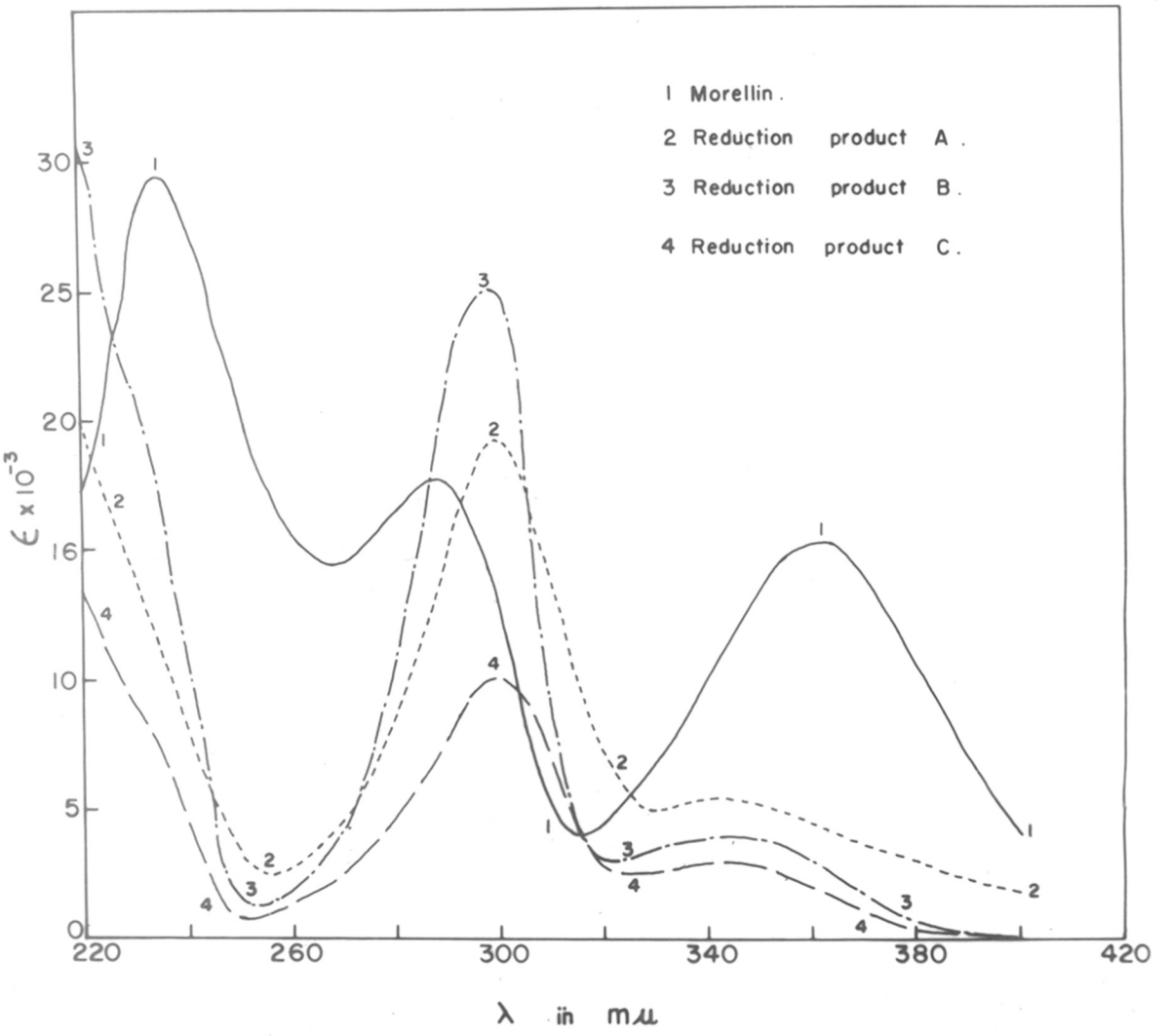
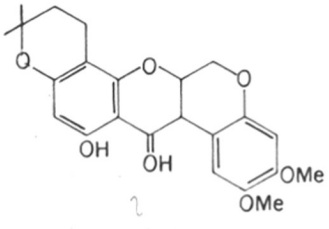


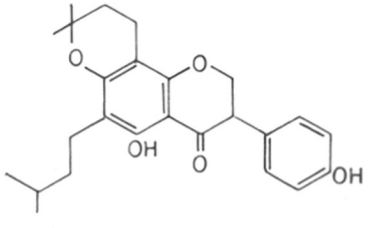
Fig. 1.

Solvent

The chromanone (XIV) has a maximum at 293 mμ (ε, 18000) and a broad weak band at about 345 mμ. The general similarity in the shape of the absorption curves showed the chromanone nature of reduction products A and C, although the intensity of the peak at 300 mμ in the RPC spectrum is only about half that of the corresponding peak in the RPA spectrum. The spectrum of RPB broadly resembled the chromanone type of spectrum, but the high intensity (ε, 25000) of the band at 300 mμ was in better agreement with that of an isoflavanone, for instance dihydrotoxicarol (XV)[19] and hexahydro-osajin (XVI).[15]



XV



XVI

The curves of RPB, dihydrotoxicarol and hexahydro-osajin are shown in Fig. 2. Although there is a marked similarity in the absorption curves, an isoflavanone structure (XVII) is not consistent with the observed chemical facts and in particular the molecular formula.

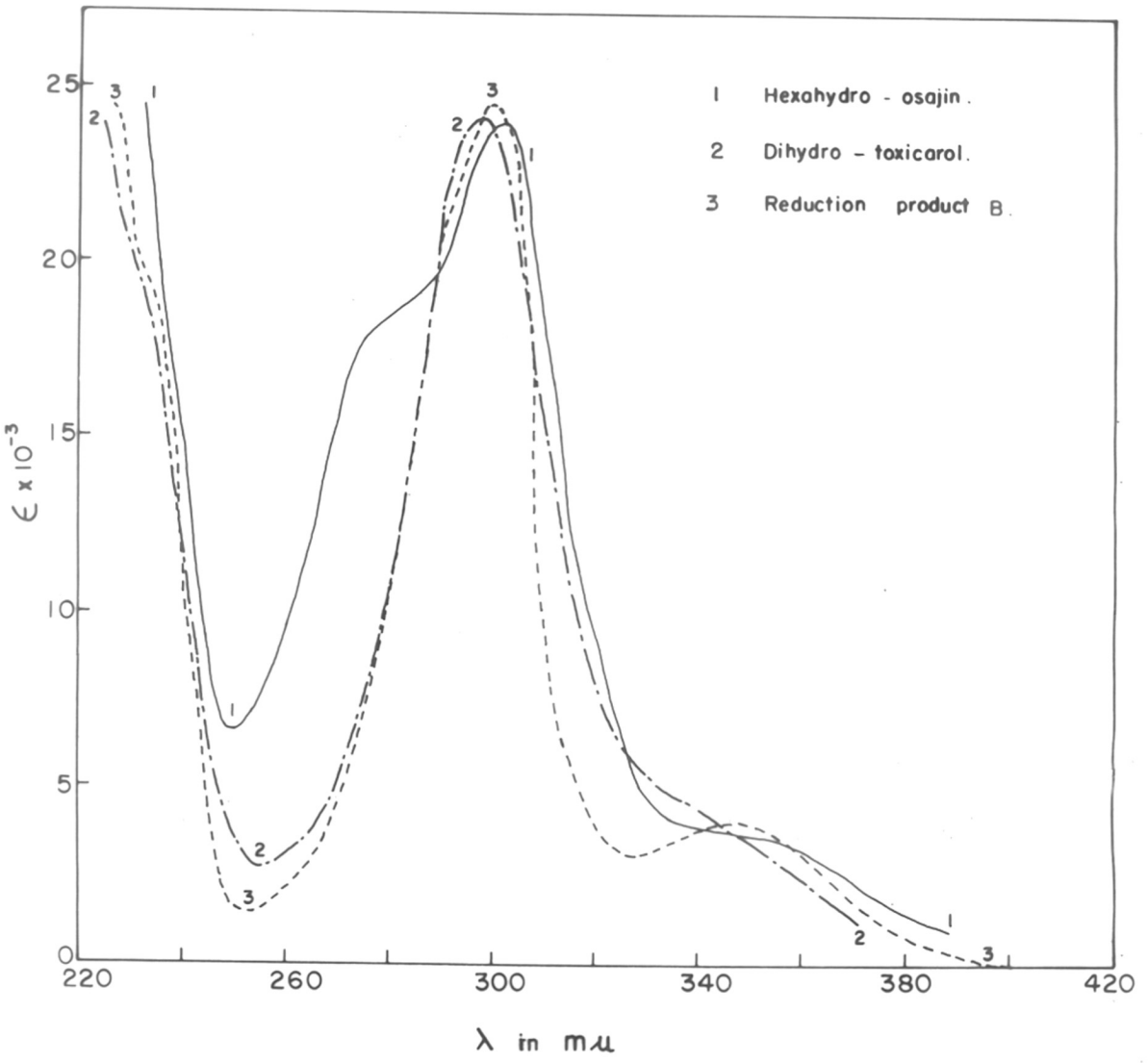
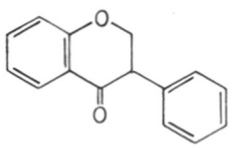

1 Hexahydro - osajin.
2 Dihydro - toxicarol.
3 Reduction product B.
ε x 10⁻³
λ in mμ
0
5
10
15
20
25
220
260
300
340
380
420

Fig. 2.



XVII

RPB did not undergo further aromatization to an isoflavone under the usual conditions, such as treatment with iodine and sodium acetate in alcohol, iodine in nitrobenzene, dichlorodicyano-p-benzoquinone, or palladized charcoal in Dowtherm.

The high intensity of the band at 300 mμ in the RPB spectrum could therefore arise by the fusion of two γ-pyranone rings to a benzene ring, for which there is considerable chemical evidence to be discussed later.

The frequencies in the double bond region of the infra-red spectra of morellin and its reduction products are tabulated in Table III and the curves are shown in Fig. 3.

Check ir of RPB - 1742 almost disappeared

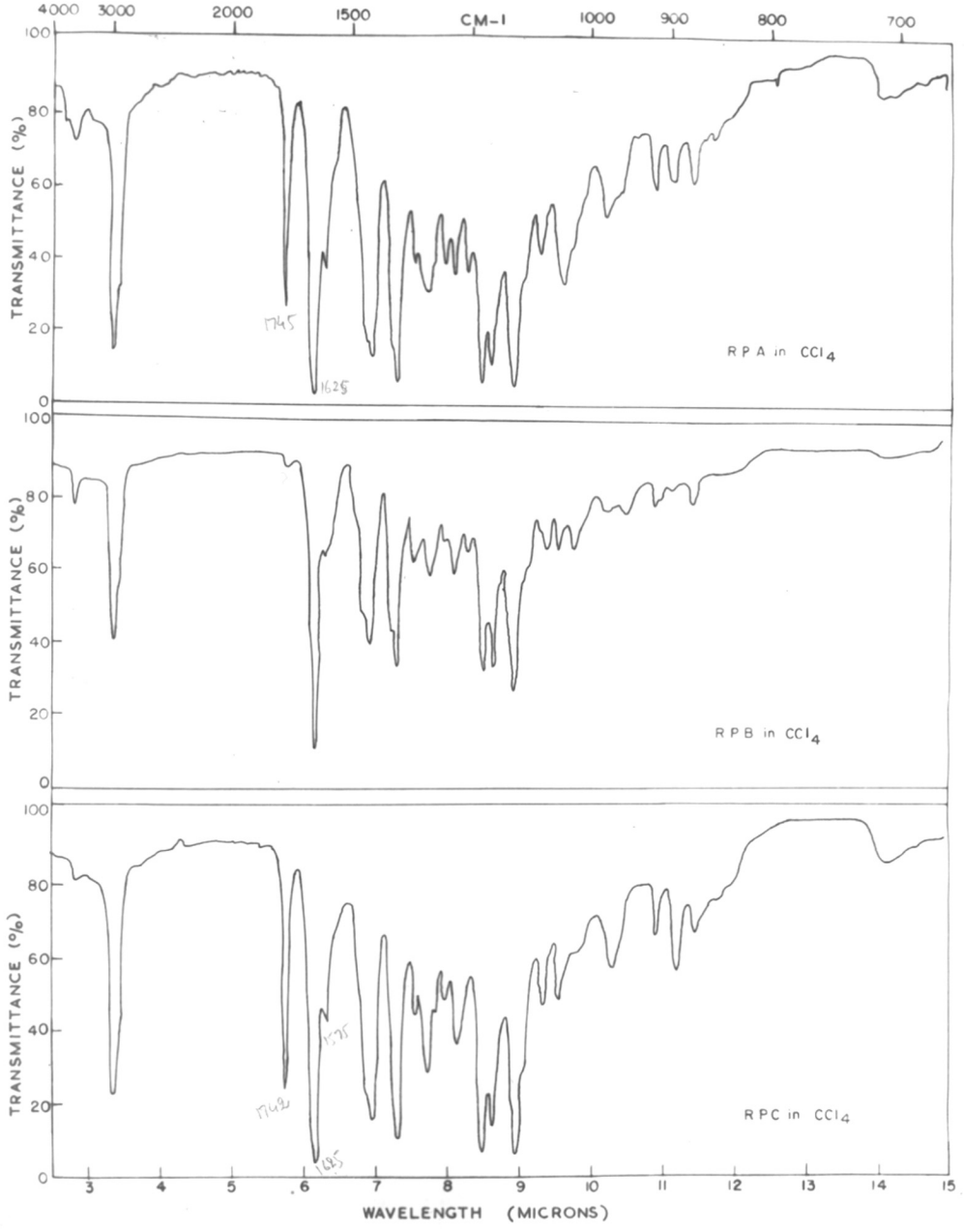


Fig. 3.

Table III

Bands (cm^{-1}) in the double bond region, the I.R. spectra of morellin and its hydrogenated products

Morellin	Tetrahydro-morellin	Octahydro-morellin	RPA	RPB	RPC
1730	1745	1745	1745	1742	1742
1667	1682	-	-	-	-
1626	1650	1640	1625	1625	1625
	1630				
1575	-	-	-	-	1575

The band at 1742-1745 cm^{-1} due to an unconjugated carbonyl group is present in all reduction products. The usual assignments for a band at about 1740 cm^{-1} are a 5-membered ring ketone and a δ-lactone. Since the products are obtained after a vigorous Raney nickel reduction, a ketone carbonyl, as in an open chain or cyclic ketone, appears to be ruled out; since the reduction products do not answer the hydroxamic acid test, a lactone is also excluded. It has been observed in this laboratory[16] that 5-hydroxychromanones are unaffected by treatment with Raney nickel under the conditions used by Mozingo; but it is obviously

the band at 1625 cm^{-1} in morellin as well as the reduction products which are to be assigned to a chelated carbonyl group as in a 5-hydroxychromanone. However, some model di-dihydro-γ-pyrones were synthesized according to the method developed by Bhat,[16] and they show typical bands in the 1730-1740 cm^{-1} region. We shall revert to a fuller discussion of this point at a later stage.

Stepwise degradations of RPB were then undertaken. In view of the structure which we have been using as a guiding hypothesis (see Introduction), the first point to decide was whether a long side-chain with 8 or more carbon atoms was present. The very poor yields of methyl heptenol obtained by the alkali fusion of morellin was one of the factors which did not support the presence of a single long chain, and evidence for the presence of two or more shorter side-chains was sought. An alkaline permanganate oxidation of RPB gave rise to n-valeric acid. This showed the presence of a normal C_5 side-chain, but no other fraction of the molecule was isolable. Considering the possibility of the attachment of the side-chain through a carbonyl or secondary hydroxyl

group, a vigorous hypoiodite oxidation of B was carried out. An acid fraction was isolated in 35 per cent yield, but only as an amorphous pale-yellow powder, and there was no steam-volatile fraction. The product gave a green ferric colour similar to the starting material, did not contain iodine, and did not respond to a magnesium and hydrochloric acid colouration for a γ-pyrone nucleus. However, it gave a red-violet colouration in the hydroxamic acid test, showing that it is a carboxylic acid. A crystalline neutral product, m.p. 179°, was also isolated from the reaction mixture; it had a molecular weight (491 ± 3) determined by the thermistor method. This compound did not dissolve in boiling 5 per cent aqueous sodium hydroxide. It gave a deep wine-red colouration with magnesium and hydrochloric acid, showing the presence of a chromone or chromanone nucleus. The ultra-violet spectrum showed bands at 252 mμ (ε 25000), 300 mμ (ε 8000) and 325 mμ (ε 4000), compatible with a chromone structure, as chromones show typical absorption at 252, 300 and 325 mμ. A similar absorption is shown by eugenin, a chromone from phloroglucinol which shows a main band at 290 mμ (log ε 2.9) and selective absorption in the region

230-260 mμ (log ε 4.2). The introduction of an alkyl group in position 6 brings about a bathochromic shift of about 5 mμ. Substitution in the 8-position produces a new maximum at 330 mμ (log ε 3.2). Likewise the maximum at 290-300 mμ is weakened and the band at 250-260 mμ becomes strong, and such a spectrum is shown by the oxidation product. A similar behaviour is shown by the attachment of a dihydrofuran ring in the 6,7 or 7,8 positions as in dihydrovisnagin and dihydrokhellin.[17] The compound was not susceptible to alkaline hydrolysis, as the hydrolysis product still responded to γ-pyrone colour reactions.* In the infra-red it showed bands at 1710 cm^{-1} (unconjugated ketone), 1649 cm^{-1} (chelated carbonyl), 1560 cm^{-1} (enolizable β-diketone). The band at 1560 cm^{-1} is not present in the starting material showing that a CHOH group probably has been oxidized to CO by hypoiodite. Only traces of iodoform could be obtained after steam distillation of the neutral fraction, which showed that no $COCH_3$ group has been oxidized quantitatively.

An attempted nitric acid oxidation[18] of RPB with ammonium venadate as catalyst readily gave a neutral

* Rules out chromone; but check props of 1/4-xanthones

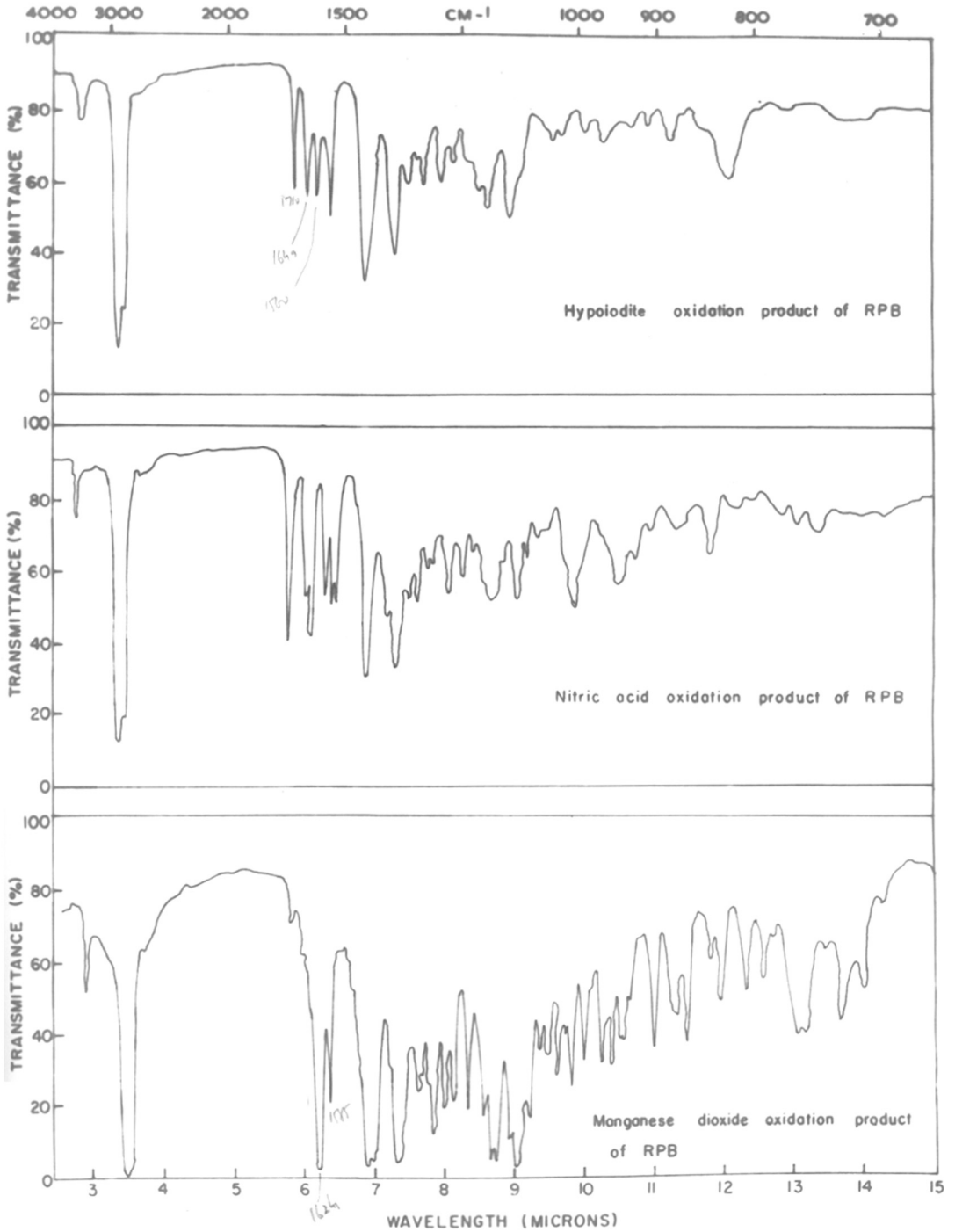


Fig. 4.

(I.R. spectra in Nujol)

product, m.p. 187°, which had a molecular weight of 488 (Rast), showing that no appreciable portion of the molecule was broken off. In the infra-red it showed bands at 1710 cm^{-1}, 1652 cm^{-1}, 1628 cm^{-1}, 1575 cm^{-1}, 1560 cm^{-1} and 1541 cm^{-1}. On oxidation with active manganese dioxide in boiling benzene RPB gave a crystalline compound, $C_{33}H_{44}O_6$, m.p. 178°, the infra-red spectrum of which did not contain any additional carbonyl band. It showed bands at 1746 cm^{-1} (unconjugated carbonyl), 1624 cm^{-1} (chelated carbonyl), 1585 cm^{-1} (phenyl band). The infra-red spectra of the hypoiodite, nitric acid and manganese dioxide oxidation products are shown in Fig. 4.

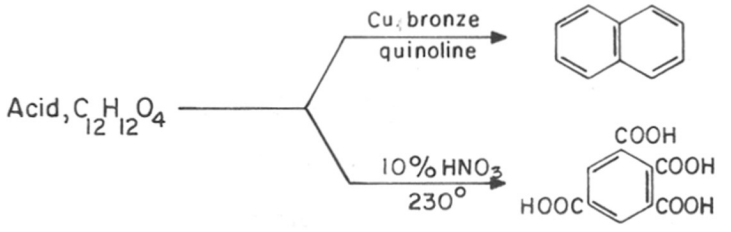
Alkali fusion of RPB

Reduction product B was fused with five times its weight of potassium hydroxide at 290° and the product separated into (a) bicarbonate-soluble, (b) carbonate-soluble, (c) sodium hydroxide-soluble, and (d) neutral fractions. The bicarbonate-soluble portion led to 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. 162-163°, was obtained from the carbonate as well as sodium hydroxide fractions.

The neutral fraction gave a volatile alcohol and a crystalline compound, m.p. 128°, obtained after high-vacuum distillation.

Constitution of the acid $C_{12}H_{12}O_4$

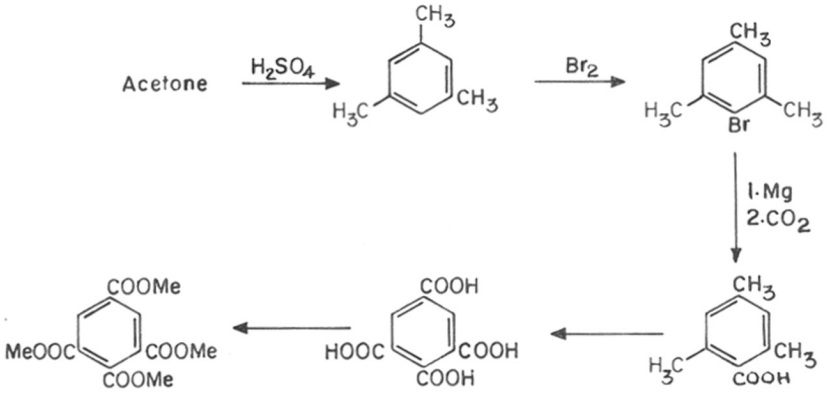
The acid analysed for $C_{12}H_{12}O_4$ and the neutralization equivalent (112) showed that it is a dicarboxylic acid. Treatment of the acid with copper bronze in boiling quinoline yielded naphthalene; it was therefore clear that decarboxylation was accompanied by dehydrogenation. The dehydrogenating effect of copper bronze in boiling quinoline was shown by the conversion of tetralin to naphthalene. Oxidation of the acid with 10 per cent nitric acid in a sealed tube at 230° yielded mellophanic acid (benzene-1,2,3,5-tetracarboxylic acid; XVIII),



XVIII

the identity of which was confirmed by direct comparison of its tetramethyl ester with an authentic sample of tetramethyl mellophanate prepared as follows by a

known series of reactions.



The conversion of the C_{12} acid to naphthalene and mellophanic acid indicated its structure to be 1,2,3,4-tetrahydronaphthalene-5,7-dicarboxylic acid. This was further supported by the close similarity of its ultra-violet absorption spectra with that of isophthalic acid (Fig. 5). Phthalic acid showed two bands: a broad band at 284 mμ (ε 1770) and a sharp peak at 230 mμ (ε 8400). Terephthalic acid showed a broad band at 282 mμ (ε 1900) and a second band at 240 mμ (ε 16,450). A comparison of the ultra-violet spectra of the alkali fusion acid with benzoic acid, phenylacetic acid, ac-tetrahydronaphthalene-1-carboxylic acid, ar-8-methyltetrahydronaphthalene-1-carboxylic acid and ar-tetrahydronaphthalene-1-carboxylic acid (Fig. 6)

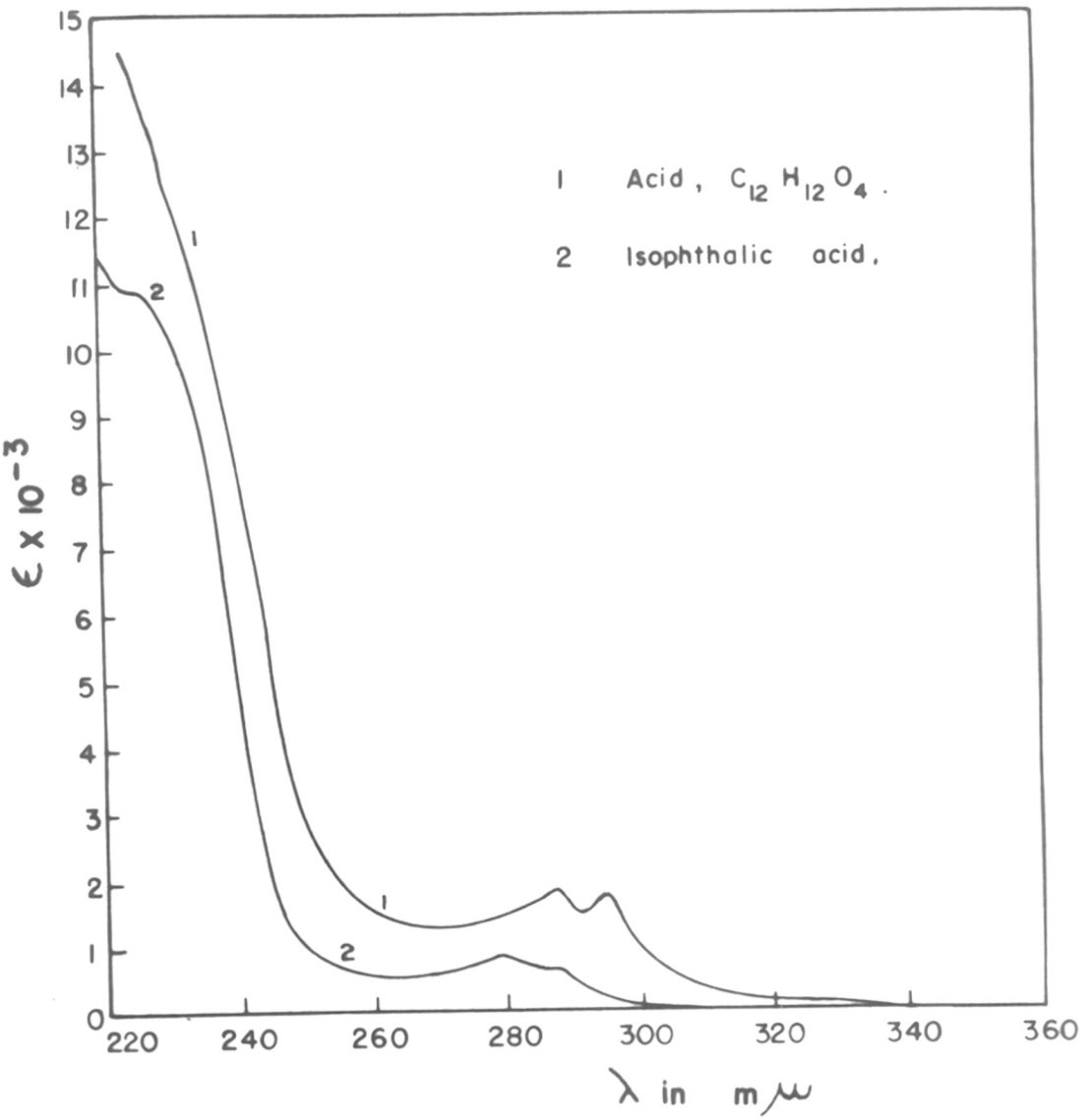

1 Acid, $C_{12}H_{12}O_4$.
2 Isophthalic acid.
$\epsilon \times 10^{-3}$
λ in mμ
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
220
240
260
280
300
320
340
360

Fig. 5.

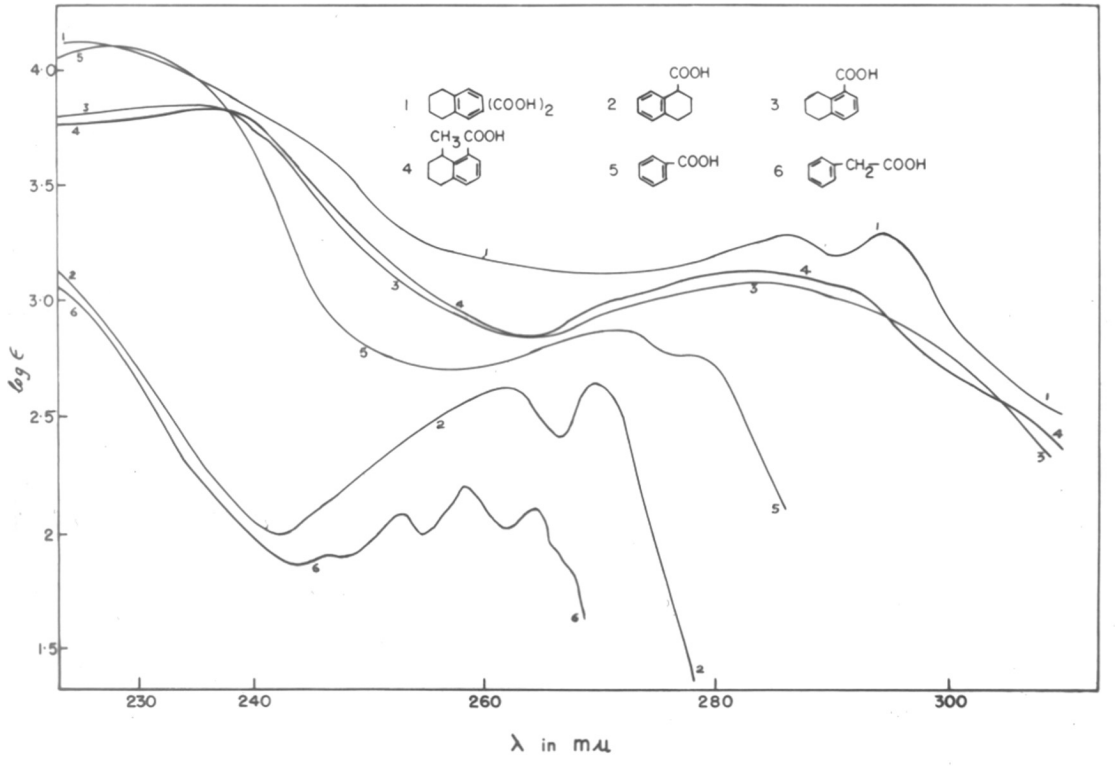
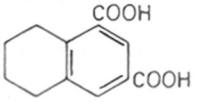

log ε
4·0
3·5
3·0
2·5
2
1·5
230
240
260
280
300
λ in mμ
1 (COOH)2
2 COOH
3 COOH
4 CH3 COOH
5 COOH
6 CH2 COOH

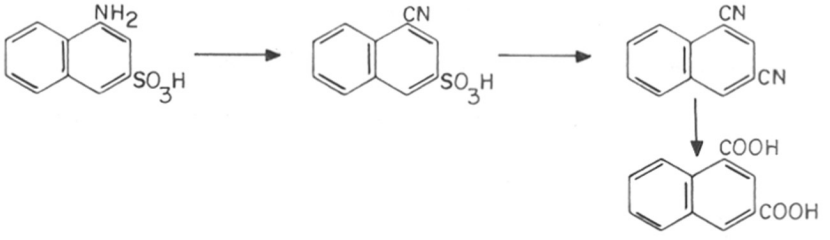
Fig. 6.

showed its similarity with ar-tetrahydronaphthalene carboxylic acids and not the ac-tetrahydronaphthalene carboxylic acid.



XIX

A synthesis of acid (XIX) was then undertaken. Naphthalene-1,3-dicarboxylic acid was synthesized as follows by a known series of reactions.



The naphthalene-1,3-dicarboxylic acid was recovered on an attempted Mozingo reduction with Raney nickel or catalytic hydrogenation with platinum oxide. Reduction with Raney nickel-alcohol under pressure in a Paar hydrogenator[20] however gave an acid, m.p. 270-290°, which was obviously a mixture of the ac- and ar-tetrahydronaphthalene-5,7-dicarboxylic acids. But its ultra-violet spectrum showed that it was mostly (XIX). It had bands at 230 m . (18500), 287 m (3000), 296 m (3100) in the U.V.

spectrum. The isolation of the acid (XIX) proved conclusively the presence of a naphthalene skeleton in the morellin molecule.

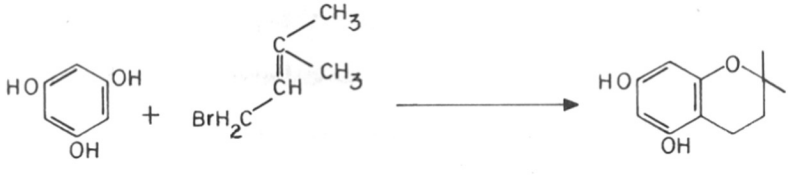
Among the volatile fatty acids, n-valeric acid was identified as the p-bromophenacyl ester as well as its β-anthraquinonoylamide. An X-ray powder pattern of the silver salt of the crude fatty acid mixture showed the presence of traces of isobutyric acid in addition to n-valeric acid. n-Valeric acid was also isolated from the oxidation of RPB with alkaline permanganate; because of possible migration of alkyl groups in a vigorous Raney nickel reduction, no attempt is made at this stage to interpret this result.

Tetrahydronaphthalene-1,3-dicarboxylic acid and n-valeric acid together account for 17 carbon atoms in morellin, leaving 16 carbon atoms, the pattern of which is elucidated by the constitution of the C_{16}-phenol.

Constitution of the phenol, $C_{16}H_{24}O_4$, and re-examination of the postulated 2,2-dimethylchromene system in morellin

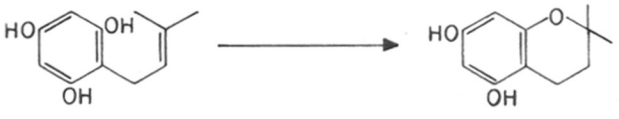
Bringi[1] regarded the production of acetone by mild alkaline hydrolysis of morellin as evidence for the presence of a 2,2-dimethylchromene nucleus in morellin. Toxicarol for instance readily gives acetone on hydrolysis with 5 per cent potassium hydroxide. However, final confirmation of its presence requires the isolation of

5,7-dihydroxy-2,2-dimethylchroman from the products of alkali fusion of octahydromorellin or other hydrogenated morellin. The chroman (XX) was thus isolated by alkaline degradation of tetrahydrorottlerin (XXI) and dihydrotoxicarol (XXII). On catalytic hydrogenation the dimethylchromene moiety absorbs a mole of hydrogen and becomes stable, not only to mild alkaline hydrolysis, but also to drastic alkali fusion. Because of the obvious difference in the properties of the C_{16}-phenol and those of 5,7-dihydroxy-2,2-dimethylchroman,[24] it was necessary to ensure that the chroman (XX) in the alkali fusion products had not eluded isolation. With this idea in mind the synthesis of 5,7-dihydroxy-2,2-dimethylchroman (XX) was undertaken in order to examine its colour reactions, solubility characteristics, and other properties. Among the earlier methods known for its synthesis, one due to Wolfrom[23] involves the condensation of γ,γ-dimethylallylbromide with phloroglucinol in presence of zinc chloride.

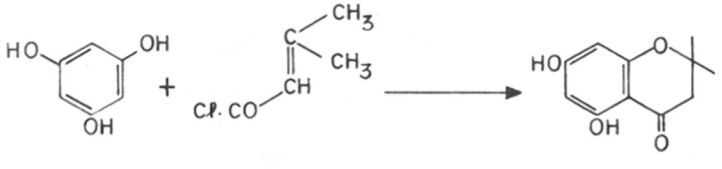


XX

A recent variation of this method is to prepare γ,γ-dimethylallylphloroglucinol by the prenylation of phloroglucinol and then cyclize it to (XX) by means of potassium hydroxide.

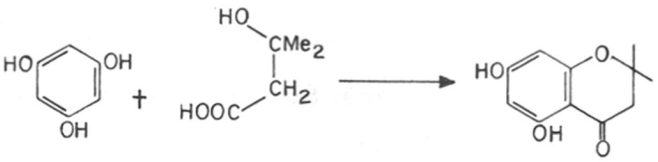


Robertson[25] carried out a Friedel-Crafts reaction between β,β-dimethylacrylyl chloride and phloroglucinol; the resultant 2,2-dimethyl-5,7-dihydroxychromanone (XXI),



XXI

obtained was then subjected to a Clemmensen reduction to give the chroman (XX). The yield in the reduction step was 61 per cent. Bhat[16] has recently synthesized 5,7-dihydroxy-2,2-dimethylchromanone by the condensation of β-hydroxyisovaleric acid with phloroglucinol in presence of boron fluoride.



XXI

The chromanone was then subjected to sodium borohydride reduction[26] in diglyme in presence of boron fluoride, when a 70 per cent yield of 5,7-dihydroxy-2,2-dimethyl-chroman was obtained. The ultra-violet spectrum of 5,7-dihydroxy-2,2-dimethylchroman[16] has a maximum at 272 mμ (ε 6000) and a minimum at 252 mμ (ε 2000). It is crystallizable from water or benzene. Robertson et al. observed a faint blue colour with aqueous ferric chloride, but the synthetic product now obtained was devoid of ferric colour reaction. The chroman (XX) readily coupled in aqueous sodium carbonate medium with diazotized aniline to give a bis-benzeneazo derivative, m.p. 258° (decomp), useful for characterizing the chroman and for isolating it from dilute aqueous solutions.

In the alkali fusion of RPB the sodium carbonate-soluble and the sodium hydroxide-soluble fractions yielded only the C_{16}-phenol. Since the chroman (XX) was carbonate soluble, the corresponding fraction from the alkali fusion of RPB was closely examined. The sodium

carbonate solution as well as the mother liquor obtained after the removal of the C_{16}-phenol did not couple with diazotized aniline. Special methods of diazonium coupling, such as those involving the use of pyridine, dimethylformamide, acetone and ammonia, or diazoaminobenzene in acetic acid, applied to the crude products isolated from the sodium carbonate and hydroxide extracts, gave negative results. The presence of the chroman unit (XX) in the morellin molecule therefore became open to serious doubt, unless the phloroglucinol ring carried a C_5 substituent.

Because of its amorphous character octahydromorellin was not further investigated in the earlier programme; but for attempts to isolate and characterize the phenolic moiety in morellin, the readily available octahydromorellin was a suitable raw material. Since only the ethylenic bonds of morellin have been reduced in the preparation of octahydromorellin and no other structural changes have occurred, alkali fusion of octahydromorellin should lead to the isolation of the chroman (XX) or a C-alkyl derivative if the C_{16}-compound is again obtained as the sole phenolic product. Octahydromorellin formed a clear melt with five times its weight of potassium hydroxide at 245°. After cooling, acidification, and extraction with ether, the fractions soluble in sodium bicarbonate,

carbonate, and hydroxide, and a neutral fraction were separated. Acidification of bicarbonate extract, ether extraction, removal of ether and steam distillation of the residue gave a mixture of fatty acids, characterized by paper chromatography in butanol-ammonia system[27] to be a mixture of traces of formic, acetic, isobutyric, iso or n-caproic acids along with a major amount of n-valeric acid. Acidification of the carbonate extract gave an oil, from which the C_{16}-phenol could be readily isolated by crystallization from a mixture of acetone and hexane with cooling at 0° for a few days. The mother liquor from the crystallization of the C_{16}-phenol, using chromatographic procedures or diazonium coupling, gave no indication of the presence of the chroman (XX). When the residue from the mother liquor was sublimed at $210^{\circ}/1.9 \times 10^{-4}$ mm, a yellow crystalline sublimate was obtained, which crystallized from acetone-hexane in yellow needles, m.p. 228°, analysing for $C_{24}H_{30}O_6$. The substance exhibited a light-green ferric colour, and the ultra-violet absorption showed two maxima at 252 mμ and 270 mμ; chroman (XX) shows only one maximum at 270 mμ (ε 6000).

The sodium hydroxide-soluble fraction, chromatographed on Florex, separated into two bands, from one of which more of the C_{16}-phenol was obtained. The other band

could not be induced to crystallize. It did not couple with diazotized aniline and the ultra-violet spectrum was not that of the chroman (XX).

It was clear beyond doubt that the chroman (XX) was not produced in the alkali fusion of octahydro-morellin, and therefore that the structure of the C_{16}-phenol had to be elucidated for determining the structure of the phloroglucinol moiety in morellin.

The C_{16}-compound, m.p. 163°, was colourless and it crystallized from benzene, hexane or water.

Repeated estimations of carbon, hydrogen and molecular weight 278, 285 (Rast) agreed with the formula, $C_{16}H_{24}O_4$. Confirmation of the molecular weight by the X-ray method was not possible as the crystals could not be obtained in large enough size. The active hydrogen values (lithium aluminium hydride method) indicated three hydroxyl groups. It did not exhibit any colouration with alcoholic or aqueous ferric chloride, indicating the absence of an o-hydroxycarbonyl or 5-hydroxychromone group; but oxidation with manganese dioxide in boiling benzene or the Oppenauer oxidation with aluminium isopropoxide gave an oily product which showed a purple ferric colour like 5,7-dihydroxy-2,2-dimethylchromanone (XXI). It appeared probable that

the C_{16}-compound was a 5-hydroxychromanol, formed by the reduction of a 5-hydroxychromanone during the alkali fusion.

The C_{16}-compound dissolved in 10 per cent aqueous sodium carbonate freely and it gave a purple colouration (λ_{max} at 545 mμ) in the Gibbs quinone-chloroimide test[28] for a free position *para* to phenolic hydroxyl group, very similar to resorcinol and orcinol (both of which show bands at 547 mμ). For the quinone-chloroimide test, King *et al.*[28] used pyridine as solvent. According to their procedure a small quantity (1-3 mg) of the liquid or the powdered substance to be tested was dissolved in pyridine (1 ml), treated with a freshly prepared solution of Gibbs reagent in pyridine (4-5 mg in 5 ml of pyridine), and finally diluted to 20 ml to sodium borate buffer (pH 9.2). The colour that developed was measured spectroscopically within 10-20 minutes of mixing using the reagent (chloroimide in pyridine-borate) as reference. A band in the region 500-700 mμ indicated a positive test. It was found that the quinone-chloroimide itself became dark green in pyridine solution in 10 minutes, which interfered in the colour reaction. However when pyridine was replaced with dimethylformamide (DMF), the reagent could be preserved for 24 hours without any change in colour. Using DMF as solvent, it was found that resorcinol

and orcinol gave a violet colour (547 mμ in the ultra-violet spectrum. The C_{16}-compound was therefore a m-dihydric phenol. Since evidence has already been adduced for the probable presence of a chromanol group, all the four oxygen atoms are accounted for, and the C_{16}-compound may be formulated as a 5,7-dihydroxychromanol.

Methylation of the C_{16}-compound did not give a crystalline ether.

The prominent bands in the infra-red spectra of 5,7-dihydroxy-2,2-dimethylchromanone, 5,7-dihydroxy-2,2-dimethylchroman and the C_{16}-phenol are shown in Table IV.

Table IV

Prominent bands (cm^{-1}) in the infra-red spectra of 5,7-dihydroxy-2,2-dimethylchromanone, 5,7-dihydroxy-2,2-dimethylchroman and the C_{16}-phenol

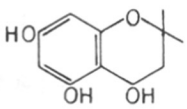
Paraffin mull

Compound	Prominent bands (cm^{-1})				
5,7-Dihydroxy-2,2-dimethylchromanone	3050	1633	1593	1014 1059 1084 1123	875
5,7-Dihydroxy-2,2-dimethylchroman	3240	1620	1604	1057 1052 1033 1119	870
C_{16}-phenol	3480	1627 1654	1575	1060 1048 1024 1117	860

gem-Me_2 ? 1380; 1365 in fusan.

In the carbonyl region the C_{16}-phenol shows a doublet at 1627, 1654 cm^{-1}. 5,7-Dihydroxy-2,2-dimethylchromanone shows a band at 1633 cm^{-1} (chelated carbonyl). Although the corresponding chroman does not have a chelated carbonyl group, it shows a band at 1620 cm^{-1}. These anomalous bands in the chroman and the C_{16}-phenol are discussed later. All the three compounds show four typical bands in the region 1000-1150 cm^{-1} and these can be attributed to a pyran or dihydropyrone ring; and the bands near 875 cm^{-1} may be assigned to a $>C-C$ group.
O

In the ultra-violet spectrum of the C_{16}-phenol two bands at 252.5 $m\mu$ (ε 9000) and 306 $m\mu$ (ε 3600) were seen. The alkylphloroglucinols have only one peak near 270 $m\mu$ (ε ca. 6000). The absorption spectrum of a chromanol (XXII) derived from phloroglucinol should have an alkylphloroglucinol spectrum.



XXII

In spite of the chemical evidence which indicated the possibility of a chromanol structure for the C_{16}-phenol, the difference between its ultra-violet spectrum and the spectra of phloroglucinol, isoamyl phloroglucinols and

5,7-dihydroxychroman made it necessary to consider other possibilities.

As stated earlier, the C_{16}-phenol did not exhibit a ferric colouration and did not form a dinitrophenylhydrazone; but after oxidation under conditions which would convert a CHOH to CO, the product gave a ferric colour like an o-hydroxyacetophenone or a 5-hydroxychromone or 5-hydroxychromanone. However, the ultra-violet spectrum of the C_{16}-phenol resembled that of o-hydroxyacetophenone. The ultra-violet absorption spectra of the C_{16}-phenol, 5,7-dihydroxy-2,2-dimethylchroman and o-hydroxyacetophenone are shown in Fig. 7. The C_{16}-phenol and o-hydroxyacetophenone show bands at 252 mμ (ε 9000) and 252.5 mμ (ε 9330) respectively. Besides, the C_{16}-phenol shows a weak band at 306 mμ (ε 3600) and a similar band is shown at 327 mμ (ε 3550) by o-hydroxyacetophenone. The 327 mμ band in o-hydroxyacetophenone is typical of a ketone. The occurrence of a low-intensity band at a shorter wave-length (306 mμ) in the spectrum of the C_{16}-phenol provides somewhat equivocal evidence in favour of an o-hydroxyacetophenone structure.

The ultra-violet absorption maxima of some phenolic ketones are listed in Table V.

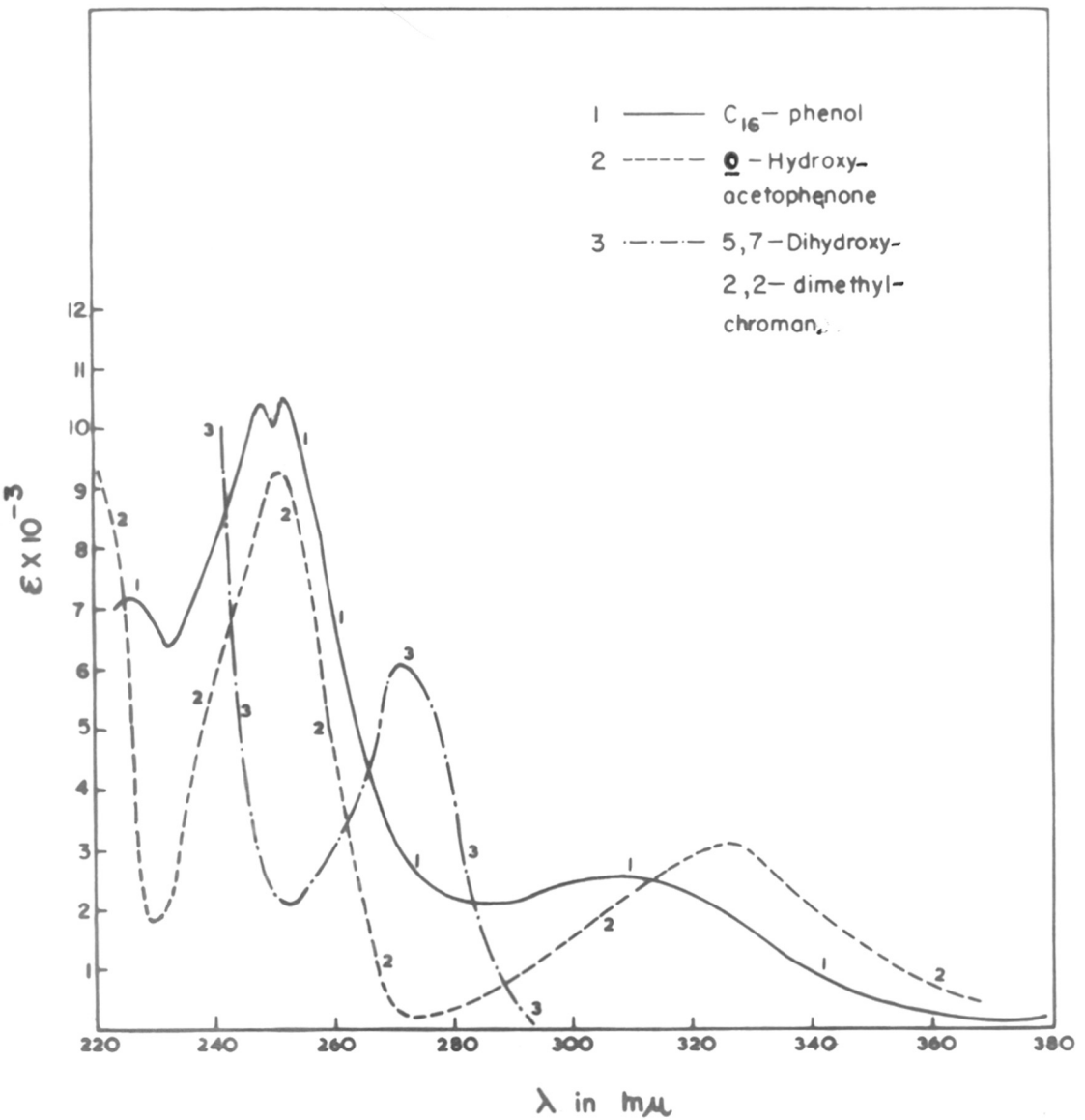

1 —— C_{16} – phenol
2 ------ o – Hydroxy-acetophenone
3 -·-·- 5,7 – Dihydroxy-2,2– dimethyl-chroman.
ε X 10⁻³
λ in mμ
220
240
260
280
300
320
340
360
380

Fig. 7.

Table V

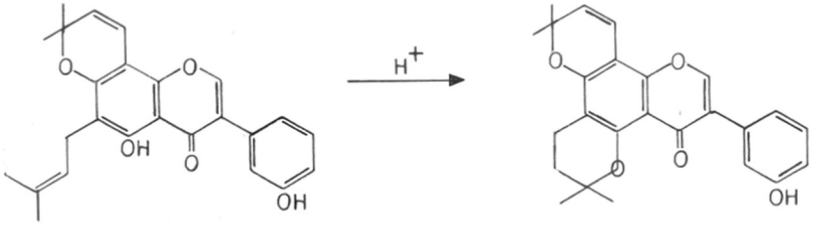
Wave-lengths and intensities of the maxima in the ultra-violet spectra of o- and p-hydroxyacetophenones, resacetophenone, C-methylphloracetophenone and C_{16}-phenol

Compound	λ_{max}	ε_{max}	λ_{max}	ε_{max}	λ_{max}	ε_{max}
o-Hydroxyacetophenone	327	3550	251.5	9330		
p-Hydroxyacetophenone	330	60	277	13500	221	10950
Resacetophenone	315	7890	278	13780	236	7980
					231	8460
C-Methylphloraceto-phenone	~333	2510	291	17800	223	12600
C_{16}-phenol	306	3600	252.5	9000	-	-

In C-methylphloracetophenone the broad band at 333 mμ corresponds to the 327 mμ band of o-hydroxyaceto-phenone, and the 291 mμ band to the 277 mμ band of p-hydroxyacetophenone. Acetophenones with a hydroxyl ortho or meta to the carbonyl group absorb at about 250 mμ; and with a p-hydroxyl they absorb around 270-280 mμ.[29] This consideration would eliminate a p-hydroxyacetophenone or phloracetophenone structure for the C_{16}-phenol.

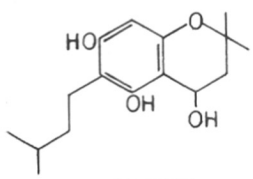
Before making further attempts to reconcile the conflicting spectral and chemical evidence, an examination of model synthetic compounds was undertaken.

5,7-Dihydroxy-2,2-dimethylchroman (XX) was not isolable from the alkali fusion products of octahydromorellin or RPB. If an isoamyl chain is attached to the benzene ring of a 2,2-dimethylchroman, as in hexahydroosajin, the formation of 6- or 8-isoamyl-5,7-dihydroxy-2,2-dimethylchroman should be expected. There was considerable evidence for the presence of an isomylene chain in morellin: the formation of isovaleric acid in the alkali fusion of morellin (cf. osajin) and the formation of acetone on ozonolysis. It was also observed that a product devoid of ferric colour was obtained from morellin on treatment with boron fluoride-etherate; apparently a cyclization similar to Wolfrom's cyclization of osajin to iso-osajin,[30] in which an isopentenyl group adjacent to a phenolic hydroxyl group isomerized to a chroman in presence of acid, had taken place (XXIII)



XXIII

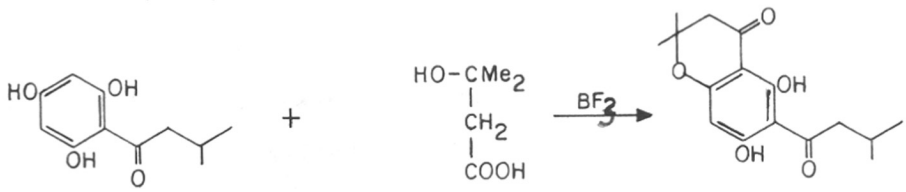
The molecular formula, $C_{16}H_{24}O_4$, and the chemical properties of the C_{16}-phenol described earlier can be explained by assigning to it the structure of 6- or 8-isoamyl-5,7-dihydroxy-2,2-dimethylchromanol (XXIV).



XXIV

With this idea in view, the synthesis of the two isomers (XXIV) and the related chromans was attempted.

Following the procedure of Bhat[16] phloroisovalerophenone (XXV) was condensed with β-hydroxyisovaleric acid (XXVI); the product was 6- or 8-isovaleroyl-5,7-dihydroxy-2,2-dimethylchromanone (XXVII).

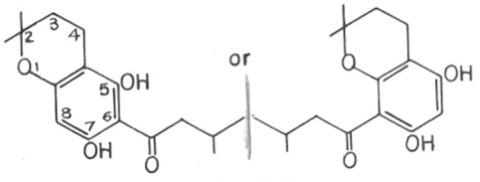


XXV XXVI XXVII

Compound (XXVII) showed a cherry-red ferric colouration; and it gave no colour with magnesium and hydrochloric acid. Clemmensen reduction of (XXVII) with zinc-amalgam and hydrochloric acid in acetic acid-ethanol solution

gave a crystalline compound which had the properties of 6- or 8-isoamyl-5,7-dihydroxy-2,2-dimethylchromanone: violet ferric colour and ultra-violet absorption at 297 mμ and 335 mμ (293.5 mμ and 335 mμ for 5,7-dihydroxy-2,2-dimethylchromanone). The possibility of a 6- or 8-isovaleroyl-2,2-dimethyl-5,7-dihydroxychroman (XXVIII) for this reduction product was excluded since the condensation product[16] of 5,7-dihydroxy-2,2-dimethylchroman and isovaleric acid in presence of boron fluoride showed bands at 278 mμ and 335 mμ. It was clear that reduction had not proceeded farther than the reduction of the side-chain CO to $-CH_2-$. However, when the Clemmensen reduction was prolonged with a larger amount of zinc amalgam, until the reaction product did not show a ferric reaction, a pale-brown oily substance was isolable from the reaction mixture which had the same ultra-violet absorption of the C_{16}-phenol. On crystallization from acetone-hexane mixture, colourless needles, m.p. 162°, were obtained in a yield of 10 per cent, which proved to be identical with the C_{16}-phenol in all respects: m.p. and mixed m.p.; superposable ultra-violet and infra-red spectra; absence of ferric colour and development of a purple ferric colour after oxidation with manganese dioxide. The Clemmensen reduction product and the C_{16}-phenol were therefore assigned the constitution

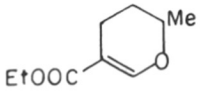
(XXIV). The isoamyl group was placed in the 6-, and not in the 8-position, because of the positive Gibbs reaction of the C_{16}-phenol mentioned earlier.



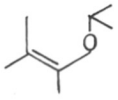
XXVIII

The permanganate oxidation to give isovaleric acid and a residue which gave a magnesium hydrochloric acid colouration was also consistent with the above structure.

Reverting back to the infra-red spectrum of the C_{16}-phenol (Fig. 8), the following assignments are made in the context of the structure (XXIV): 3480 cm^{-1} (free hydroxyl); 1060, 1048, 1024, 1117 cm^{-1} (pyran or dihydropyrone type of ring); 875 cm^{-1} due to $Me_2C(=O)-C$; and finally the 1627 cm^{-1} band to C=C-O- type of linkage. A band is shown at about 1620 cm^{-1} by some substituted furans and pyrans.



XXIX



XXX

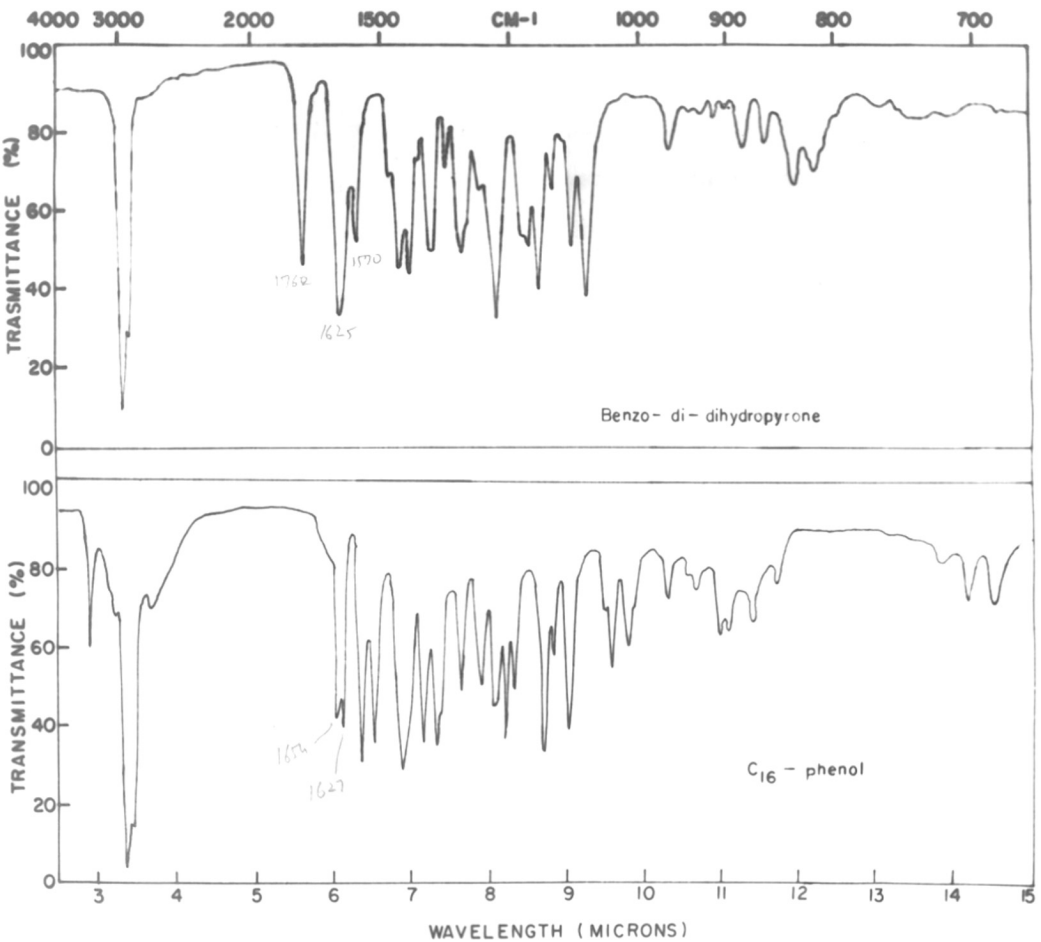


Fig. 8.

(I.R. spectra in Nujol)

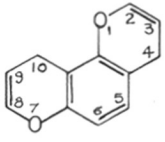
Fig. for chromon?

The dihydropyran (XXIX)shows a band at 1627 cm^{-1}, attributed to C=C-O.[31] This provides a close analogy to the C_{16}-phenol which has the part structure (XXX).

On oxidation with permanganate in acetone at room temperature, the C_{16}-compound gave acetic and isovaleric acids, which were identified by paper chromatography. Sublimation of the residue after the removal of the fatty acids gave a pale-yellow powder, m.p. 130-135°, which could not be crystallized; but the sublimate gave a distinct purple colouration with magnesium and hydrochloric acid, indicating a γ-pyrone or pyranone group. The magnesium-hydrochloric acid test, like the sodium amalgam test, is limited in scope, and dependable interpretation requires direct comparison with authentic samples. 5,7-Dihydroxychromanone (XXI) gives a wine-red colour with magnesium and hydrochloric acid, but the 6- or 8-isoamyl derivative exhibits no colouration. Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) gives a red colour with magnesium and hydrochloric acid, and it would appear that the C-isoamylphloroglucinol nucleus in the C_{16}-compound (XXIV) undergoes degradation, leaving the dihydropyrone ring intact.

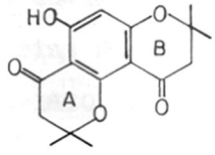
Bringi's work, supported by the present experiments,

has shown that the main framework of the morellin molecule consists of phloroglucinol linked to a partially hydrogenated naphthalene ring by a γ-pyrone nucleus. The isolation of a chromanol and not a chroman from the alkali fusion of morellin reduction products indicated the possibility of the phloroglucinol unit being fused to two γ-pyrone or dihydro-γ-pyrone rings, rather than one, morellin therefore being a derivative of benzodi-γ-pyran ring system such as (XXXI), which is 4H, 10H-benzo(1,2-b: 3,4-b')dipyran.[32] To examine such a possibility



XXXI

the synthesis of model compounds of the type (XXXII) was undertaken. Condensation of 5,7-dihydroxy-2,2-dimethylchromanone with β-hydroxyisovaleric acid (10 moles) in presence of boron trifluoride-etherate readily gave the benzo-di-dihydro-γ-pyrone (XXXII). The condensation



XXXII

product (XXXII) was assigned the angular orientation due

to the fixation of double bonds in the benzene ring as shown in the structure. Also the compound (XXXII) was the major product.

Compound (XXXII) showed two bands in the ultra-violet at 286 mμ (ε 15400) and at 345 mμ (ε 3240). The ultra-violet absorption spectra of C-methyl-phloracetophenone, 5,7-dihydroxy-2,2-dimethylchromanone, compound (XXXII), and reduction product A, are shown in Fig. 9. Though there is a general similarity in the shape of the spectra, there is considerable bathochromic shift in the spectra of the reduction products of morellin compared to compound (XXXII), but this may be the effect of the alkyl substituents and the partially or fully hydrogenated naphthalene ring fused to one of the dihydropyrone rings in the reduction products.

The infra-red spectrum of compound (XXXII) (Fig. 8) in the double bond region was of considerable interest. It showed bands at 1570, 1625 and 1762 cm^{-1}. The 1570 cm^{-1} band is the phenyl band, and the 1625 cm^{-1} band is due to the chelated carbonyl in ring A. The carbonyl group in ring B should be expected to show a band in the 1700-1680 cm^{-1} region, but it is apparently responsible for the strong band at 1762 cm^{-1}. This is of unusual interest, because in all the Raney nickel reduction products of morellin as well as octahydro-morellin a band near 1740 cm^{-1} is observed.

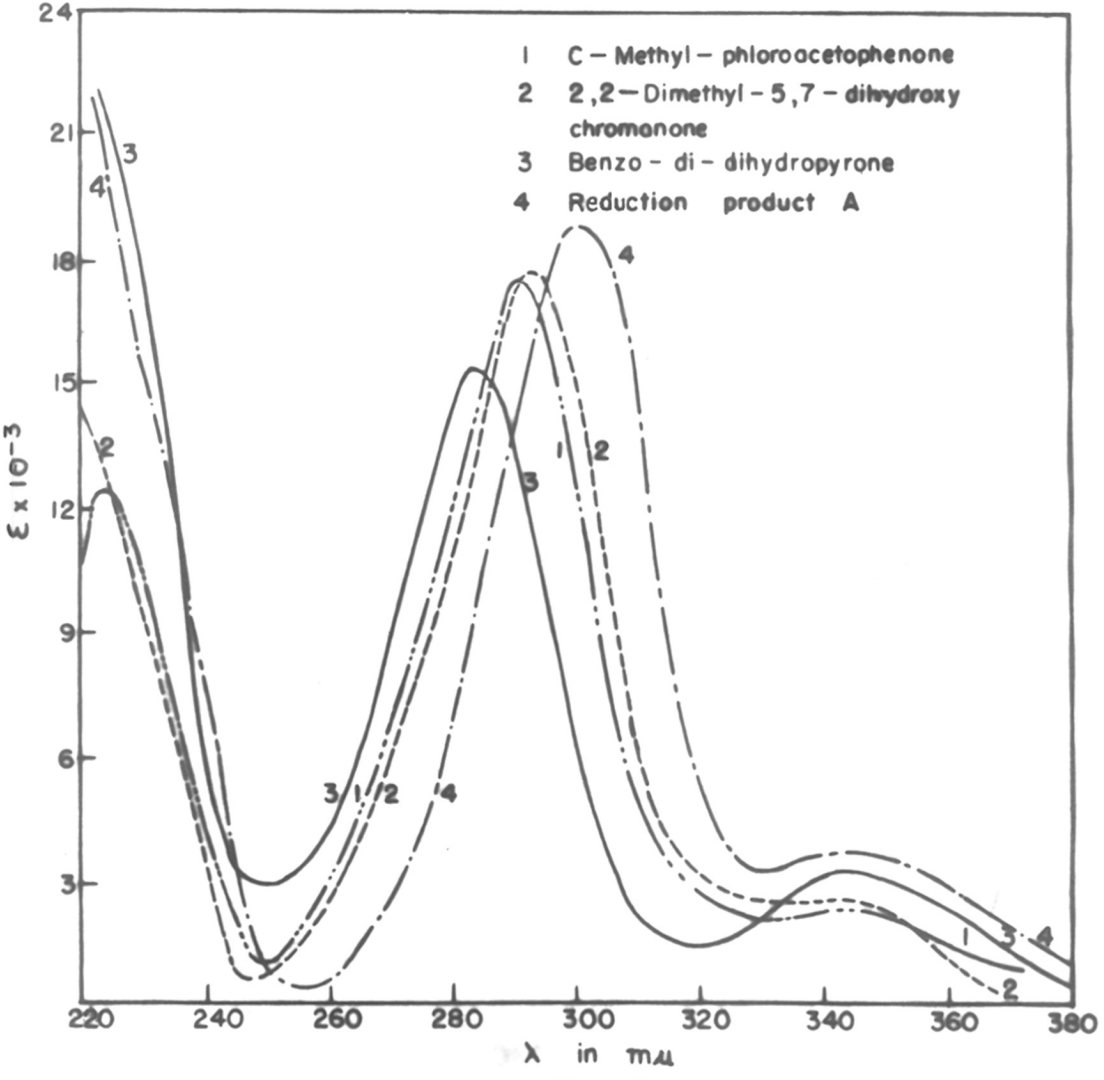
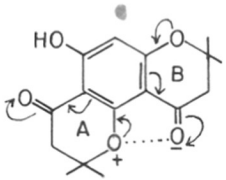

1 C – Methyl – phloroacetophenone
2 2,2 – Dimethyl – 5,7 – dihydroxy chromanone
3 Benzo – di – dihydropyrone
4 Reduction product A
ε x 10⁻³
λ in mμ
24
21
18
15
12
9
6
3
220
240
260
280
300
320
340
360
380

Fig. 9.

The appearance of this band at a frequency much higher than 1700 cm^{-1} may be the result of a dipole interaction between the carbonyl group in ring B with the ether oxygen in ring A, as shown in (XXXIIB).

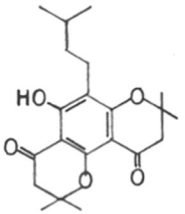


XXXII B

The carbonyl frequencies in the benzodipyranone (XXXII) appear at 1626 cm^{-1} and 1762 cm^{-1}. The band at the lower frequency is evidently due to the chelated carbonyl group and the high-frequency band has to be assigned to the second carbonyl group. The abnormally high frequency observed for the latter may most probably have its origin in the dipolar interaction with the ethereal oxygen of ring A. The thoroughly investigated α-bromoketones provide an analogy. The interatomic distance between the oxygen atom in ring A and the carbonyl oxygen in ring B in compound (XXXIIB) is smaller than the distance between the bromine and oxygen atoms in α-bromoketones, and dipolar interaction would be expected to be stronger in (XXXIIB). The ether dipole in ring A induces a polarization opposite to the normal polarization in the carbonyl group of ring B, causing an increase in its

double bond character and a consequent increase in its vibration frequency.

The carbonyl vibration of ring B appears to be influenced by the introduction of a large alkyl group in the benzene ring (XXXII). Thus the C-isoamyl derivative (XXXIII) shows a band at 1729 cm^{-1} instead of 1762 cm^{-1}. A possible explanation is that the



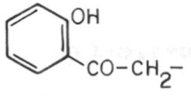
XXXIII

polarization in ring A in compound (XXXII) is strengthened by chelation of the carbonyl and hydroxyl groups, and this chelation is less effective in (XXXIII) because of the alkyl group adjacent to the hydroxyl.

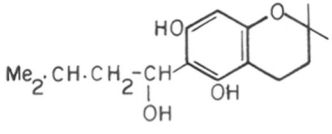
In the light of the constitution of the C_{16}-phenol obtained by alkali fusion of octahydromorellin and the anomalous high-frequency absorption in the carbonyl region of the infra-red spectra of the benzodipyranone (XXXII) as well as octahydromorellin, it was clear that octahydromorellin, which was neglected in the earlier work of Bringi because of its amorphous character, would repay attempts at stepwise degradation. The behaviour of morellin and octahydromorellin towards o-phenylenediamine and the colour of the condensation products with hydrochloric acid had shown that morellin and octahydromorellin

contained a 1,3-diketone group of the type -CO-CHR-CO-. Alkaline hydrolysis of octahydromorellin under mild conditions was therefore studied in the hope of effecting a cleavage of the β-diketone group.

Since large amounts of octahydromorellin were required, the reduction of morellin was carried out in a Paar hydrogenation apparatus, using 30 per cent by weight of 12 per cent palladium-charcoal and about 40 lb. pressure. The amorphous octahydromorellin yielded a crystalline monodinitrophenylhydrazone, m.p. 135°, $C_{39}H_{50}O_{10}N_4$, when an alcoholic solution was treated with dinitrophenylhydrazine-phosphoric acid reagent[33] and the orange-yellow precipitate was chromatographed on Florex, using benzene as solvent. Octahydromorellin also condensed with ethyl orthoformate in presence of pyridine and piperidine. This reaction, developed by Sathe and Venkataraman[34] for the cyclization of o-hydroxyphenyl benzyl ketones to isoflavones, was carried out as a diagnostic test for the group (XXXIV) at a stage of the investigation when one of the alternatives under consideration for the structure of the C_{16}-phenol was (XXXV).



XXXIV

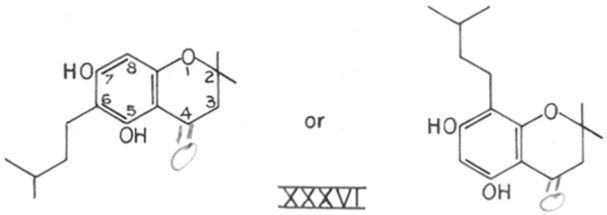


XXXV

The crystalline product, m.p. 86°, obtained after chromatography on Florex, analysed for $C_{36}H_{50}O_8$, which showed that a -CHOEt group has been introduced at a reactive methylene centre in the molecule. The product gave an olive-green ferric colour similar to octahydromorellin.

Hydrolysis of octahydromorellin with 15 per cent ethanolic potassium hydroxide in a water-bath for four hours gave formic acid and a crystalline phenol, $C_{26}H_{36}O_6$, m.p. 132°, in 60 per cent yields (M 445, 446 by the Rast method). It exhibited a blue-green ferric colour. It was soluble in aqueous sodium carbonate, but insoluble in bicarbonate. The iodide-iodate test and the hydroxamic test were negative, showing that it was not a carboxylic acid, ester or lactone. The carbonate solubility and the ferric colour therefore indicated that it contained two phenolic hydroxyl groups and was probably a 2-acylresorcinol. By the lithium aluminium hydride method it was found to contain two active hydrogen atoms, but positive evidence of 2 hydroxyl groups could not be obtained. It failed to couple with diazotized aniline, but this could not be regarded as proof that there was no free coupling position, because it was observed that 6- or 8-isoamyl-5,7-dihydroxy-2,2-dimethylchromanone (XXXVI) failed to couple with diazotized aniline under a variety of conditions. Unlike octahydromorellin, it

gave a negative sodium amalgam test.



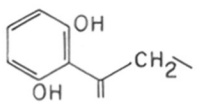
or

XXXVI

The C_{26}-phenol could also be obtained by hydrolysing octahydromorellin with 5 per cent ethanolic potassium hydroxide, the yield in fact being slightly improved. In the 15 per cent alkaline hydrolysis of octahydromorellin a functional separation was necessary to isolate the pure C_{26}-phenol which appeared in the aqueous sodium carbonate extract of an ether solution of the fission products; but in the 5 per cent alkaline hydrolysis, acidification of the alkaline solution gave the C_{26}-phenol as a yellow precipitate which readily crystallized from methanol.

The C_{26}-phenol underwent an ethyl orthoformate pyridine, piperidine condensation, and the crystalline product, m.p. 102°, analysed for $C_{27}H_{34}O_6$, which indicated that a chromone was formed. The condensation product still gave an olive-green ferric reaction. There was no colour reaction with magnesium and hydrochloric acid. On heating the C_{26}-phenol with acetic anhydride and sodium acetate for 15 hours (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 (XXXVII) to a 2-methylchromone by acetylation of a hydroxyl group. Acetyl estimation showed the presence of one acetoxy group. Since the C_{26}-phenol underwent both the ethyl orthoformate and the Kostanecki cyclizations, the presence of the group (XXXVII) was clearly proved.

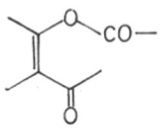


XXXVII

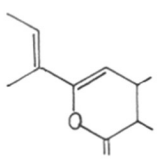
The C_{26}-phenol gave a positive iodoform test, and quantitative estimation of iodoform spectroscopically[35] showed the formation of 1 mole of iodoform and therefore the presence of one $-CO-CH_3$ group. C_{26} absorbs no H_2 on Pt/C-

Since the above evidence pointed towards the presence of the group (XXXVII) in the C_{26}-phenol, attempts were made to degrade it to the chromanone corresponding to the C_{16}-phenol and a carboxylic acid by vigorous alkaline hydrolysis. Under the conditions used for tetrahydrorottlerone, which involves hydrolysis with 75 per cent aqueous alkali at reflux, the C_{26}-phenol was recovered unchanged. Similar stability was observed on conducting the hydrolysis in ethylene glycol at reflux (b.p. 198°). However, alkali fusion with five times the weight of

potassium hydroxide at 220° gave a crystalline acid, m.p. 140°, analysing for $C_{25}H_{34}O_6$ (M 450 by the Rast method) in a yield of 50 per cent. This compound readily responded to the iodide-iodate and the hydroxamic acid tests for a carboxylic acid. Alkali titration to determine the neutralization equivalent gave very erratic values, and was very slow. The liberation of iodine was very slow when an attempt was made to determine the carboxyl content by titration with potassium iodide-iodate solution. The analysis did not agree for the conversion of a $-COCH_3$ group to a -COOH or a benzylic acid rearrangement $-\overset{OH}{\underset{|}{C}}-\overset{}{\underset{}{C}}\overset{COOH}{}$ at the $-CO-CH_2-$ centre in the molecule. However, the formation of a lactone (XXXVIII) or a stable isocoumarin (XXXIX) by cyclization would be in conformity with the analysis.



XXXVIII

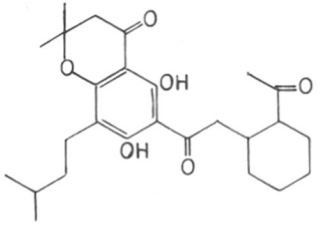


XXXIX

Alkali fusion of the C_{26}-phenol at 300° with five times its weight of potassium hydroxide for 20 minutes gave the C_{16}-phenol obtained earlier by the alkali fusion of octahydromorellin as well as reduction product B.

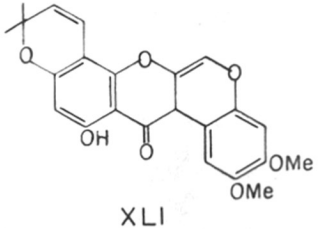
Since the C_{16}-phenol is formed by vigorous alkali fusion of the C_{26}-compound, and in view of its properties

and reactions already discussed, the C_{26}-phenol may be assigned the structure (XL). Although in the C_{16}-phenol

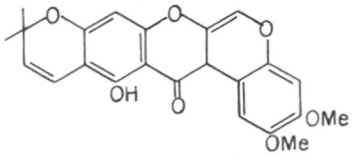


XL

the isoamyl group was located in the 6-position of the chromanone because of the positive Gibbs test, the isoamyl group is placed in the 8-position in the C_{26}-compound (XL) to account for the blue-green ferric colour which is very similar to that of 2-acetylresorcinol; resacetophenone shows a wine-red ferric colouration. The shift of the isoamyl group to the 6-position in the C_{16}-phenol, which takes place during the alkali fusion of the C_{26}-phenol, has an analogy in the isomerization of α-toxicarol (XLI) to β-toxicarol (XLII).



XLI

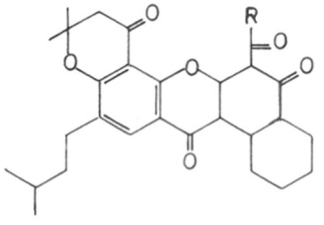


XLII

The 2,6-dihydroxyketonic structure assigned to the C_{26}-phenol, rather than the 2,4-dihydroxyketonic structure which will result from a direct derivation of the C_{16}-phenol from the C_{26}-phenol, is further supported by

the formation of a benzene-soluble red copper complex by morellin and not by octahydromorellin.

The ready hydrolysis of octahydromorellin with 5 per cent alcoholic potassium hydroxide would support the postulation of a 1,3-diketone group as in 2-acetylcyclohexanone. The structure of octahydromorellin could therefore be expanded to (XLIII)..

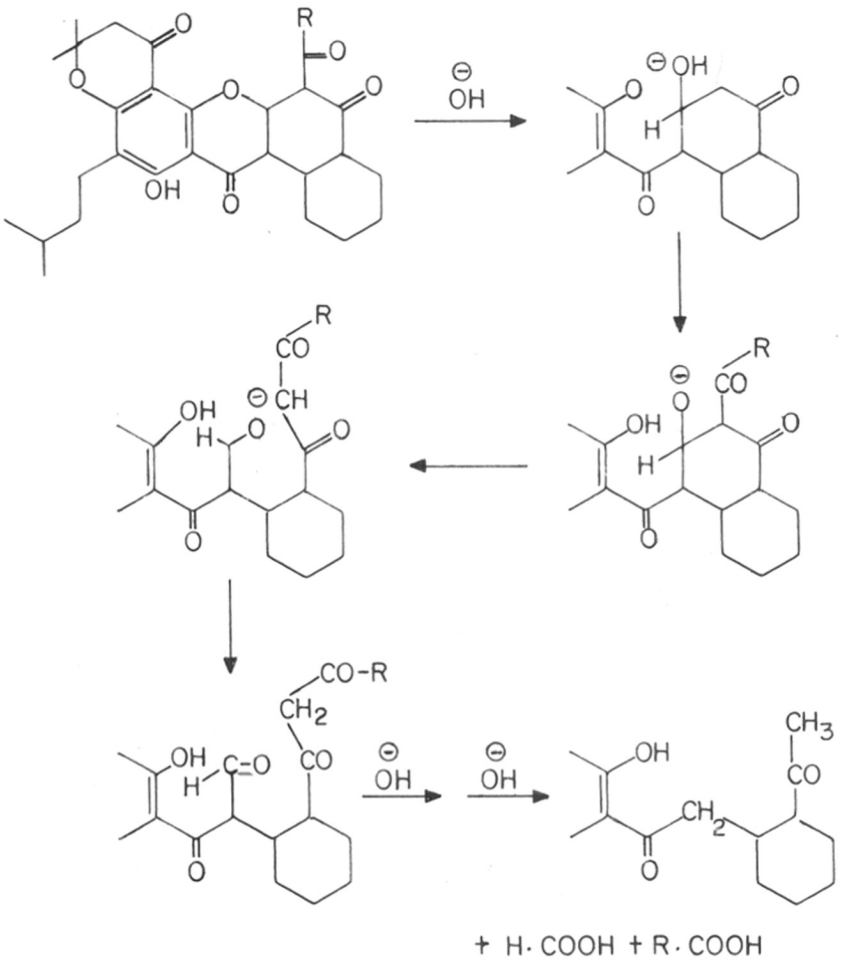


XLIII

The mechanism of the smooth hydrolysis of octahydromorellin to give formic acid and the C_{26}-compound may be represented as in Chart II.

Although there are gaps in the evidence, the proposed structure for the C_{26}-compound appears to be supported by its infra-red spectrum and the infra-red spectra of its derivatives presented in Fig. 10. The frequencies in the double bond region are shown in Table VI.

CHART II


R
O
O
O
O
OH
O
⊖
OH
⊖ OH
H
O
O
R
CO
⊖ CH
OH
H
O
O
O
R
CO
⊖
O
OH
H
O
O
CO-R
CH2
OH
H
C=O
CO
O
⊖
OH
⊖
OH
CH3
CO
OH
CH2
O
+ H·COOH + R·COOH

Make the nmr bicarbs
Repeat in CCl_4

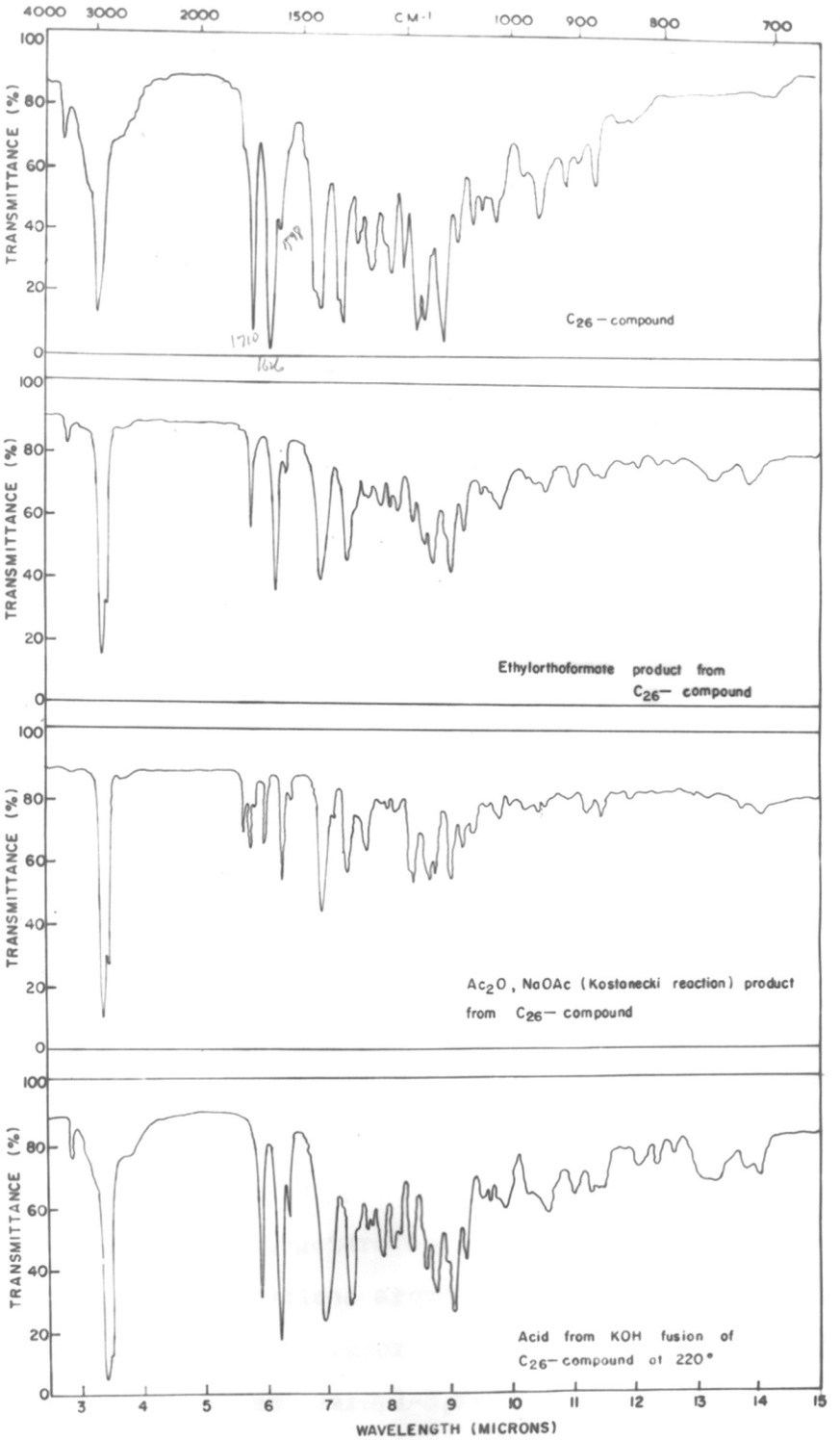


Fig. 10.

(I.R. spectra in Nujol)

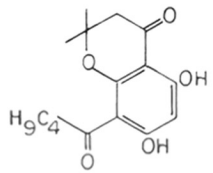
Table VI

Prominent bands (cm^{-1}) in the infra-red spectra of C_{26}-compound, its ethyl orthoformate and Kostanecki products and the C_{25}-acid from the KOH fusion of C_{26} compound.

Compound	Frequencies (cm^{-1})		
C_{26}-compound	1710	1626	1598
Ethyl orthoformate product from C_{26}-compound	1726	1622	1580
Kostanecki reaction product from C_{26} compound	1754 1732 1708	1671	1568
C_{25}-acid from KOH fusion of C_{26}-compound	1700	1620	1575

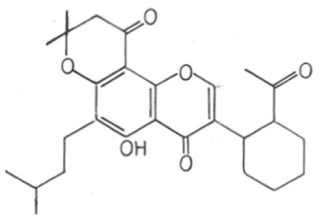
The C_{26}-compound shows a hydroxyl band at 3560 cm^{-1} and two bands at 1710 cm^{-1} and 1626 cm^{-1} in the carbonyl region. The 1710 cm^{-1} band, which is that of an unconjugated -CO- group, is assigned to the free carbonyl group in the acetyl cyclohexane part. The 1626 cm^{-1} band is assigned to the chelated carbonyl. Only one band at 1626 cm^{-1} is seen, although the structure would demand two distinct chelated carbonyl vibrations around 1635 cm^{-1} (a 5-hydroxychromanone) and 1624 cm^{-1} (o-hydroxyacetophenone). However, the synthetic 8-isovaleroyl-5,7-dihydroxy-2,2-dimethylchromanone

(XLIV) showed only one band at 1624 cm^{-1}, although it has two distinct types of chelated carbonyl groups.



XLIV

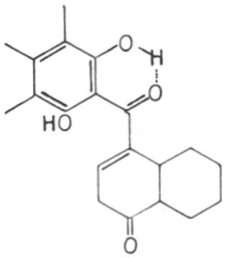
Repeated estimations of carbon, hydrogen, oxygen, molecular weight and active hydrogen are in agreement with the proposed structure for the C_{26}-compound. The structure is of the desoxybenzoin type, and there should be three active methylene centres in the molecule. If this is so, the crystalline compound (m.p. 97°), obtained by the action of ethyl orthoformate on the C_{26}-compound, should be a simple 3-substituted chromone (XLV). The structure should show three distinct carbonyl functions: a chelated carbonyl, an



XLV

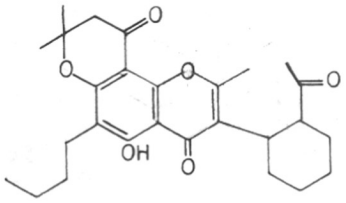
α,β-unsaturated carbonyl, and an unconjugated carbonyl as in acetone. In the infra-red the ethyl orthoformate product showed a hydroxyl band at 3500 cm^{-1} and only two bands in the carbonyl region, one at 1624 cm^{-1} (chelated carbonyl) and the other at 1726 cm^{-1} (an unconjugated

carbonyl). There is no band in the 1670-1680 region for an α,β-unsaturated carbonyl function. This evidence renders the chromone structure for the ethyl orthoformate product unlikely, although the condensation product on treatment with 10 per cent methanolic sodium hydroxide gives formic acid. The structure (XLVI) is a possibility to be considered.



XLVI

The infra-red spectrum of the Kostanecki reaction product from the C_{26}-compound was in agreement with the structure (XLVII). In the carbonyl region four distinct bands were seen: 1754 cm^{-1}, 1732 cm^{-1}, 1708 cm^{-1} and at 1671 cm^{-1}. The 1732 cm^{-1} and 1708 cm^{-1} bands may be



XLVII

assigned to the O-acetyl and the C-acetyl groups respectively. If an analogy with the benzodipyranone may be permitted, the 1754 cm^{-1} band can be assigned to the chromone and the 1671 cm^{-1} band to the chromanone carbonyls.

The absence of the 1754 cm^{-1} band in the ethyl orthoformate product would then furnish additional evidence for not giving it a chromone structure.

The ultra-violet spectra of the C_{26}-phenol, its ethyl orthoformate reaction product, the Kostanecki reaction product, and the C_{25}-acid are shown in Fig.11.

Table VII

Wave-length maxima in the ultra-violet spectra of C_{26}-compound, its ethyl orthoformate and Kostanecki products and the C_{25}-acid from KOH fusion of C_{26}-compound

Compound	λ_{max}, mμ	ε_{max}	λ_{max}, mμ	ε_{max}
C_{26}-compound	301	24160	347	4800
Ethyl orthoformate product from C_{26}-compound	302	20120	347	4000
Kostanecki reaction product	289	18640	348	1400
C_{25}-acid obtained from the fusion of C_{26}-compound with KOH at 220°	301	36800	347	4800

The ultra-violet spectra of the C_{26}-compound and its derivatives are shown in Fig. 11. The close similarity of the spectra of the ethyl orthoformate compound and the parent C_{26}-compound would suggest that the former was not

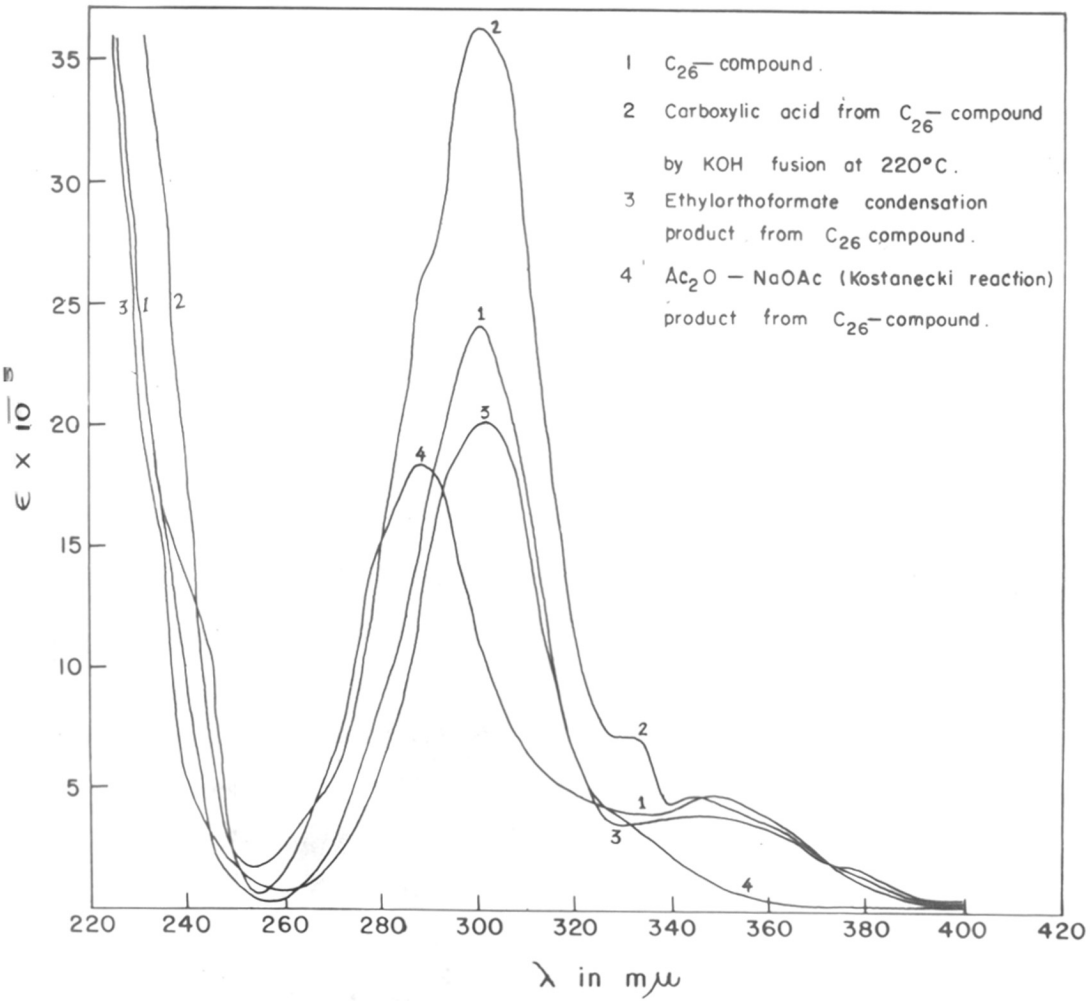
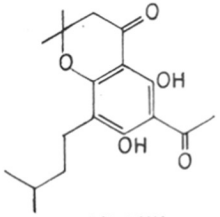

1 C_{26}— compound.
2 Carboxylic acid from C_{26}— compound by KOH fusion at 220°C.
3 Ethylorthoformate condensation product from C_{26} compound.
4 Ac_2O — NaOAc (Kostanecki reaction) product from C_{26}— compound.
$\epsilon \times 10^{-3}$
λ in mμ
35
30
25
20
15
10
5
220
240
260
280
300
320
340
360
380
400
420

Fig. 11.

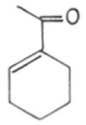
a 3-substituted chromone. On the other hand, the ultra-violet absorption of the Kostanecki product shows a weak band between 235-245 mμ, a strong band at 285 mμ, and a broad band at 325 mμ. Absorption at these wave-lengths are shown by chromones.[17] The acid obtained by the potassium hydroxide fusion of the C_{26}-compound has the same ultra-violet spectrum as the C_{26}-compound. This is in conformity with the observed chemical data; the acid analyses for $C_{25}H_{34}O_6$ and shows greenish-blue ferric colour similar to the C_{26}-compound. The molecular formula shows that only one carbon atom has been lost.

The structure (XL) postulated for the C_{26}-compound agrees with many of its properties, reactions and spectra, but these are facts which remain to be explained. Although the structure (XL) contains a normal exocyclic carbonyl group, the C_{26}-compound did not yield a dinitrophenylhydrazone with Brady's reagent or an oxime under the usual conditions of refluxing an ethanolic solution with hydroxylamine hydrochloride and sodium acetate. Further work on the constitution of the C_{26}-compound is in progress, and attempts are being made by hypohalite, alkaline peroxide and other oxidations to cleave the molecule into the chromanone and cyclohexane moieties. The structure (XL) is that of a 1,5-diketone, and attempts are also being made to effect a reversed Michael reaction by unassisted or base-catalysed pyrolysis,

breaking down the molecule to (XLVIII) and 1-acetylcyclohexene (XLIX).[36]

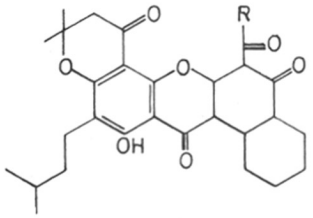


XLVIII



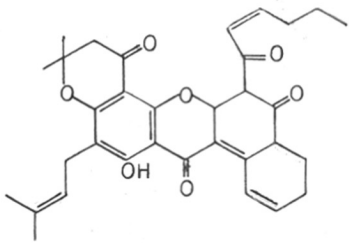
XLIX

The structure of octahydromorellin (L)



L

may now be expanded to a complete structure (LI) for morellin.

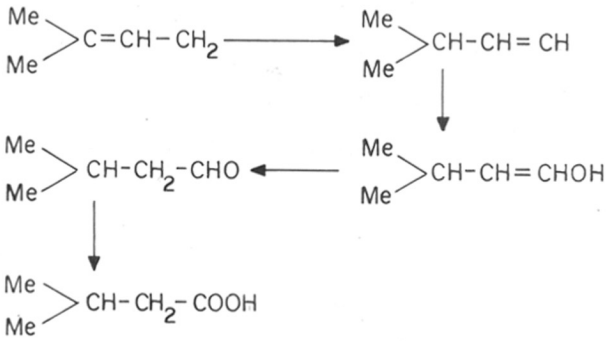


LI

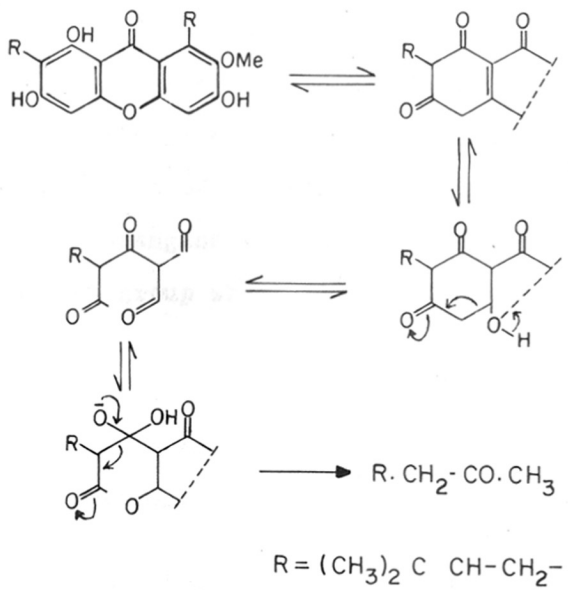
The structure (LI) explains the main products of the drastic alkali fusion of morellin. Phloroglucinol is a normally expected product. Isovaleric acid results from the isoamylene chain by the migration of the double bond towards the aromatic ring, a retro-aldol cleavage, methylheptenol may be formed from isopentenylphloroglucinol

by a retro-aldol cleavage as Yates has suggested in his work on mangostin.

Mechanism for the formation of isovaleric acid by the KOH fusion of morellin



Yates' mechanism for the formation of methyl heptenol from mangostin[37]

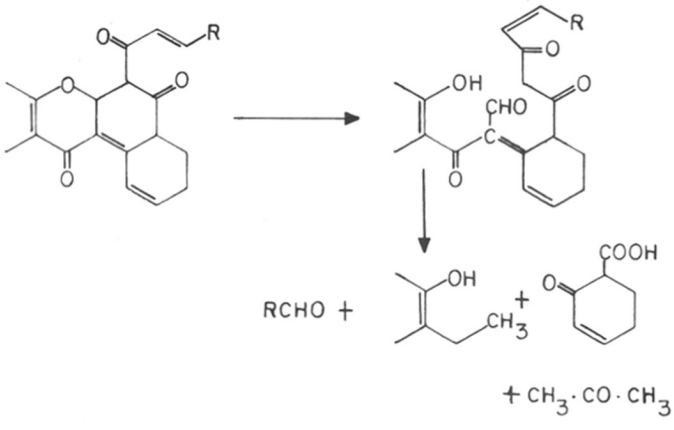


Homophthalic acid is obtained from the naphthalene part of the molecule, possibly via the C_{26} type of compound and aromatization of a hydrogenated benzene ring.

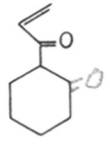
On reduction with Raney nickel and cyclohexanol the CO group in the naphthalene part gets reduced to CH_2, and after alkali fusion of reduction product B ar-tetrahydronaphthalene-1,3-dicarboxylic acid is obtained by cleavage of the central γ-pyranone ring and partial aromatization of the hydrogenated naphthalene part. From the same reduction product B as well as octahydromorellin and the C_{26}-compound the C_{16}-phenol is obtained, representing the separation of the isoamyl-chromanone moiety. The formation of a chromanol, rather than a chromanone, is not difficult to explain, because instances are known of the reduction of a carbonyl group to CHOH during drastic alkali treatment.

The formation of α-hydroxyisobutyric acid from morellin by permanganate oxidation is explained by the isopropylidene group which first undergoes hydroxylation across the double bond and then cleaves. The formation of acetone by ozonolysis of an isoamylene group is naturally to be expected. The easy formation of acetone by alkaline hydrolysis of morellin can be schematically

represented as:



Pyruvic aldehyde on ozonolysis probably comes from the same part (LII), but a definite interpretation

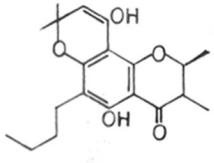


LII

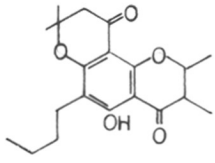
of the source of this aldehyde cannot be given at this stage. The double bonds in the naphthalene moiety are placed in two rings and conjugated; morellin does not form a maleic anhydride adduct. The double bonds are so placed, in order to explain the orange-yellow colour of morellin and the long wave-length absorption at 360 mμ in the ultra-violet spectrum of morellin. Rottlerin, which has a cinnamoyl group attached to phloroglucinol, also shows a similar long wave-length absorption.

The isoamylene side-chain has been linked to position 8 in the phloroglucinol moiety.
This change in the position of the side-chain is necessary to explain the formation of copper complex which requires the presence of a hydroxy group, a carbonyl group and an ethylenic bond in conjugation with the carbonyl group.

The complex spectrum of morellin methyl ether and diacetate (Fig. 12) may be tentatively explained as follows. In the spectrum of morellin monomethyl ether considerable hypsochromic shift is observed. This may partially be due to the known hypsochromic shifts observed on methylation of a 5-hydroxychromone or chromanone, and also the formation of a chromone consequent on the shift of the ethylenic bond attached to the central γ-pyrone ring. Also the methyl ether of morellin is pale yellow in colour. The formation of a diacetate, but only a monomethyl ether under normal conditions, is explained by an enolic group. On acetylation the whole system probably changes into a pyreno-benzo-dihydropyrone (LIII) instead of benzo-di-dihydropyrone (LIV) as in morellin and hence the pronounced change in spectrum.



LIII



LIV

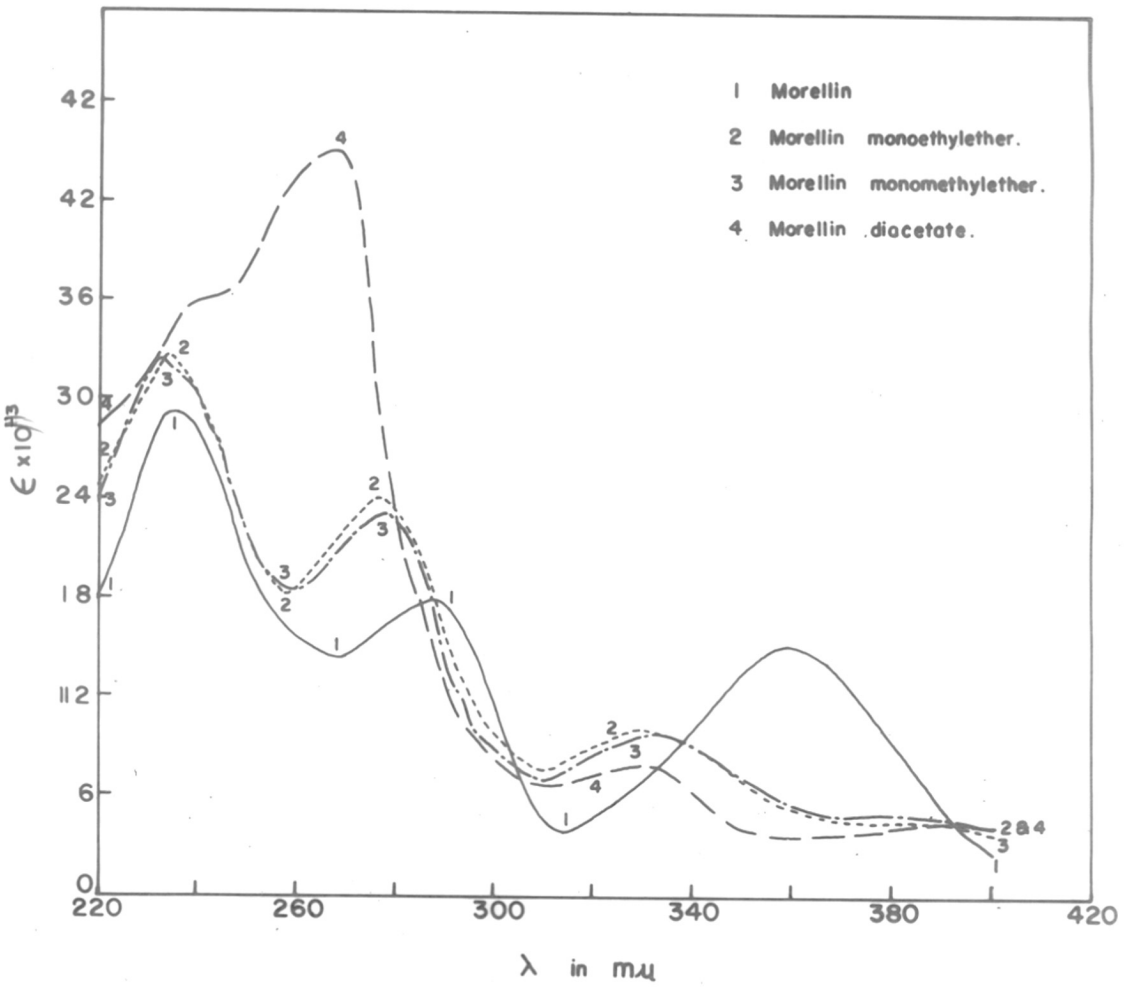

1 Morellin
2 Morellin monoethylether.
3 Morellin monomethylether.
4 Morellin diacetate.
ε x 10^-3
λ in mμ
0
6
12
18
24
30
36
42
42
220
260
300
340
380
420
2 & 4

Fig. 12.

The NMR spectra of morellin and its derivatives

The nuclear magnetic resonance spectra of morellin, isomorellin, morellin methyl ether and tetrahydromorellin methyl ether in deuterochloroform at 60 Mc are shown in Fig. 13. The standard from which the chemical shifts were measured is benzene. The small peak at -51 cps in these spectra is due to the chloroform present in the deuterochloroform. The observed chemical shifts of the low-field absorptions for morellin were converted to the τ-scale with the help of the chloroform peak and its known separation (438 cps) from the absorption due to tetramethylsilane and are given in Table VIII.

The absorption at the extreme left (τ -2.7) in morellin and isomorellin is easily assigned as due to a chelated hydroxyl group. Toxicarol also shows a similar absorption in this region. This hydroxyl resonance disappears in the monomethyl ethers.

The single proton resonance at about 0.4 can be due to a proton as unshielded as in a hydroxyl group. This absorption splits into a doublet in morellin methyl ether. It is shifted to 0.81 in isomorellin. The isomerization could conceivably be an epimerization involving the proton concerned.

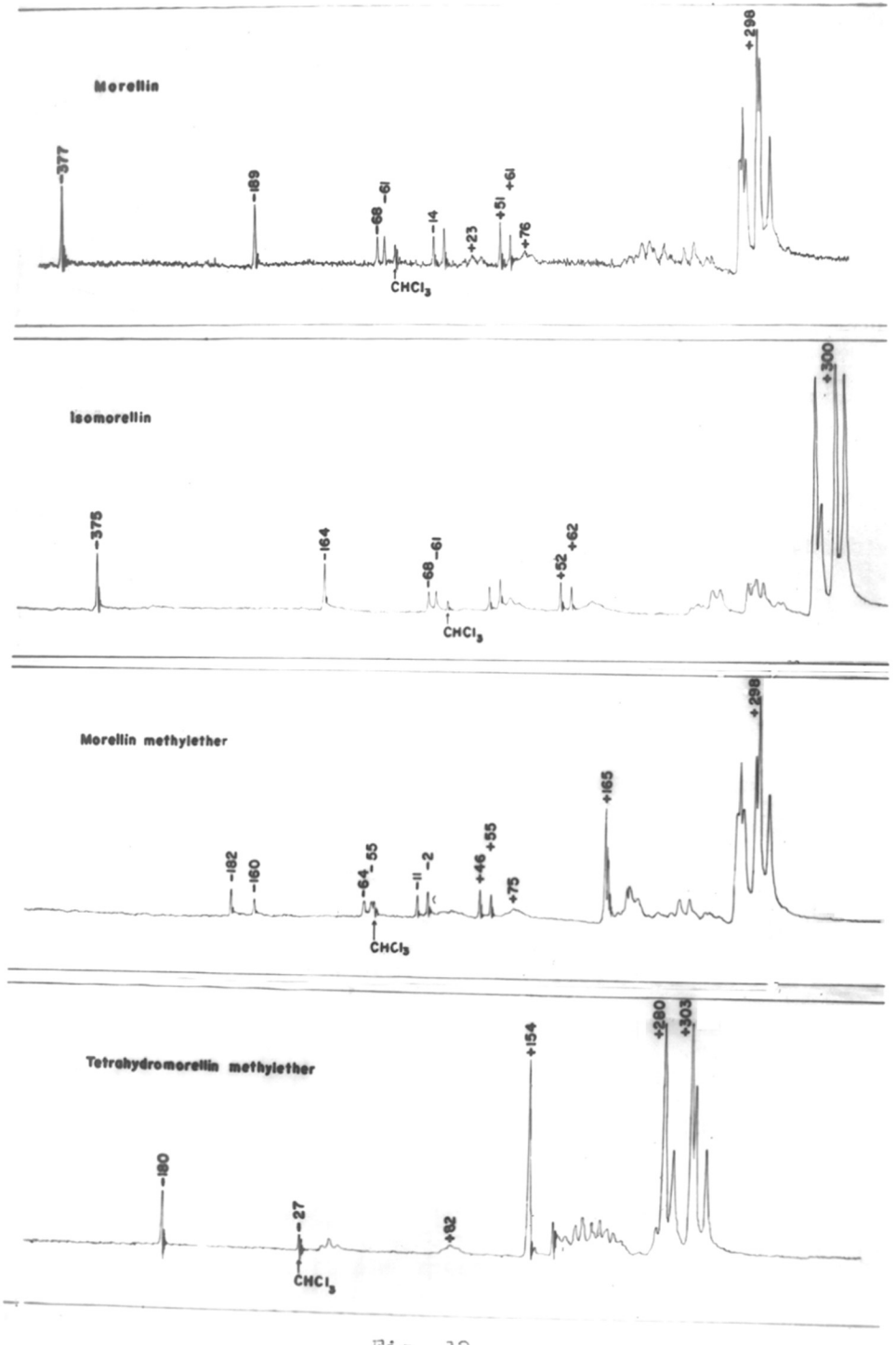

Morellin
-377
-189
-68
-61
-14
+23
+51
+61
+76
CHCl3
+298
Isomorellin
-375
-164
-68
-61
CHCl3
+52
+62
+300
Morellin methylether
-182
-160
-64
-55
-11
-2
+46
+55
+75
CHCl3
+165
+298
Tetrahydromorellin methylether
-180
-27
CHCl3
+82
+154
+280
+303

Fig. 13.

The spectra indicate that both morellin and isomorellin have five vinyl protons each. Three of these give doublet absorptions at 2.48, 3.40 and 4.39 with J = 7, 10 and 10 cps respectively. The pair at 3.40 and 4.49 with J = 10 cps may be due to the cis hydrogens on a double bond that is conjugated to an aromatic system or a carbonyl group. The absorption at 2.48 (J = 7 cps) shows that the double bond concerned is attached to a strongly electron withdrawing group which is probably cis oriented to the hydrogen involved.

The triplet vinyl absorptions of morellin at 3.95 and 4.82 are shifted to 3.10 and 4.92 in tetrahydromorellin methyl ether. The signal at the higher τ value is probably due to the vinyl hydrogen of the $\gamma\gamma$-dimethyl allyl group attached to an aromatic ring. The other absorption indicates a double bond conjugated to an electron withdrawing group. The shift of this absorption to the more shielded side in tetrahydromorellin methyl ether can be due either to the disappearance of cross conjugation of the electron withdrawing group or a shift of a double bond during the hydrogenation or methylation.

A C-methyl determination has not been possible with the help of the spectra obtained so far.

Table VIII

Low field absorptions in the NMR of morellin

Absorption positions	Chemical shifts in τ units	No. of protons
-377	-2.73	1
-189	+0.40	1
-68	+2.48	1
-61		
-14	+3.40	1
- 4		
+23	+3.95	1
+51	+4.49	1
+61		
+75	+4.82	1

EXPERIMENTAL

The Raney nickel-alcohol reduction product was prepared according to the procedure described by Bring *et al*.

Palladium charcoal dehydrogenation of reduction product A (RPA)

The reduction product (3 g) was dissolved in *p*-cymene (50 ml), palladium-charcoal (1.5 g, 12%) added and contents refluxed for 48 hr. Palladium charcoal was filtered off and the filtrate after the removal of *p*-cymene by steam distillation gave a light brown solid which crystallized from methanol in colourless needles (1.3 g, m.p. 155-157°). Two more crystallizations from the same solvent raised the m.p. to 172°. (Found: C, 72.8, 73.0; H, 8.7, 8.9; *M* by the Rast method, 505, 520. $C_{33}H_{48}O_6$ requires: C, 73.3; H, 8.9%. *M*, 540). The product gave an olive green ferric colour; produced no change in colour with magnesium and HCl; gave on reduction with 4% sodium amalgam a pink colour deepening to wine red on keeping for 24 hr.

Raney nickel-cyclohexanol reduction of morellin: reduction product B (RPB)

Morellin (10 g) was added to Raney nickel (100 g) in cyclohexanol (200 ml) and toluene (25 ml) and contents refluxed for 24 hr under stirring. Nickel was filtered

off and washed with boiling toluene, and steam distillation of the filtrate gave a pale yellow residue which was crystallized from methanol in colourless needles (m.p. 171-172°, 6.3 g). The crude product was recrystallized from methanol, m.p. 172°, alone or mixed with the dehydrogenated product obtained from RPA. The infra-red and ultra-violet spectra were identical. (Found: C, 73.0; H, 9.1. M by X-ray method, 517. $C_{33}H_{48}O_6$ requires: C, 73.3; H, 8.9%. M, 540).

MW by MS — 552

Raney nickel-cyclohexanol on reduction product A (RPA)

To Raney nickel (10 g) in cyclohexanol (15 ml) and toluene (5 ml) the reduction product A (1 g) was added and the mixture refluxed with stirring for 15 hr. Nickel was filtered off, washed with boiling toluene and the combined filtrates steam distilled to give a yellow solid (0.95g) which was crystallized from methanol, m.p. 172°, alone or mixed with reduction product B.

Raney nickel-cyclohexanone reduction of morellin: reduction product C (RPC)

To a suspension of Raney nickel (10 g) in cyclohexanone (25 ml) and toluene (5 ml), morellin (1 g) was added and contents refluxed for 24 hr under stirring. Nickel was filtered, washed with warm toluene, and the combined filtrates steam distilled off to a light brown

semi-solid (0.95 g). This semi-solid came out of methanol as a pale yellow solid (0.55 g, m.p. 132° with softening at 124°). On recrystallization from the same solvent the compound (RPC) came out as needles (long flat plates under the microscope) (m.p. 145-146°, 0.45 g). This was purified to constant m.p. 148°. (Found: C, 73.7; H, 8.0. $C_{33}H_{44}O_6$ requires: C, 73.9; H, 8.3%). It gives an olive green ferric colour; very pale pink colour with magnesium and hydrochloric acid after 24 hr. The compound gave a typical C-methylphloracetophenone spectrum (vide Fig.2).

Dehydrogenation of octahydromorellin with palladium charcoal

Octahydromorellin (amorphous powder, 0.5 g) was dissolved in p-cymene (5 ml), palladium charcoal (0.25 g, 12%) added and the mixture refluxed with mechanical agitation in a metal bath maintained at 180-185° for 15 hr. The mixture was then cooled, filtered, the palladium charcoal washed repeatedly with warm toluene, and finally the combined filtrates steam distilled to give a soft residue which was filtered and dried under suction (0.47 g). The precipitate crystallized from methanol in long flat plates (210 mg, m.p. 139-140°). One more crystallization raised the m.p. to 147-148°. The mixed m.p. with RPC (m.p. 148°) prepared by the cyclohexanone reduction of morellin was undepressed. (Found: C, 74.2;

H, 8.1; active hydrogen, 0.39% and 0.34% i.e. 2. $C_{33}H_{44}O_6$ requires: C, 73.9; H, 8.3%). The ultra-violet spectrum and colour reactions of this compound was the same as RPC.

Hydrolysis of RPB under flavonol conditions

RPB (50 mg) was dissolved in 10% alcoholic alkali (2 ml) and refluxed for 6 hr. The clear yellow solution on dilution with water gave a colourless solid (m.p. 155-170°) which on crystallization from methanol gave colourless needles, m.p. 173-174°, undepressed on admixture with RPB. It had the same olive green ferric colouration.

Hydrolysis of RPB with zinc and potassium hydroxide

RPB (100 mg), zinc dust (100 mg), potassium hydroxide (0.6 ml of 4% solution) were added to alcohol (95%, 3 ml) and contents refluxed for 1 hr. The clear yellow solution after filtering of zinc dust was diluted with water (6 ml), carefully acidified to Congo Red with ice cold 50% H_2SO_4 and cooled when a pale yellow solid (95 mg) separated out (m.p. 127-129°). The product was crystallized from methanol in colourless needles, m.p. and mixed m.p. with RPB, 169-170°.

Hydrolysis of RPC

The reduction product C (200 mg) was dissolved in

alcohol (10 ml), 10% potassium hydroxide (10 ml) added, refluxed for 8 hr and cooled. On pouring into water (100 ml) a pale yellow precipitate separated, which was filtered and crystallized from methanol (170 mg), m.p. and mixed m.p. with RPC 147°.

Iodine, alcohol-potassium acetate on RPB

RPB (100 mg) and potassium acetate (175 mg) were dissolved in hot absolute alcohol (20 ml) and iodine (140 mg) dissolved in 3 ml absolute alcohol added portionwise and the flask left at room temp for 40 hr. On cooling to 5° a colourless crystalline compound separated out, m.p. and mixed m.p. with RPB (m.p. 172°) was 166-167°. The solid did not give any test for iodine; did not answer the magnesium hydrochloric acid test.

Action of copper bronze-quinoline on RPB

To a mixture of RPB (200 mg) and copper bronze (100 mg), quinoline (10 ml) was added, and the mixture refluxed for 2 hr. The mixture was then poured into ice and HCl was added to destroy the quinoline. The resulting deep brownish red solution was ether extracted and removal of solvent gave a thick red oil which was dried, dissolved in pet ether and chromatographed on Florex XXX. The homogeneous yellow band that came down was collected, hexane distilled

off and the yellow residue crystallized from methanol (m.p. 171-172°, 45 mg), mixed m.p. with RPB was 172°.

Palladium-charcoal dehydrogenation of RPB

The reduction product B (100 mg) and palladium charcoal (12%, 0.1 g) were mixed thoroughly and heated to 250° in a metal bath for 6 hr. A pale yellow crystalline compound sublimed on the cooler sides of the flask. The sublimed product was scooped out (45 mg). On crystallization from methanol it had a m.p. 172°, undepressed on mixing with an authentic sample of the reduction product. The residue that was left behind after sublimation was found to be charred and insoluble in organic solvents.

A similar result was observed on carrying out the aromatization in a high boiling solvent dowtherm. The compound was recovered.

Sodium borohydride reduction of morellin

Morellin (1 g), sodium borohydride (3 g), methanol (25 ml) were mixed together and kept at room temp for 2 hr. A vigorous gas evolution was observed and a pale yellow, slightly turbid solution was formed on standing. This was filtered and poured into dil HCl. A colourless precipitate (nearly 100% yield) was obtained which

exhibited bluish green ferric reaction. It resinified when crystallization was attempted. It was purified by dissolving in methanol and subsequent precipitation by the addition of saturated ammonium sulphate solution.

Hydrogen absorption of the sodium borohydride reduction product of morellin

The borohydride reduction product (0.1606 g) was dissolved in cellosolve (15 ml), 30% palladized carbon (100 mg) added and shaken with hydrogen at 3-4 cm above atm pressure. (Hydrogen absorbed, 22.5 ml. Required for 3 moles, 21.9 ml). The first mole was absorbed in 10 min, the second in 95 min, and the third in 5 hr and 30 min. After hydrogenation the solution was filtered through Hyflo-supercel and diluted with water, when an emulsion was formed which was ether extracted. Removal of ether gave an oil which was purified by dissolving in methanol and precipitating with a saturated solution of ammonium sulphate when a yellow solid separated. The substance softened at 110° and melted at 120-125°. It had three active hydrogens.

Catalytic hydrogenation of sodium borohydride reduction product of tetrahydromorellin

Tetrahydromorellin was reduced by sodium borohydride as described for morellin. The reduction product from

tetrahydromorellin (99 mg), palladium charcoal (75 mg) and cellosolve (10 ml) were mixed together and hydrogenated as above. (Hydrogen absorbed, 8.8 ml. Required for 2 moles, 8.6 ml). The first mole was absorbed in 12 min and the second mole in another 1½ hr. Worked up as in the case of morellin, an oily substance was obtained which was purified by dissolving in methanol and precipitating with ammonium sulphate solution. The yellow amorphous substance softened at 110° and melted between 115-125°. It had three active hydrogens.

Copper complex of morellin

Copper acetate solution was prepared by dissolving copper acetate monohydrate (50 g) and crystalline sodium acetate (200 g) in 2 litres of water. The solution was filtered and used as such. A solution of morellin (0.2 g) in dioxane (2 ml) was taken and an excess of Cu acetate solution (25 ml) was added. A red brown precipitate separated out which was filtered and crystallized from chloroform in blood red needles. (Found: C, 69.0; H, 6.0. $C_{33}H_{37}O_7$ Cu/2 requires: C, 68.9; H, 6.4%).

Hydrolysis of copper complex

The copper complex (0.1 g) was dissolved in acetone and conc HCl (5 ml) was added. The precipitate that separated on dilution was filtered and crystallized from

minimum amount of methanol to give orange yellow needles, m.p. 120-121°, which was identified as isomorellin as was shown by the non-depression of the mixed m.p. with an authentic sample of isomorellin.

o-Phenylenediamine condensation of morellin, tetrahydromorellin and octahydromorellin

The compound (1 g) and o-phenylenediamine (1 g) were dissolved in alcohol (20 ml) and left at room temp for 48 hr. On dilution with water (20 ml) a yellow solid separated out which was filtered and washed (0.7-0.8 g). The amorphous yellow compounds obtained in all the cases did not crystallize, but on dissolving in acetone and treating with a few drops of HCl they exhibited deep red colouration, like heptazines.

o-Phenylenediamine condensation of copper complex of morellin

The Cu complex (0.1 g) and o-phenylenediamine (0.1 g) were dissolved in alcohol separately and mixed and kept at room temp for 48 hr. A black precipitate separated out which was filtered and washed with acetone and dried. The product, when suspended in acetone and treated with HCl gave a deep red colouration.

Ozonolysis of morellin

Morellin (1 g) was dissolved in alcohol free and dry chloroform (35 ml) and after cooling the solution to -60°

in a dry ice-acetone bath, ozonized oxygen was passed for two hours at the end of which no more ozone was absorbed. 10 ml of ice cold water was then added to the ozonide and the contents were left overnight at the same bath temp. After the ozonide was decomposed, the mixture was distilled on a steam bath and the distillate collected in Brady's reagent. The distillate was extracted with chloroform (5 x 25 ml), washed with water to remove any 2,4-dinitrophenylhydrazine sulphate, dried over sodium sulphate, and finally distilled to an orange crystalline residue of dinitrophenylhydrazone (410 mg, m.p. 130-150). The residue was extracted with pure dry benzene and chromatographed on alumina. The major orange band was collected, benzene distilled off, and the residue crystallized from alcohol (320 mg, m.p. 125-126°). This was identified as acetone-2,4-dinitrophenylhydrazone as shown by the non-depression of m.p. on mixing with an authentic sample of acetone-2,4-dinitrophenylhydrazone.

The non-volatile fraction gave typical aldehyde tests, formed a dinitrophenylhydrazone, but it could not be crystallized.

Ozonolysis of tetrahydromorellin

Tetrahydromorellin (1 g) was dissolved in alcohol free and dry chloroform (35 ml), and after cooling the solution to -60° in dry ice bath, ozonized oxygen was

passed for 2 hr, at the end of which no more ozone was absorbed. Ice-cold water (10 ml) was then added and the contents were left overnight. After the ozonides were decomposed, the mixture was steam distilled and the distillate collected in Brady's reagent. The distillate was extracted with chloroform, washed with water, dried and distilled to give an orange crystalline residue (m.p. 95-97°). The residue was extracted with purified and dry benzene and chromatographed on alumina. A single orange band moved down which was eluted with more benzene, distilled off and finally crystallized from alcohol, m.p. 121°, undepressed on mixing with an authentic sample of acetone-2,4-dinitrophenylhydrazone.

Prolonged ozonolysis of morellin

Morellin (1.5298 g) was dissolved in purified ethyl acetate (60 ml) cooled to -60° in a solid carbon dioxide-acetone bath, and ozonized oxygen was bubbled for 6 hr, although the starch-iodide paper turned blue in 3 hr and was left aside in solid carbon dioxide overnight. In a 250 ml hydrogenation flask palladium charcoal (0.5 g, 10%) was saturated with hydrogen and to the saturated catalyst the ozonide was added, and hydrogenated at atm pressure. (Volume of hydrogen absorbed, 186 ml; theoretical volume for tetraozonide is 180 ml). After hydrogenation the ethyl acetate solution was filtered, washed with ethyl acetate and the filtrate steam distilled

and the distillate collected in Brady's reagent (3.5 g of 2,4-dinitrophenylhydrazine in 230 ml of 10% H_2SO_4). The mixed dinitrophenylhydrazones were extracted with benzene (600 ml), washed with water, dried and distilled. The residue was dissolved in 25 ml of benzene and chromatographed on alumina. Elution of the lower band and evaporation gave an orange yellow crystalline residue (m.p. 76-95°, 0.56 g). The upper band was eluted out with 750 ml of benzene and removal of solvent gave reddish orange crystals, m.p. 245-254° (decomp) (.1374 g). Recrystallization from ethyl acetate afforded the pure dinitrophenylhydrazone, m.p. 293-295°(decomp), the mixed m.p. of which with authentic pyruvic aldehyde di-dinitrophenylhydrazone was undepressed.

The first band on paper chromatography was found to be a mixture of formaldehyde (R_f 0.19), acetaldehyde (R_f 0.26) and acetone (R_f 0.37).

50% nitric acid-ammonium venadate oxidation of RPB

50% nitric acid (20 ml) was heated nearly to boil and a pinch of ammonium venadate (10 mg) was added, when the catalyst went into solution and the colour became slightly red. Then a few milligrams of RPB were added, and as soon as the reaction was initiated as shown by evolution of nitrous fumes, the temp was brought down to 60°, and the remaining portion of RPB (ca. 500 mg) added

in one lot. The mixture was stirred for 1½ hr at 60°, and finally poured into water.

The aqueous mixture was then saturated with sodium chloride and ether extracted. The bicarbonate soluble and insoluble portions were separated.

Bicarbonate soluble portion. A brown oil (100 mg) was obtained. It was soluble in benzene or alcohol or water; could not be crystallized; did not contain nitrogen. An aqueous solution decolourized aqueous potassium permanganate on warming.

Bicarbonate insoluble portion. The yellow ether solution on distilling of ether gave a yellow semi-solid which on thorough drying solidified (320 mg). The solid was crystallized from cyclohexane and subsequently from aqueous acetone-water. Aqueous acetone was however more suitable as the compound separated out as beautiful yellow plates, m.p. 187°. It gave an olive green ferric colour and did not answer magnesium-hydrochloric acid test. (Found: C, 63.6; H, 7.1. M by the Rast method, 488. $C_{26}H_{36}O_9$ requires: C, 63.4; H, 7.3%. M, 492).

Sodium hypoiodite oxidation of reduction product B (RPB)

RPB (1 g) was dissolved in dioxane (100 ml) and alkali (50 ml of 5% NaOH) added, followed by excess iodine solution (100 ml containing 10 g of potassium

iodide and 5 g of iodine), the whole reaction being carried out in a steam bath with mechanical agitation for 3 hr. The contents were then cooled and diluted with water when a strong iodoform odour was perceived. After acidification with 50% H_2SO_4, excess iodine was destroyed with sodium bisulphite and the mixture was ether extracted after saturation with NaCl. The bicarbonate soluble and neutral portion were separated.

Bicarbonate soluble portion. On acidification with 50% H_2SO_4 it gave a yellow solid (350 mg). It did not contain iodine; gave a green ferric colour; did not answer magnesium-HCl test; and did not contain any steam volatile portion.

Neutral fraction. On removal of ether a brown oil was left, which was steam distilled to remove iodoform (25 mg). The non-steam volatile residue was filtered (520 mg). On adding cyclohexane the resinous matter dissolved and a pale yellow crystalline mass separated out. The cyclohexane was carefully decanted off into another flask, a fresh lot of cyclohexane added and the compound crystallized. The compound came out as yellow plates in almost pure form (m.p. 177-178°, 250 mg). It was crystallized again from cyclohexane and analyzed. (Found: C, 71.2, 71.0; H, 8.1, 8.2; active hydrogen, 4.3. M by the Rast method, 491. 493 (thermistor). $C_{32}H_{42}O_7$ requires: C, 71.1; H, 8.1%. M, 522).

It gave olive green ferric colour; a pink colour deepening to wine red after 24 hr with magnesium and HCl; was insoluble in carbonate and 5% sodium hydroxide (boil); and did not answer hydroxamic acid test for acids. On hydrolysis with 30% alcoholic sodium hydroxide for 8 hr it yielded an oil which gave a much deeper magnesium and HCl colour than the oxidation product.

Alkali-permanganate oxidation of RPB

RPB (3 g) was suspended in alkali (3% NaOH, 90 ml), heated in a steam bath, and permanganate (5% solution, 300 ml) was added in an hr, and heating combined for 15 hr. The mixture was then cooled, acidified, and heated with bisulphite to destroy the manganese dioxide precipitate. The solution became yellow and a considerable amount of an insoluble crystalline material was found, and the mixture smelt strongly of fatty acid.

(i) Fatty acid was separated by steam distillation and converted to the sodium salt by titration with N/10 sodium hydroxide (320 mg). The sodium salt of the fatty acid (200 mg) was dissolved in water (4 ml), carefully acidified with dil HCl till it was just acidic to litmus. p-Bromophenacyl bromide (270 mg) dissolved in alcohol (4 ml) was added, when turbidity appeared. More alcohol (4 ml) was added and contents refluxed for 3 hr, filtered

and cooled to 5°. Colourless glistening plates separated out. The crystalline derivative was filtered and recrystallized from alcohol-water (2:1.5, m.p. 57-58°); mixed m.p. with p-bromophenacyl ester of n-valeric acid (m.p. 59°) was 58-59°.

(ii) The non-steam volatile portion was filtered to give a colourless and crystalline compound (1.5 g, found to be recovered RPB as shown by non-depression of m.p. of the recrystallized sample (m.p. 172°). The filtrate was extracted with ether using a liquid-liquid extracter. The ether extract on removal of solvent gave practically nothing.

Manganese dioxide oxidation of RPB

Active manganese dioxide prepared according to Harfenist et al. or manganese dioxide (James Wooley and Sons) was used. In either case the manganese dioxide was heated for 48 hr between 250-300° and used. The RPB (100 mg) was dissolved in dry toluene or benzene (15 ml), active manganese dioxide (400 mg) added, and contents refluxed for 40 hr. Manganese dioxide was filtered, washed with boiling toluene and the combined filtrates on steam distillation gave a pale yellow solid which was filtered, dried, and crystallized from pet ether (60-80°,

m.p. 177-180°, 80 mg). Recrystallization from pet ether gave the pure compound 178°. (Found: C, 73.6, 73.7, 74.0; H, 8.6, 8.3, 8.4; active hydrogen, 2.5. $C_{33}H_{44}O_6$ requires: C, 73.9; H, 8.3%). It gave a green ferric colour; a yellow colour on treatment with magnesium and HCl. Unlike the starting material, it was very sparingly soluble in normal hexane. The mixed m.p. with RPB (m.p. 172°) was 165-166°.

Alkali fusion of RPB

RPB (6 g) was thoroughly mixed with potassium hydroxide pellets (30 g). The intimate mixture was then transferred to a pyrex tube fitted with a mechanical agitator, a nitrogen inlet and an outlet with an arrangement to collect the volatile matter of the fusion. The tube was dipped into a metal bath at 150° and temp was raised to 290° and maintained at the same temp for 20 min, after which the contents were allowed to cool gradually to room temp. The fusion melt was extracted with water (6 x 25 ml) and the clean yellow solution was cooled and acidified with ice-cold 50% H_2SO_4, when considerable turbidity developed and an offensive fatty acid odour was perceived. The mixture was cooled overnight when an oil separated out. The mixture was finally ether extracted after thorough saturation with common salt and then washed successively

with sodium bicarbonate, sodium carbonate and sodium hydroxide solutions.

Bicarbonate soluble fraction. The bicarbonate soluble fraction was acidified, saturated with common salt and ether extracted. The ether was dried and distilled off to give a brown oil. The oil, which smelt strongly of fatty acid, was then steam distilled to give a steam volatile portion.

Steam volatile fraction. The aqueous solution (500 ml) containing the steam volatile acid was titrated with N/10 sodium hydroxide and then evaporated to dryness when the sodium salt of the fatty acid (480 mg) was left. The sodium salt was treated as follows:

(i) 30 mg of the sodium salt was treated with phosphorus oxychloride (6 drops) and left overnight. The acid chloride that was formed was taken up in toluene (50 ml) and treated with 30 mg of β-aminoanthraquinone and refluxed for 2 hr. The contents were then filtered and distilled. The residue was dissolved in benzene and the light orange solution was chromatographed on alumina. The lowermost small brown band was discarded. The major lemon yellow band was collected, concentrated to a very small bulk, diluted with petrol (b.p. 100°) until turbidity developed and then warmed and cooled, when the

β-anthraquinonoyl amide of the fatty acid crystallized out in yellow needles, m.p. 210°, undepressed on admixture with an authentic sample of the n-valerylamido-anthraquinone (m.p. 210°). The mixed m.p. with isovaleryl-amidoanthraquinone (m.p. 232°) was 194°.

(ii) The p-bromophenacyl ester of the fatty acid was prepared as follows: The sodium salt (100 mg) was dissolved in water (3 ml) and p-bromophenacyl bromide (135 mg) dissolved in 3 ml of alcohol was added. The white precipitate that was formed was dissolved by adding more alcohol (3 ml) and contents refluxed for 2½ hr. The aqueous alcoholic solution was then filtered and cooled to 5°, when colourless glistening needles separated out (85 mg, m.p. 56-57°). The crystalline ester was recrystallized from aqueous methanol (1.5 ml water and 2 ml methanol). The m.p. was 60°, undepressed on admixture with an authentic sample of the p-bromophenacyl ester of n-valeric acid (m.p. 63°).

Non-steam volatile fraction. The pale yellow aqueous solution left after the steam distillation of the bicarbonate soluble oil was filtered while hot into a flask and cooled, when a colourless crystalline acid separated out. The water insoluble oily resin was treated separately. The acid (430 mg, 278-279°) was crystallized twice more from chloroform, filtered, dried and analysed. (Found: C, 64.4,

64.5, 65.3; H, 6.4, 6.2, 5.6. M by the Rast method, 223, 216. N.E., 112, 113. Average analysis: C, 64.8; H, 6.0. $C_{12}H_{12}O_4$ requires: C, 65.4; H, 5.5%. M, 220. N.E., 110). The water insoluble fraction on treatment with benzene gave a white crystalline powder (m.p. 236°) which on recrystallization from water gave more of the acid, m.p. 281°.

Decarboxylation of the acid $C_{12}H_{12}O_4$ (m.p. 281°)

The acid (150 mg) was dissolved in quinoline (3 ml, b.p. 238°), Cu-bronze (30 mg) added and contents refluxed for 3 hr. The mixture, which was dark brown in colour, was then poured into ice and quinoline destroyed with HCl, when a strong odour of naphthalene was perceived. The aqueous mixture was then ether extracted thoroughly and removal of the solvent gave a red-brown oil. The oil was transferred to a bulb tube by means of ether and distilled under vacuum. Fraction I (80-90°/30 mm) was redistilled and crystallized from alcohol to give pure naphthalene, m.p. 80°, undepressed on mixing with pure naphthalene. (Found: C, 93.4; H, 6.2. $C_{10}H_{10}$ requires: C, 93.7; H, 6.3%).

Permanganate oxidation of the acid (m.p. 281°)

The acid (100 mg) was added to aqueous alkali (72 mg in 2.2 ml of water, ca. 3%) and heated with

stirring nearly to boiling point. The heating was discontinued and powdered potassium permanganate (143 mg, 2 moles) were added gradually till the pink colour persisted and finally the mixture was refluxed for 2 hr. The excess permanganate was destroyed with alcohol, the precipitated manganese dioxide filtered off and the resulting colourless filtrate acidified with 50% H_2SO_4 when a colourless crystalline substance separated out (85 mg, m.p. 280°, undepressed on mixing with the starting material).

Oxidation of the acid (m.p. 281°) with 10% HNO_3 in a sealed tube

The acid (0.1 g) was suspended in 10% HNO_3 (10 ml) and heated in a sealed tube at 240° for 6 hr. The tube was cooled to room temp and cooled overnight at 5° when no crystalline solid separated. The reaction mixture was therefore exhaustively extracted with ether, washed with saturated sodium bicarbonate solution, acidified and re-ether extracted. Evaporation of the dried ether extract gave a solid which crystallized in colourless plates from water (m.p. 228-229° decomp, 45 mg). The above acid was totally methylated with diazomethane. The acid (45 mg) was added to a solution of diazomethane in dry ether (85 mg in 20 ml, 10 moles) and the mixture left overnight (15 hr). As methylation proceeded, the acid which was in

suspension started dissolving in ether. Finally, ether was distilled off to give a crystalline residue (m.p. 107°, 50 mg). This ester was crystallized from hexane (60-80°) twice (m.p. 108-109°) and analysed. (Found: C, 53.9, 54.1; H, 4.1, 4.4. M, 282, 284. $C_{14}H_{14}O_8$ requires: C, 54.1; H, 4.5%. M, 310). The mixed m.p. with authentic melliphanic acid tetramethyl ester (m.p. 109°) was undepressed.

Copper bronze-quinoline on tetralin

Tetralin (0.3 g) was mixed with Cu-bronze (0.15 g), quinoline (6 ml) added, and contents refluxed for 3 hr. On pouring the reaction mixture into ice and destroying the quinoline with HCl, a strong odour of naphthalene was observed. The mixture was saturated with sodium chloride, ether extracted and ether removed to give a red-brown oil, which was subsequently bulb distilled and the fraction 85-90°/30 mm was collected, crystallized, and identified as naphthalene (m.p. and mixed m.p. 80°, 230 mg).

Carbonate soluble fraction in the alkali fusion of reduction product B (RPB)

After the removal of the bicarbonate soluble fraction, the ether extract was washed with 10% Na_2CO_3 solution. The carbonate extract was then acidified with 50% H_2SO_4 and re-ether extracted after saturating with sodium chloride.

The ether extract was dried over anhydrous sodium sulphate and distilled off to give a brown oil which was crystallized from benzene-hexane. The pale-yellow crystalline compound (m.p. 159°, 670 mg) was then crystallized from benzene and subsequently from water as colourless plates (m.p. 163°, 600 mg) and analysed. (Found: C, 68.8, 68.9, 68.8; H, 8.4, 8.2, 8.5. M by the Rast method, 278, 285. Active hydrogen, 2.8, 2.9. $C_{16}H_{24}O_4$ requires: C, 68.5; H, 8.6%. M, 280).

Sodium hydroxide soluble fraction in alkali fusion of reduction product B (RPB)

The 10% sodium hydroxide soluble fraction was acidified with 50% H_2SO_4, and ether extracted. The dark-brown oil that was left on removal of the ether crystallized from pet ether (b.p. 100°) in fine colourless plates (m.p. 154-156°, 320 mg). The mixed m.p. with the phenol, $C_{16}H_{24}O_4$, obtained from the carbonate soluble fraction (m.p. 163°) was undepressed.

Neutral fraction from the alkali fusion of RPB

The oil that was obtained from the ether solution after removal of the sodium bicarbonate, sodium carbonate and sodium hydroxide soluble fractions, was distilled under high vacuum 10^{-5}/160-170°, to give a pale-yellow oil which crystallized from hexane (60-80°) in colourless needles

(m.p. 128°). (Found: C, 67.5; H, 8.7. $C_{11}H_{16}O_3$ requires: C, 67.2; H, 8.2%). The U.V. absorption showed only one band at 252 mμ (ε, 7300). It gave no ferric colouration.

2,4-Dinitrophenylhydrazone of octahydromorellin

Octahydromorellin (100 mg) was dissolved in alcohol (10 ml) and treated with dinitrophenylhydrazine-phosphoric acid reagent (2 ml, 0.25 M), when a copious orange precipitate separated. The precipitate was filtered, dried (140 mg), and chromatographed on Florex, using benzene as solvent. The major orange band was collected and crystallized from alcohol as orange plates (m.p. 135°). (Found: C, 63.9; H, 6.5; N, 7.5. $C_{39}H_{50}O_{10}N_4$ requires: C, 64.0; H, 6.8; N, 7.7%).

Ethyl orthoformate reaction on octahydromorellin

Octahydromorellin (0.5 g), pyridine (7.5 ml), piperidine (15 drops, i.e. 0.75 ml), and ethyl orthoformate (5 ml) were refluxed for 12 hr, when the initial almost colourless solution turned orange red. The solution was poured into HCl and crushed ice, when a sticky brown mass (0.43 g) was obtained. The solid was dissolved in benzene and chromatographed on Florex (25 g). The eluate (500 ml) on concentration gave a pale yellow oil (0.31 g) which crystallized from methanol (0.065 g, m.p. 86°).

(Found: C, 71.3; H, 8.2. $C_{36}H_{50}O_8$ requires: C, 70.8; H, 8.1%).

Alkali fusion of octahydromorellin

An intimate mixture of octahydromorellin (16 g) and caustic potash (80 g, E. Merck) was taken in a pyrex tube provided with a mercury sealed stirrer, an inlet for nitrogen and an outlet leading to an adsorption device to collect the volatile matters of fusion. The tube was immersed in a metal bath at 100° and temp was slowly raised. At 150° a chocolate coloured resinous mass floating on a milky-white liquid was observed. At this stage the sticky mass was found to be slightly mobile and stirring was started. At 200° a rubber like mass started spreading out. The viscid mass then turned coarse and at 240° started melting. At 245° a complete melt yellowish brown in colour was formed. A peppermint odour was also perceived in the trap to collect the volatiles. As soon as the melt was formed, heating was stopped and contents cooled to room temp (30°). The melt was dissolved in water (3 x 100 ml) and extract left overnight in the frig. The alkaline solution, cooled to 5°, was then acidified with ice-cold 50% H_2SO_4. Fatty acid odour developed and an insoluble red sticky mass separated out which slowly solidified. The whole mixture was thoroughly saturated with salt and ether

extracted. The bicarbonate soluble fraction (A), the sodium carbonate soluble fraction (B), the sodium hydroxide soluble fraction (C), and the neutral fraction (D) were separated.

(A) - The sodium bicarbonate soluble fraction was acidified with 50% H_2SO_4 and ether extracted. Removal of ether gave a thick red oil (4.4 g) which was steam distilled to remove the fatty acids. The steam distillate was saturated with ammonium sulphate and ether extracted. The ether extract on drying and removal of solvent gave free fatty acid (1.34 g). The sodium salt of the fatty acid was converted to its p-bromophenacyl ester. The sodium salt (0.5 g), p-bromophenacyl bromide (0.75 g), alcohol 20 ml) and water (10 ml) were refluxed in a steam-bath for 3 hr, filtered and cooled. A colourless crystalline solid, m.p. 43-65°, separated out. Recrystallization did not give a sharp melting derivative. The analysis (C, 478; H, 3.5%) showed that it was a mixture of probably isobutyric and iso- or n-valeric acids. However paper chromatography of the fatty acid mixture showed that it was essentially n-valeric acid along with smaller amounts of acetic, propionic, isobutyric, isocaproic and octanoic acids.

The steam non-volatile fraction was filtered hot,

and on cooling the aqueous solution a colourless acid (400 mg, m.p. 246°) separated out. The amorphous substance was again separated from water (m.p. 252°). It was not crystallizable. It gave a purple ferric colour.

The water insoluble portion of the non-steam volatile and residue gave a gummy-brown product (2.5 g) which could not be crystallized.

(B) - The aqueous sodium carbonate soluble fraction was acidified and re-ether extracted and distillation of the ether after drying over anhydrous sodium sulphate gave a red oil (1.6 g) which crystallized from acetone-hexane as almost colourless needles (320 mg, m.p. 161-162). The mixed m.p. with C_{16}-phenol obtained by the alkali fusion of RPB was undepressed. It was re-crystallized and analysed. (Found: C, 68.9; H, 8.5. Active hydrogen, 2.6. $C_{16}H_{24}O_4$ requires: C, 68.5; H, 8.6%).

The mother liquor, obtained after the removal of the C_{16}-phenol, gave on sublimation at 210/1.9 x 10^{-4} cm a pale-yellow crystalline sublimate (90 mg). This was crystallized from acetone-hexane mixture (m.p. 228°). (Found: C, 70.2; H, 6.9. M, 405. $C_{24}H_{28}O_6$ requires: C, 70.0; H, 6.8%).

(C) - The aqueous sodium hydroxide (10%) soluble fraction was acidified and re-ether extracted. Removal of the solvent gave an oil (3.8 g) which did not

exhibit a ferric reaction. The oily matter was dissolved in dry benzene and chromatographed on alumina. Elution with benzene led to a readily movable band, which gave (2.1 g) of an oil. This fraction on crystallization from acetone-hexane gave more of C_{16}-phenol (0.1 g). The more strongly held band was eluted out with alcohol and the oily matter obtained therefrom was not crystallizable.

(D) - The neutral ether extract from the fusion gave a resinous mass (5 g) which gave an olive green ferric reaction similar to octahydromorellin, and a red colour with magnesium and HCl.

Acetone-permanganate oxidation of C_{16}-phenol

The compound (200 mg) was dissolved in acetone (25 ml) and finely powdered potassium permanganate was added with stirring at room temp, slowly as decolourization proceeded. Stirring was continued for 3 hr and contents were left overnight. Excess permanganate was destroyed with alcohol (10 ml) warmed and filtered. The precipitate was washed with warm alcohol (10 ml), and the combined filtrates distilled to dryness (brown oil). Water (5 ml) was added, the mixture rendered acidic with 50% H_2SO_4, and finally steam distilled to remove the fatty acids. The steam

distillate was treated with ammonia and evaporated to dryness. The ammonium salt (ca. 20 mg) on paper chromatography showed the presence of acetic and isovaleric acids. The non-steam volatile fraction was extracted with ether. Worked up the usual way, it gave a brown oily matter which sublimed at 250°/2 mm. The yellow sublimate (m.p. 130-135°) could not be crystallized. It gave a purple ferric colour similar to 5,7-dihydroxy-2,2-dimethylchromanone and gave a deep red magnesium and HCl colouration similar to chromanone.

Aluminium isopropoxide oxidation of C_{16}-phenol

Dry toluene (50 ml) was taken in a 3-neck flask provided with a stirrer, and a part of it (15 ml) was distilled to remove any moisture. Cyclohexanone (13 ml) was added and some more toluene (5 ml) was distilled off. The C_{16}-phenol (200 mg) was added, followed by aluminium isopropoxide (80 mg in 5 ml of toluene) dropwise with the simultaneous distillation of toluene. The addition was carried over a period of 30 min and the final volume of toluene was 15 ml. The contents were cooled, a saturated solution of sodium potassium tartrate was added to clarify the inorganic layer, and the contents extracted

with ethyl acetate. The extract was washed with water, dried and distilled to give a brown oil (0.17 g), which could not be induced to crystallization. The oil gave a purple ferric reaction.

A similar observation was made on an attempted manganese dioxide oxidation of C_{16}-phenol. The phenol (100 mg), manganese dioxide (1 g) and benzene (10 ml) were refluxed for 24 hr. Filtration and evaporation gave a red oil, which exhibited a purple ferric colour, but resisted all attempts at crystallization.

Manganese dioxide oxidation of C_{16}-phenol at room temp in chloroform

The phenol (50 mg), active manganese dioxide (500 mg) and chloroform (5 ml) were shaken at room temp for 36 hr. The mixture was filtered, and the pale yellow solution on evaporation gave an oily matter devoid of ferric colouration, which crystallized from acetone-hexane (10 mg), m.p. 160-161°, undepressed on admixture with starting material.

Preparation of 5,7-dihydroxy-2,2-dimethylchromanone according to the procedure of Bhat[16]

In a 25 ml flask fitted with a calcium chloride guard tube, phloroglucinol (2.1 g), β-hydroxyisovaleric acid (2.1 g) and boron fluoride etherate (5 ml) were

heated for 2½ hr in a steam bath. Water (10 ml) was then added and the mixture heated on the steam bath for 15 more min. After cooling it was extracted with ether, washed with aqueous sodium bicarbonate and water, and finally the ether extract was dried and distilled to give an oil which crystallized from dil ethanol (m.p. 186-195°). A re-crystallized sample melted at 198° (lit. 198°).

Reduction of 5,7-dihydroxy-2,2-dimethylchromanone to the corresponding chromane according to the procedure of Bhat[16]

The chromanone (300 mg) was dissolved in 10 ml of a molar solution of sodium borohydride in diglyme, boron fluoride etherate (1 ml) added and the solution heated on a steam bath for 1½ hr. The deep red solution was cooled, acetic acid (1 ml) added to destroy the excess borohydride, and finally the mixture was ether extracted. The ether extract was washed with sodium bicarbonate, and the main ether extract after washing with water, drying and distilling to dryness, gave an oil which readily crystallized from benzene (m.p. 163°). The 5,7-dihydroxy-2,2-dimethylchromane formed a bis-benzeneazo derivative on coupling with diazotized aniline. The derivative crystallized from ethanol, m.p. 258° (decomp).

Synthesis of 6-isovaleroyl-5,7-dihydroxy-2,2-dimethylchromanone

Phloroisovalerophenone (m.p. 142°, 2 g), β-hydroxyisovaleric acid (1 g) and boron fluoride etherate (5 ml) were heated on a steam bath for 2 hr. The orange-red solution was diluted with water, when a yellow solid precipitated, which was filtered, washed with water and dried. The dried pale-yellow solid readily crystallized from dil alcohol (norit) as colourless needles (m.p. 112°, 750 mg). It was recrystallized and analysed. (Found: C, 65.3; H, 6.7. $C_{16}H_{20}O_5$ requires: C, 65.7; H, 6.9%).

It gave a cherry-red ferric colour.

Clemmenson reduction of 6-isovaleroyl-2,2-dimethyl-5,7-dihydroxychromanone

Zinc amalgam was prepared by shaking zinc wool (25 g) with aqueous mercuric chloride (2 g in 50 ml water) and conc HCl (2 ml) for 15 min. The liquor was decanted and the amalgam washed with water three times.

Isovaleroyl chromanone (400 mg), alcohol (3 ml), acetic acid (2 ml), water (14 ml) and conc HCl (12 ml) were added and left overnight (24 hr). Next day conc HCl (7 ml) and alcohol (10 ml) were added and again left overnight (24 hr). Then HCl (7 ml) was added and

the mixture refluxed for 7 hr, until the mixture exhibited no ferric colouration. The contents were cooled, extracted with ether, the extract washed with water and sodium bicarbonate solution. The ether extract was then washed with distilled water, dried and distilled to give a brown oil (350 mg) which crystallized from acetone-hexane on keeping at 0° for 3 days. The m.p. 161-162° was undepressed on mixing with an authentic sample of C_{16}-phenol (m.p. 163°).

Like C_{16}-phenol it showed bands at 252 mμ and 306 mμ in the U.V. spectrum and in Gibbs quinone-chloroimide test it gave a purple colour (U.V. band at 547 mμ like the C_{16}-phenol).

Synthesis of benzo-di-dihydro-γ-pyrone

2,2-Dimethyl-5,7-dihydroxychromanone (200 mg) and β-hydroxyisovaleric acid (1 g) were heated with boron fluoride-etherate for 2½ hr and the red solution diluted with water. The mixture was ether extracted and washed with 10% sodium hydroxide (2 x 10 ml). The alkaline extract was acidified, when a semi-solid was obtained, which was filtered, dried and finally crystallized from methanol (m.p. 125-150°, 80 mg). It was crystallized twice more from methanol (m.p. 149-151°) and analysed. (Found: C, 66.3; H, 6.2. $C_{16}H_{18}O_5$ requires: C, 66.1; H, 6.1%).

Hydrolysis of octahydromorellin with 15% alcoholic potassium hydroxide

Octahydromorellin (2 g) was dissolved in alcohol (35 ml), caustic soda solution (30%, 10 g in 35 ml of H_2O) added and the solution refluxed for 4 hr. The initial yellow solution turned deep red. The alcohol was then removed as much as possible (until slight turbidity), and the solution cooled with ice and acidified with 50% H_2SO_4. The mixture did not have a fatty acid odour, and a brown sticky mass separated. The mixture was saturated with common salt and ether extracted. The ether extract was separated as sodium bicarbonate, sodium carbonate and sodium hydroxide soluble fractions and a neutral fraction.

Sodium bicarbonate soluble fraction. - The bicarbonate extract was acidified with 50% H_2SO_4. A yellow solid (0.2 g) was obtained. The solid gave a positive iodide-iodate test for a carboxylic acid and a blue green ferric colour. It was not crystallizable. The filtrate did not contain any fatty acid.

Sodium carbonate soluble fraction. - The sodium carbonate soluble fraction on acidification came out as a yellow oily mass which solidified partly on treating with crushed ice. The mixture was ether extracted, extract washed with water, dried and distilled to give an oil

which crystallized from dil methanol in fine pale-yellow needles (m.p. 131-132°, 1.05 g). This was crystallized from methanol to constant m.p. 132° and analysed. (Found: C, 69.9, 70.3; H, 8.7, 8.5; O, 22.2, 22.4. Active hydrogen, .35, .42 and .43%, i.e. 2. M by the Rast method, 445, 451. M, 436. N.E. 535, 570. $C_{26}H_{38}O_6$ requires: C, 70.0; H, 8.5; O, 21.6%. M, 446. $C_{26}H_{36}O_6$ requires: C, 70.3; H, 8.1; O, 21.6%. M, 444). It gave a greenish blue ferric colour; did not produce any change in colouration with magnesium and HCl; did not produce a wine-red colour on treatment with sodium amalgam and subsequent acidification; did not answer the hydroxamic acid test for acids, esters, lactones and coumarins; did not answer the iodide-iodate test for carboxylic acids.

Sodium hydroxide (10%) soluble fraction. - Acidification of the extract gave a yellow precipitate which was filtered, washed and dried (0.21 g). It separated as an amorphous yellow solid from methanol. It gave a green ferric reaction.

Neutral fraction. - On distillation of the ether extract a brown oily matter was obtained (0.31 g). This oily resin gave an olive-green ferric reaction similar to octahydromorellin.

Alcoholic hydrolysis of octahydromorellin with 5% potassium hydroxide

Octahydromorellin (2.0 g) and 5% alcoholic alkali (80 ml, 4 g potassium hydroxide in 40 ml of water and 40 ml of alcohol) were refluxed for 5 hr. The initial lemon-yellow colour turned orange red at the end of the reaction. The solution was cooled and poured into water (100 ml) and acidified with HCl, when a yellow precipitate separated out, which was filtered, washed and dried (1.6 g). The yellow solid crystallized from methanol (1.1 g), m.p. 131-132°, undepressed on mixing with an authentic sample of C_{26}-phenol. It gave a bluish-green ferric colour. The aqueous filtrate was steam distilled, titrated with N/10 NaOH (3.75 ml) and evaporated to give the sodium salt which answered the chromotropic acid test for formic acid. It did not form a dinitrophenyl hydrazone. On saturation and ether extraction of the non-steam volatile fraction no compound was isolable.

Action of ethyl orthoformate on C_{26}-compound

C_{26}-compound (0.1 g), ethyl orthoformate (1 ml), pyridine (2.5 ml) and piperidine (3 drops) were refluxed gently for 6.5 hr, poured into dil HCl and left overnight. The brown semi-solid that was obtained was filtered, washed free of acid, and finally treated with saturated

sodium carbonate solution. The residue (0.95 g) was crystallized from dil alcohol. It was crystallized to constant m.p. 97°, and analysed. (Found: C, 70.7, 70,5; H, 8.5, 8.6. $C_{27}H_{37}O_6$ requires: C, 71.0; H, 8.1%). The product gave a grey ferric colour, and the U.V. spectrum was similar to the C_{26}-compound.

Treatment of C_{26}-compound with sodium acetate and acetic anhydride (Kostanecki reaction)

The C_{26}-compound (0.2 g) was refluxed gently on a hot plate with fused sodium acetate (2 g) and acetic anhydride (5 g) for 12 hr. The orange-yellow solution was poured into water. An oily mass separated. The aqueous solution was decanted off and fresh water added and stirred, when the oily mass solidified to a grey powder, which was filtered, washed and dried (180 mg). The crude product was extracted with hexane (60-80°) and filtered to remove a black tarry mass. The filtrate was evaporated and the gummy residue dissolved in methanol (3 ml), a few drops of water added, warmed and allowed to cool. A colourless crystalline product separated (80 mg, m.p. 218-222°). It was crystallized from dil methanol twice more when the pure product, m.p. 230°, was obtained. (Found: C, 70.8, 70.8; H, 7.5, 7.6; -OAc, 9.6, 9.1. M, 488, 495. $C_{30}H_{40}O_7$ requires C, 70.3; H, 7.8; -OAc, 8.4%. M, 512). The product did

not exhibit a ferric colouration.

Hydrolysis of the ethyl orthoformate product from C_{26}-compound

The ethyl orthoformate product (3.20 mg) was refluxed with 10% aqueous potassium hydroxide (10 ml) for 2 hr. Since a sparingly soluble potassium salt separated out, methanol (8 ml) was added and again refluxed for 1½ hr. After cooling and acidifying with 50% phosphoric acid, the aqueous mixture was steam distilled. The steam distillate answered the positive chromotropic acid test for formic acid.

Oxidation of C_{26}-compound with HCl in a sealed tube

The C_{26}-compound (1 g) and conc HCl (5 ml) were mixed and heated in a sealed tube for 15 hr at 170°. The tube was allowed to come to room temp (2 hr), cooled with ice and opened. The contents containing an insoluble red mass was diluted with water (10 ml) and extracted with ether. The extract was separated into the sodium bicarbonate soluble, sodium carbonate soluble, the sodium hydroxide soluble and the neutral fraction. The bicarbonate soluble fraction on acidification gave hardly 10 mg of a yellow solid, which answered the iodide-iodate test for carboxylic acids. The carbonate soluble fraction was acidified

and re-ether extracted. Removal of the solvent gave a brown oily substance (720 mg) which sublimed at 0.1 mm/250° to give a yellow sublimate (190 mg). The crystalline sublimate readily crystallized from methanol (m.p. 196°). (Found: C, 71.2; H, 7.6. M, 471. $C_{24}H_{33}O_5$ requires: C, 71.8; H, 8.0%). M.W.

The sodium hydroxide soluble fraction on acidification, ether extraction and subsequent cooling up, gave a thick red oily substance (200 mg). The oil gave a green ferric colour, but was not crystallizable.

The neutral fraction did not contain any compound.

Hydrolysis of C_{26}-compound with 75% potassium hydroxide according to tetrahydrorottlerone conditions

The C_{26}-compound (1 g) was dissolved in 75% potassium hydroxide solution and heated in an oil bath at 140° for 4 hr. At first as temperature rose, the potassium salt started separating as an oily mass, which was seen throughout the heating period. Finally the mixture was cooled and acidified with HCl when a light-brown gritty mass separated. The whole mixture was then ether extracted after thorough saturation with sodium chloride and functional separation effected.

The bicarbonate soluble fraction gave a negligible amount (20 mg) of yellow oil which answered tests for

a carboxylic acid.

The carbonate soluble fraction on acidification, ether extraction and removal of the solvent gave a brown oil (820 mg) which crystallized from methanol in colourless needles, m.p. 131-132°, undepressed on mixing with an authentic sample of C_{26}-compound.

Worked up as above the 10% sodium hydroxide soluble fraction gave 80 mg of an oily mass which crystallized from methanol (m.p. 131°), undepressed on admixture with C_{26}-compound.

The neutral fraction did not contain any substance.

Hydrolysis of C_{26}-phenol with 75% potassium hydroxide and ethylene glycol

The C_{26}-compound (1 g) was dissolved in ethylene glycol (150 ml), potassium hydroxide (75%, 100 ml) added and contents refluxed in a nitrogen atmosphere for 16 hr. After keeping overnight at room temp (25°), the solution was poured into ice and acidified with HCl. A colourless fluffy solid separated along with a brown oil. The contents were ether extracted and separated into sodium bicarbonate soluble, sodium carbonate soluble and sodium hydroxide soluble fractions.

The bicarbonate soluble fraction was acidified with HCl. Appreciable turbidity was observed and on leaving at room temp for 2 days a yellow amorphous powder (50 mg)

separated, which gave a ferric colour and responded to the iodide-iodate test for carboxylic acids. On contact with solvents it resinified.

The carbonate soluble fraction on acidification with HCl gave a pale-yellow solid which was filtered (700 mg). It crystallized readily from methanol (m.p. 130°), undepressed on mixing with C_{26}-compound. It gave a greenish-blue ferric colour.

Likewise the sodium hydroxide soluble fraction gave a solid (90 mg) which crystallized from methanol and gave more of C_{26}-compound.

Fusion of C_{26}-compound with potassium hydroxide at 220°

An intimate mixture of C_{26}-compound (4 g) and KOH pellets (20 g) was placed in a nickel crucible, water (4-5 drops) added and the crucible dipped in a metal bath at 100°. The temp was raised to 220° during 20 min. The crucible was maintained at this temp for 20 more min, and finally cooled to room temp. The melt was dissolved in water (5 x 50 ml) and then acidified with HCl. A pale-yellow solid separated, which was filtered (3.2 g) and the filtrate worked up separately.

The yellow solid was dissolved in saturated sodium bicarbonate solution (complete solution), filtered and acidified. The purified sample (3.1 g) was dissolved in methanol (7 ml), ether (2 ml) added and cooled to 0°

when pale-yellow crystals separated (m.p. 134-136°). Repeated crystallization from methanol gave the pure compound (m.p. 139-140°) as snow-white needles. (Found: C, 69.7; H, 8.0. M, by the Rast method, 467. $C_{25}H_{34}O_6$ requires: C, 69.7; H, 7.9%. M, 430).

It answered the hydroxamic acid and the iodide-iodate tests for carboxylic acids. However it titrated with alkali slowly and the neutralization equivalent gave erratic values. Two determinations gave the values 650 and 670. The filtrate and washings obtained after the removal of the acid, smelt of fatty acid. It was ether extracted and removal of ether gave a liquid (50 mg), which was converted into the ammonium salt. Paper chromatography of the ammonium salt showed the presence of n or isovaleric acid.

Fusion of C_{26}-compound with KOH at 300°

The C_{26}-compound (1 g) was mixed thoroughly with potassium hydroxide pellets (5 g) in a mortar and heated in a pyrex flask provided with mechanical agitation in a metal bath. The temp was raised from 100° to 300° during 15 min and maintained at 300° for 15 min. The contents were cooled, extracted with water (4 x 25 ml) and acidified with HCl. The mixture was saturated thoroughly with common salt and ether extracted. The extract was separated

into sodium bicarbonate soluble, sodium carbonate soluble and neutral fractions.

The bicarbonate soluble fraction on acidification, ether extraction, and removal of solvent, gave a thick red oil (600 mg). The oil was extracted with benzene to give a soluble fraction (330 mg) which was extracted with water (20 ml), filtered and cooled, when a colourless solid separated out (40 mg), m.p. 208-210°. This was crystallized from water (210-211°) and analysed. (Found: C, 61.3; H, 6.3. $(C_5H_6O_2)_x$ requires: C, 61.3; H, 6.1%). The compound answered the iodide-iodate test for a carboxylic acid.

The carbonate soluble fraction on acidification, ether extraction, and subsequent working up, gave a brown oily residue (220 mg). It was dissolved in acetone (3 ml), diluted with hexane (7 ml), filtered and cooled in deep freeze, when an almost colourless crystalline solid separated out (m.p. 160°). The mixed m.p. with an authentic sample of C_{16}-phenol was 160-161°. It had no ferric colouration.

Hypoiodite oxidation of C_{26}-compound: Quantitative estimation of iodoform

In a 500 ml separatory funnel, sodium hydroxide solution (20%, 33 ml) was taken and iodine solution (iodine and potassium iodide, 10%) was added gradually

as decolourisation proceeded. Addition was continued until the solution was distinctly orange yellow (250 ml). Finely powdered C_{26}-compound was then added to the hypoiodite solution and the separating funnel stoppered and agitated for 20 min. A distinct smell of iodoform was perceived. The alkaline solution was then extracted with chloroform (50 + 10 + 10 ml). The combined extracts were washed with water, dried over anhydrous sodium sulphate, and finally filtered into a 100 ml flask, through a bed of sodium sulphate. The sodium sulphate was washed with dry chloroform, and finally the chloroform solution was made up to 100 ml.

The solution was then estimated spectroscopically by measuring the absorbance at 347 mμ.[35] From the standard iodoform curve described,[35] the amount of iodoform present in the chloroform extract was calculated as 38 mg. (Theoretical weight for one mole of iodoform is 37 mg).

Manganese dioxide oxidation of C_{26}-compound

The C_{26}-compound (200 mg) was dissolved in chloroform (10 ml) by slight warming, active manganese dioxide (1 g) was added and the mixture agitated for 24 hr at room temp. Filtration and evaporation of the solvent gave a brown residue which was extracted with

hexane (60-80°, 25 ml), filtered, concentrated to 10 ml and cooled. Colourless needles separated out, M.p and mixed m.p. with C_{26} compound was 132°.

Reduction studies of naphthalene-1,3-dicarboxylic acid (m.p. 281°)

Naphthalene-1,3-dicarboxylic acid was prepared by the known series of reactions. [38]

Papa reduction of naphthalene-1,3-dicarboxylic acid

The acid (100 mg) was dissolved in 10% sodium hydroxide (10 ml) and heated to 80°. Raney alloy (1 g) was added to the stirred hot solution gradually during 45 min, and the contents were then heated in a boiling water bath for 2 hr. The solution was filtered and acidified with 20 ml of conc HCL. The precipitated acid was left overnight and filtered (85 mg). It was crystallized from water, m.p. 200-220, undepressed on admixture with parent acid, m.p. 267-268°.

Kenner-Murray reduction of naphthalene-1,3-dicarboxylic acid

The acid (100 mg) was dissolved in aqueous sodium bicarbonate (40 mg in 10 ml of water), nickel (1 g) added and hydrogen passed with stirring at room temp for 3 hr. After filtering the nickel, the filtrate was acidified with 50% H_2SO_4, when a light-brown solid separated out. The mixture was ether extracted, washed

with water and dried. Removal of ether gave a pale-yellow acid (60 mg, m.p. 237-240°). After two crystallizations from water, the m.p. rose to 252-253° which melted at 230-232° on mixing with acid, m.p. 281°, obtained from the fusion of RPB with potassium hydroxide.

Reduction of naphthalene-1,3-dicarboxylic acid under pressure in a Paar hydrogenator

The acid (200 mg) was dissolved in alcohol (20 ml), Raney nickel (4 g) added and hydrogenated in a Paar apparatus under 40 lb. pressure for 6 hr. The nickel was then filtered, and the alcoholic solution evaporated to give an acid. Crystallized from water, it had a m.p. range of 270-290°.

U.V. absorption showed bands at 285 and 230 mμ; and the general similarity in the curves showed that it was mainly ar-tetrahydronaphthalene-1,3-dicarboxylic acid.

Attempted dehydrogenation of acid, $C_{12}H_{12}O_4$ (m.p. 281°) obtained from the KOH fusion of RPB

Palladium-charcoal dehydrogenation of acid, $C_{12}H_{12}O_4$, m.p. 281°.

The acid (60 mg) was dissolved in diphenyl oxide (8 ml), palladium-charcoal (60 mg) added, and the contents refluxed for 10 hr. The mixture was then filtered free of the catalyst, steam distilled to remove the diphenyl oxide and finally the aqueous mixture was ether extracted.

On removal of ether a brown solid was obtained which on crystallization from water gave the acid (m.p. 272-274°, 15 mg) whose m.p. was undepressed on mixing with starting material.

Dehydrogenation of acid, $C_{12}H_{12}O_4$, m.p. 281°, with chloranil and diethyl carbitol in a sealed tube

The acid (75 mg), chloranil (130 mg) and diethyl carbitol (2 ml) were heated in a sealed tube at 130° for 72 hr, at the end of which a deep-brown solution was formed. After the reaction was over, the contents were poured into benzene (25 ml), when an insoluble brown powder separated out, which was filtered and crystallized from water (35 mg), m.p. 270°, undepressed on mixing with starting material. The U.V. spectrum was similar to starting material.

Iodine-alcohol on acid, m.p. 281°

The acid (50 mg) and iodine (90 mg) were dissolved in alcohol (5 ml), refluxed for 4 hr, and poured into water. Iodine was destroyed with a pinch of bisulphite and the contents were ether extracted. The bicarbonate soluble fraction of the ether extract was acidified with H_2SO_4 and re-ether extracted. The ether extract was washed with water, dried and distilled off to give a pale-yellow solid (ca. 40 mg), m.p. 277-280°

after re-crystallization from dil methanol, undepressed on mixing with acid (m.p. 281°). (Found: C, 65.2; H, 5.6. $C_{12}H_{12}O_4$ requires: C, 65.4; H, 5.5%).

p-Bromobenzenesulphonyl ester of morellin

Morellin (1 g) and p-bromobenzenesulphonyl chloride (2 g) were dissolved in acetone (10 ml), fused potassium carbonate (10 g) added and contents refluxed for 3 hr, when the initial orange solution turned pale yellow. The mixture was cooled, poured into crushed ice and the pale-yellow solid that separated was filtered (1.4 g). The solid was extracted with hexane to remove the excess sulphonyl chloride and the residue after drying crystallized from alcohol (m.p. 181°). It was recrystallized from acetone-hexane (m.p. 182°). (Found: C, 61.7; H, 5.3. $C_{33}H_{41}O_9BrS$ requires: C, 61.3; H, 5.4%).

REFERENCES

1. N. V. Bringi, K. H. Shah and K. Venkataraman, J. Sci. Industr. Res. 1955, 14B, 135.
2. M. Miyano and M. Matsui, Ber. 1958, 91, 2044.
3. E. Elisberg, H. Vanderhaeghe and T. S. Gallagher, J. Amer. Chem. Soc. 1952, 74, 2814.
4. E. C. Kornfeld, E. J. Fornefeld, G. B. Kline, M. J. Mann, R. G. Jones and R. B. Woodward, J. Amer. Chem. Soc. 1954, 76, 5256.
5. H. Heymann and L. F. Fieser, J. Amer. Chem. Soc. 1951, 73, 5252.
6. C. Djerassi, P. Sengupta, J. Herran and F. Walls, J. Amer. Chem. Soc. 1954, 76, 2966.
7. F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz, Chem. & Ind. 1954, 1482; J. K. Norymberski and Gilbert F. Woods, J. Chem. Soc. 1955, 3426.
8. R. V. Lemieux and E. von Rudloff, Can. J. Chem. 1955, 33, 1710.
9. Brockmann and Maier, Naturwiss. 1939, 27, 259.
10. S. Viebel, The Identification of Organic Compounds, 4th Ed., E. E. C. Gad Publisher, Copenhagen, 1954, 111.
11. S. B. Vaisman, Chem. Abstr. 1940, 34, 5847[7].
12. R. Mozingo, C. Spencer and K. Folkers, J. Amer. Chem. Soc. 1944, 66, 1859.
13. E. C. Kleiderer and E. C. Kornfeld, J. Org. Chem. 1948, 13, 455.

14. N. V. Bringi, M. R. Padhye and K. Venkataraman, J. Sci. Industr. Res. 1956, 15B, 128.

15. M. L. Wolfrom, P. W. Morgan and F. L. Benton, J. Amer. Chem. Soc. 1940, 62, 1484.

16. H. B. Bhat, unpublished work.

17. H. Schmid, Fortschr. Chem. org. Naturstoffe 1959, 11, 137.

18. Organic Syntheses, Vol. I, John Wiley & Sons, Inc., New York, 1941, 18.

19. J. Cason and J. D. Wordie, J. Org. Chem. 1950, 15, 611.

20. D. M. Musser and H. Adkins, J. Amer. Chem. Soc. 1938, 60, 664.

21. A. McGookin, A. B. Percival and A. Robertson, J. Chem. Soc. 1938, 309.

22. W. Bridge, R. G. Heyes and A. Robertson, J. Chem. Soc. 1937, 283.

23. M. S. Wolfrom and B. S. Wildi, J. Amer. Chem. Soc. 1951, 73, 235.

24. R. Mitteldorf and W. Riedl, Ber. 1960, 93, 309.

25. W. Bridge, R. G. Heyes and A. Robertson, J. Chem. Soc. 1937, 280.

26. D. S. Bapat, M. K. Unni and K. Venkataraman, Tetrahedron Letters 1960, 5, 15.

27. F. Brown, Biochem. J. 1950, 47, 598.

28. F. E. King, T. G. King and L. C. Manning, J. Chem. Soc. 1957, 563.

29. R. A. Morton and A. L. Stubbs, J. Chem. Soc. 1940, 1347.

30. M. L. Wolfrom, W. D. Harris, G. F. Johnson, J. E. Mahan, S. M. Moffat and B. Wildi, J. Amer. Chem. Soc. 1946, 68, 1946.

31. W. Cocker, W. J. Davis, T. B. H. McMurry and P. A. Start, Tetrahedron 1959, 7, 299.

32. Ring Index No.3552, Second Edition, ACS Monograph Series; G. Wittig, Fr. Bangert, H. E. Richter, Ann. 1925, 446, 165.

33. G. D. Johnson, J. Amer. Chem. Soc. 1951, 73, 5888.

34. V. R. Sathe and K. Venkataraman, Current Sci. 1949, 18, 373.

35. S. D. Nagare, T. O. Norris, J. Mitchell Jr. Anal. Chem. 1951, 23, 1473.

36. D. H. R. Barton, P. De Mayo and J. C. Orr, J. Chem. Soc. 1958, 2240; R. B. Turner, J. Amer. Chem. Soc. 1954, 76, 1390.

37. P. Yates and G. H. Stout, J. Amer. Chem. Soc. 1958, 80, 1691.

38. E. F. Bradbrook and R. P. Linstead, J. Chem. Soc. 1936, 1739.

SUMMARY

A SUMMARY

OF THE PRESENT WORK : STRUCTURE OF MORELLIN

The molecular formula of morellin has been confirmed by X-ray mol. wt. determinations of the methyl ether, benzenesulphonyl ester, and p-bromobenzenesulphonyl ester of morellin; the respective values, 555, 682.5 and 765, corresponded closely to the formula, $C_{33}H_{38}O_7$, suggested earlier for morellin.

The presence of four ethylenic bonds in morellin has been established. On hydrogenation in presence of palladium-charcoal morellin absorbed four moles of hydrogen. Tetrahydromorellin and octahydromorellin had one active hydrogen, and the corresponding methyl ethers none, showing that no carbonyl group was reduced during catalytic hydrogenation. Further, the product obtained by the sodium borohydride reduction of tetrahydromorellin absorbed two moles of hydrogen, which confirmed the presence of four ethylenic bonds. The sodium borohydride reduction product of morellin absorbed only three moles of hydrogen, indicating the presence of an $\alpha\beta$-unsaturated carbonyl group in morellin, which gets reduced by sodium borohydride as in dehydrorotenone which has an ethylenic bond in conjugation with a γ-pyrone carbonyl.

Ozonolysis of morellin and tetrahydromorellin under controlled conditions gave one mole of acetone; this showed the presence of an isopropylidene group in morellin, which did not undergo catalytic reduction up to the tetrahydro stage. Prolonged ozonolysis of morellin gave besides acetone, formaldehyde,* acetaldehyde* and pyruvic aldehyde. The formation of formaldehyde could not be explained by the presence of a $-\underset{|}{C}=CH_2$ group in morellin, as morellin did not undergo oxidation with periodate-permanganate to give formic acid.

Morellin gave a copper complex which crystallized from benzene and analysed for one atom of copper for two molecules of morellin. On hydrolysis with conc hydrochloric acid, the copper complex gave isomorellin. Isomorellin also gave a copper complex, while morellin methyl ether, isomorellin methyl ether, tetrahydromorellin and octahydromorellin did not give copper complexes. The formation of a copper complex therefore involved the phenolic hydroxyl group, an adjacent carbonyl group and an ethylenic bond in conjugation with the carbonyl group.

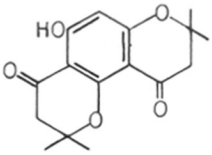
o-Phenylenediamine condensed with morellin, tetrahydromorellin, octahydromorellin and the copper complex of morellin; and the products gave colour

p. 24

* only by paper

reactions for heptazines derived from a group of the type -CO-CH(R)-CO-.

Morellin tends to resinify on treatment with solvents and is alkali-sensitive, but it was observed that stable derivatives, suitable for degradative work, could be prepared by reduction with massive amounts of Raney nickel. When such reduction was carried out in boiling ethanol, cyclohexanol or cyclohexanone, three crystalline products A, B, and C were obtained. Reduction product B was also obtained by the action of palladium-charcoal on reduction product A in boiling p-cymene; and reduction product C was also obtained by similar treatment of octahydromorellin. Reduction products A and B had 3 active hydrogens, showing that 2 carbonyl groups were reduced to secondary alcoholic groups, besides the ethylenic bonds. Reduction product C had only 2 active hydrogens, showing that one carbonyl group remained intact because the reduction was carried out in cyclohexanone, a hydrogen acceptor. Reduction products A, B and C had ultra-violet absorption spectra similar to the spectrum of the benzo-di-dihydro-γ-pyrone (I), synthesized from phloroglucinol.



I

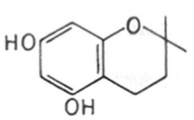
In the double bond region of the infra-red the reduction products showed bands at 1626 cm^{-1} (chelated carbonyl) and at 1745 cm^{-1}; the latter high-frequency band is discussed later.

Reduction product B was not affected by prolonged refluxing with 30 per cent ethanolic potassium hydroxide, but it was a convenient starting point for degradation under more drastic conditions. Since the isolation of 2-methylhepten-2-ol-6 by alkali fusion of morellin in the earlier work indicated the presence of a long side-chain composed of 7 or more carbon atoms in morellin, reduction product B was first submitted to oxidations. Alkaline permanganate surprisingly gave n-valeric acid, but no crystalline fraction was isolable. Vigorous hypoiodite oxidation, under conditions in which a $-CO-CH_2-CO-$ or $-CHOH-CH_2-CHOH-$ group should cleave to a mixture of carboxylic acids, gave a neutral crystalline compound, which had a molecular weight of about 500, showing that no major part was broken off. Oxidation of B

with 50 per cent nitric acid catalysed by ammonium venadate gave a crystalline compound of molecular weight 488.

Fusion of reduction product B with potassium hydroxide at 290° gave a crystalline phenol, $C_{16}H_{24}O_4$, n-valeric acid, and a dicarboxylic acid, $C_{12}H_{12}O_4$, which was shown to be 5,6,7,8-tetrahydronaphthalene-1,3-dicarboxylic acid. On treatment with copper bronze in quinoline the latter acid underwent decarboxylation as well as dehydrogenation to give naphthalene, and oxidation with nitric acid under pressure gave mellophanic acid (benzene-1,2,3,5-tetracarboxylic acid). The isolation of naphthalene thus provides direct and conclusive evidence for the presence of a partially hydrogenated naphthalene ring in morellin.

Although there was some evidence for the presence of a 2,2-dimethylchromene ring in morellin, final confirmation required the isolation of 5,7-dihydroxy-2,2-dimethylchroman (II) among the alkali fusion products of morellin after hydrogenation of two or more of the ethylenic bonds.

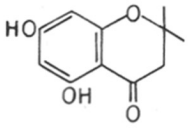


II

This chroman was synthesized by a new method developed by Bhat in this Laboratory, involving the boron fluoride condensation of phloroglucinol with β-hydroxyisovaleric acid, and the subsequent reduction of the chromanone to the chroman by sodium borohydride and boron fluoride-etherate. The synthetic chroman readily formed a bisbenzeneazo derivative on coupling with diazotized aniline, which was a useful method for isolating and identifying the chroman. The chroman (II) could not be spotted in any of the fractions from the alkali fusion of reduction product B. Alkali fusion of octahydromorellin also did not yield the chroman (II), but it gave the C_{16}-phenol obtained earlier from B, together with a crystalline compound, $C_{24}H_{30}O_6$, the ultra-violet absorption of which was not similar to that of the chroman (II).

The constitution of the C_{16}-phenol, on which the constitution of the phloroglucinol half of the morellin molecule obviously depended, was then investigated. The C_{16}-phenol was soluble in aqueous sodium carbonate, and since it was not a carboxylic acid, it was probably a dihydric phenol. It did not give a ferric colour, but on treatment with manganese dioxide or with aluminium isopropoxide oxidation of a secondary alcoholic to a ketonic group apparently took place, the

product exhibiting a purple ferric colour like 5,7-dihydroxy-2,2-dimethylchromanone (III).



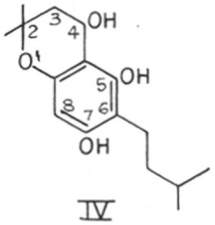
III

Thus the C_{16}-phenol was probably a chromanol. On oxidation with ~~acetone~~ in acetone permanganate the C_{16}-phenol gave acetic and isovaleric acids, and a non-steam-volatile residue which gave a purple ferric colour and a wine-red colour with magnesium and hydrochloric acid; a γ-pyrone or pyranone ring appeared to be left intact.

The ultra-violet spectrum of the C_{16}-phenol showed two bands: one at 252 mμ and a broad band at 306 mμ. This was not similar to the spectra of C-isoamylphloroglucinol or 5,7-dihydroxy-2,2-dimethylchroman (II), but to the spectrum of o-hydroxyacetophenone. However, such a structure was excluded by the absence of a ferric reaction and the failure to form a dinitrophenylhydrazone; further, the number of oxygen atoms and the derivation from morellin clearly pointed to a phloroglucinol nucleus.

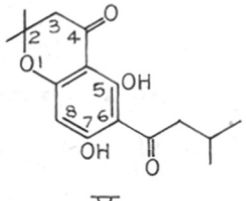
There was considerable evidence for the presence of an isoamylene substituent in morellin: the formation

of isovaleric acid in the alkali fusion of morellin, formation of acetone on ozonolysis, and the cyclization of morellin to a compound devoid of ferric colour on treatment with boron fluoride; all these properties were reminiscent of osajin and pomiferin which Wolfrom proved to be isoflavones derived from C-isoamylenephloroglucinol. The molecular formula, $C_{16}H_{24}O_4$, and the chemical properties of the C_{16}-phenol can be explained by assigning to it the structure of 6-isoamyl-5,7-dihydroxy-2,2-dimethylchromanol (IV) or the isomer with the isoamyl group in the 8-position.



IV

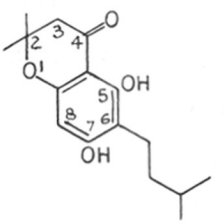
With this idea in view the synthesis of (IV) and the corresponding 8-isomer and related chromans was attempted. Condensation of phloroisovalerophenone with β-hydroxyisovaleric acid gave 6-isovaleroylchromanone (V) or the 8-isomer.



V

Clemmensen reduction under controlled conditions gave

a crystalline compound which gave a violet ferric colour, showing that only one of the two carbonyl groups had undergone reduction. The product proved to be the 6-isoamylchromanone (VI) or the 8-isomer, and not the 6-isovaleroylchroman ~~(VII)~~ or the 8-isomer obtained by the action of isovaleric acid and boron fluoride on the chroman (II). This conclusion was arrived at on the basis of the ultra-violet spectra.



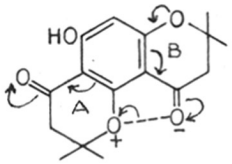
VI

In the Clemmensen reduction of (V), the side-chain carbonyl was therefore preferentially attacked. When (V) was submitted to Clemmensen reduction under more drastic conditions, using the disappearance of ferric colour as the end-point, a crystalline product was obtained, which was identical with the C_{16}-phenol in all respects. The absence of ferric colour and the molecular formula with 4 oxygen atoms could only be explained by a chromanol structure (IV).

The side-chain was placed in the 6-position since the C_{16}-phenol responded to the Gibbs quinone-chloroimide colour reaction for an unoccupied position <u>para</u> to a phenolic hydroxyl group.

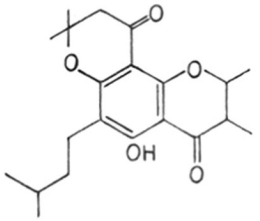
The structure of the C_{16}-phenol revealed the possibility of two γ-pyrone or dihydro-γ-pyrone rings, built round one phloroglucinol nucleus, in morellin.

For comparison with the reduction products of morellin, the unknown benzo-di-dihydropyrone (I) derived from phloroglucinol was therefore synthesized by the boron fluoride condensation of 5,7-dihydroxy-2,2-dimethylchromanone with excess of β-hydroxyisovaleric acid. The infra-red spectrum of (I) showed bands at 1762 cm^{-1} and 1626 cm^{-1} in the double bond region. The band at 1762 cm^{-1} is unusual, and appears to arise from the dipolar interaction of the pyranone carbonyl in ring B with the adjacent ether oxygen in ring A.



I B

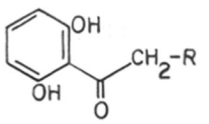
It may be recalled that reduction products A, B and C and octahydromorellin show bands at 1745 cm^{-1}, for which therefore a similar assignment may be made. The partial structure (VII) may now be written for the reduction products of morellin.



VII

It has been observed that such a carbonyl group in a chromanone is not susceptible to Raney nickel reduction under the conditions used in the present work.

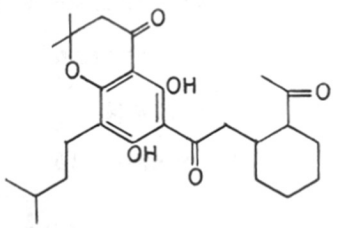
Hydrolysis of octahydromorellin with 5 or 15 per cent ethanolic potassium hydroxide gave a phenol, $C_{26}H_{36}O_6$, and formic acid. The phenol had the properties of a ketone of the type (VIII).



VIII

It condensed with ethyl orthoformate and with acetic anhydride-sodium acetate. The latter proceeded like a Kostanecki reaction to give a 2-methylchromone, but the constitution of the ethyl orthoformate product remains to be proved conclusively. The spectral and other properties are discussed.

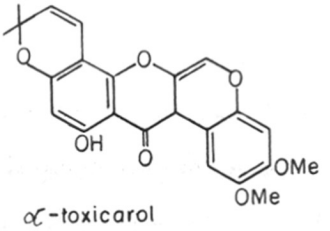
Hypoiodite oxidation of the C_{26}-compound gave one mole of iodoform; alkali fusion led to the C_{16}-phenol. It has therefore been tentatively assigned the constitution (IX). Although in the C_{16}-phenol the



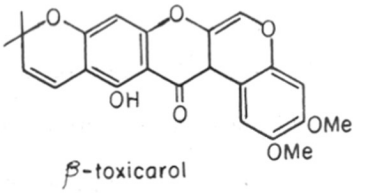
IX

isoamyl group is located in the 6-position, the structure

(IX) places the isoamyl group in the 8-position in the C_{26}-phenol because it exhibited a blue-green ferric colour, similar to 2-acetylresorcinol and different from the wine-red colour of resacetophenone. It is reasonable to assume that during the alkali fusion isomerization to the 6-isoamylchromanone takes place, probably by opening of the pyranone ring and recyclization in the alternative direction on the analogy of α- and β-toxicarol.

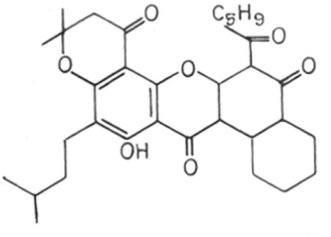


α-toxicarol



β-toxicarol

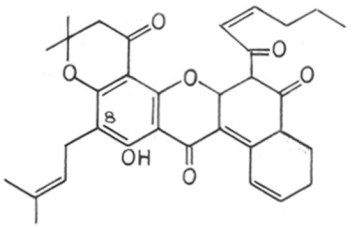
From the structure of the C_{26}-compound the structure of octahydromorellin may be developed to (X).



X

The ready alkaline hydrolysis of octahydromorellin supports the postulation of a 1,3-diketone group.

From this a complete structure for morellin (XI) is suggested and discussed.



XI

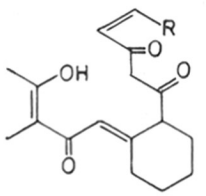
The location of the isoamylene side-chain in the indicated position follows from structure (IX) for the C_{26}-phenol; the hydroxyl group then occupies a position in the partially hydrogenated benzoxanthone system which explains the formation of a copper complex by morellin, and not by O-methylmorellin or the hydrogenated morellins. The ethylenic bonds in the naphthalene part are shown as conjugated with a chromanone carbonyl in order to explain the colour of morellin and the 360 mμ band in the ultra-violet absorption spectrum.

The structure (XI) explains the formation of the main products of the alkali fusion of morellin: phloroglucinol; isovaleric acid from the isoamylene chain by the migration of the double bond towards the aromatic ring, subsequent retro-aldol cleavage, and a Cannizzaro reaction; methylheptenol from isopentenyl-phloroglucinol moiety by a retro-aldol cleavage as in mangostin; homophthalic acid from the partially

hydrogenated naphthalene part of the molecule.

The precise structures of the Raney nickel reduction products cannot yet be formulated; but the alkaline degradation of reduction product B to the C_{16}-phenol and ar-tetrahydronaphthalene-1,3-dicarboxylic acid is understandable on the basis of structure (XI) for morellin.

Morellin yields α-hydroxyisobutyric acid by permanganate oxidation as a result of the isopropylidene group hydroxylating across the double bond. The liberation of acetone by mild alkaline hydrolysis of morellin probably involves the opening of the partially hydrogenated benzoxanthone ring system to (XII).



XII

Numerous ultra-violet and infra-red spectra of morellin and its derivatives, as well as related compounds, are recorded and discussed during the progressive development of the structure of morellin.

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

I wish to express my sincere thanks to Professor K. Venkataraman, Director, National Chemical Laboratory, Poona, for suggesting the problem and for his continued interest in the progress of the work.

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V. K. Vijayaraghavan

POONA,
14th December 1960.

(V. K. VIJAYARAGHAVAN)
CANDIDATE