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EXTRACTIVES OF *MILLETIA OVALIFOLIA*

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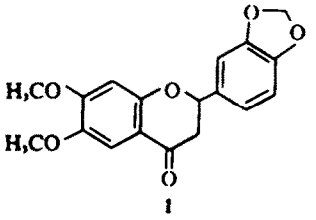
Abstract—Isolation of lanceolatin B, the flavanones 1 and 2, the flavones 3 and 4 and the dibenzoylmethane 5 from *M. ovalifolia* is reported. The structures were assigned from spectral data and confirmed by synthesis.

In continuation of our previous work on the flavonoid constituents of *M. auriculata*,[1,2] we studied the extractives of *M. ovalifolia*. This led to the identification of six compounds which are of interest because they bear no relationship to the constituents of *M. auriculata* or other *Milletia* species[1] but are related rather to the constituents of *Tephrosia lanceolata*[3] and *Pongamia glabra*.[4-6]

TLC of the leaf extract of the plant indicated the presence of three fluorescent substances, two of which could be separated by preparative TLC and are designated here as milletenins A and B.

Milletenin A (1) m.p. 178–79°, gave a blue colour with Mg/HCl (an excess of the compound was necessary for a positive test) which suggested the presence of a flavanone nucleus. Its UV spectrum is similar to that of 6,7,3',4'-tetramethoxy flavanone[7] showing substantial absorption (log ϵ 3·89) at 342 nm along with the expected flavanone maximum at 278 nm (log ϵ 4·18). The IR spectrum shows the carbonyl band at 1685 cm^{-1} but is devoid of hydroxylic absorption. The assigned structure follows from these features and the NMR and mass spectra of the compound. The former shows clearly the presence of two methoxyls and one methylenedioxy group by singlets at 6·05 (6H) and 3·92 τ(2H) respectively and the characteristic ABX system of the flavanone nucleus [4·55 (1H q), 7·08 (2H mc)]. The aromatic region is defined by the singlets of two isolated protons at 2·58 and 3·40 τ arising from protons at C-5 and C-8, and a broad three proton multiplet centred at 2·96, similar in appearance to that observed in the spectrum of 5,7-dihydroxy-3', 4'-dimethoxy flavanone.[8]

The relative positions of methoxyls and methylenedioxy groups as shown in 1 follow from the mass spectrum which shows RDA fragments at *m/e* 180 (95%) and 148 (100%) as required by structure 1. Confirmation of this was obtained by comparison with a synthetic sample prepared by condensation of 2-hydroxy-4, 5-dimethoxy acetophenone, available by persulphate oxidation,



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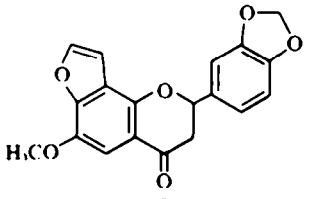
followed by partial methylation, of 2-hydroxy-4-methoxy acetophenone, with piperonal. The resulting chalcone m.p. 180–81° resisted cyclisation under basic conditions and was recovered unchanged after treatment with KOH in ethanol at room temperature for 48 h or with pyridine/piperidine for 12 h under reflux. Exposure to refluxing aqueous ethanolic H_2SO_4 brought about partial conversion to the flavanone isomer which was separated by preparative TLC. As in the case of bavachinin[9] the synthetic racemic sample had identical m.p. and gave no depression in mixture m.p. with the optically pure natural material. The IR spectra of the natural and synthetic products were also superimposable.

Milletenin B (2) m.p. 185–88° was obtained in comparatively smaller quantities and was not analysed. The molecular formula $C_{15}H_{14}O_6$ is derived from the molecular ion peak at *m/e* 338 (89%) and other spectral data. Its UV spectrum is similar to that of milletenin A having absorption at 247, 278 and 341 nm (log ϵ 4·3, 4·0 and 3·6). The presence of a furoflavanone nucleus is evident from the NMR spectrum in which, along with the quartet and multiplet centred at 4·52 and 6·98, the doublets (J = 2 c/s) of the α and β furan protons are clearly discernible at 2·40 and 3·08, the latter overlapping partially with the multiplet of the 2',5',6' protons at 3·12. Apart from these resonances the aromatic region of the spectrum shows only one singlet at 2·73 which can be assigned to the C-5 proton as it is only slightly upfield from the chemical shift of this proton in milletenin A. The NMR spectrum also

shows singlets of a methylenedioxy and an OMe 1 group at 4·0 and 6·0 respectively.



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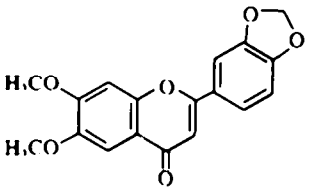

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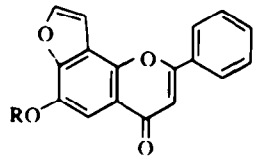
Added support for structure 2 is forthcoming from the mass spectrum in which the RDA fragments occur at *m/e* 190 (100%) and 148 (70%) which definitely places the methylenedioxy group in the B ring.

Of the four structures possible on the basis of the above spectral data 2 is strongly favoured by the occurrence of lanceolatin B in the bark extract and the oxygenation pattern of 1 and the other constituents of this plant.

The third constituent milletenin C was obtained only in traces and could not be crystallised to a sharp melting product. TLC, however, showed it to be pure and structure 3 for the compound is based on comparative TLC with the dehydrohalogenation product, m.p. 251°, of 1.

TLC of the trunk bark extract revealed the presence of at least six fluorescent substances running very close to each other. Resolution by repeated chromatography afforded lanceolatin B[3] along with two new compounds ovalifolin (**4a**) m.p. 135° and milletenone **5a** m.p. 138°. The NMR spectrum of lanceolatin B shows the singlet of the C-3 proton at an abnormally low value 3·13, though the spectrum is otherwise compatible with only this structure. Final confirmation was obtained by comparison with an authentic sample of lanceolatin B kindly provided by Prof. N. V. Subba Rao.

The UV spectrum of ovalifolin is almost superimposable on that of lanceolatin B suggesting the presence of identical chromophoric systems in the two compounds. The NMR spectrum shows the presence of a γγ-dimethylallyloxy side chain by resonances at 4·43 (1H m), 5·19 (2H d) and 8·20 (6H s). The singlet of the C-3 proton is shifted here even further downfield to 3·04 and this, therefore, seems to be a feature characteristic of furoflavones. The aromatic region of the spectrum is similar to that of lanceolatin B except for the absence of the doublet of the C-5 proton at 1·83 which provides evidence for attachment of the O-allyl side chain at C-6. Confirmation of this was obtained by hydrogenolytic or acid catalysed cleavage of the side chain followed by methylation of the phenol (**4b**), m.p. 291° with diazomethane to a product m.p. 191°. This agrees with the literature melting point of kanjone. The IR spectrum of the methyl ether is also superimposable on that of the published spectrum of kanjone **4c**.[4]



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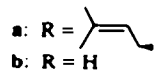
a: R = 
b: R = H
c: R = CH_3

The mass spectrum of ovalifolin does not show the molecular ion peak, the allylic side chain being eliminated, as in isoauriculatin[2] and other such compounds,[10] with hydrogen transfer from the gem-dimethyl group to give the base peak at *m/e* 278. The M^+-C_5H_8 fragment undergoes *retro* diene cleavage to give an ion at *m/e* 176 (57%) 16 mass units higher than the corresponding ion *m/e* 160 (31%) in lanceolatin B.

Milletenone **5a** m.p. 138° forms, along with lanceolatin B, the predominant constituent of the bark extract. Elemental analysis and M^+ at *m/e* 328 (78%) lead to the molecular formula $C_{18}H_{16}O_6$. It gives a green ferric colouration and a pink colour with Mg/HCl but the UV spectrum, which shows marked concentration and solvent dependence, rules out the usual flavonoid structure for the compound. The NMR spectrum provides evidence for the presence of two methoxyls and one methylenedioxy group by singlets at 6·06, 6·14 and 3·97. The aromatic region of the spectrum shows doublets of two *ortho* coupled protons (J = 8·6 c/s) at 2·08 and 3·42, the latter distorted by overlap with the signal of another proton. Signals of two other *ortho* coupled protons are discernible at 2·46 and 3·15 (J = 8·1 c/s), the former coupled further (J = 1·5 c/s) to a *meta* proton. Taken together with the sharp singlet of an isolated proton at 2·94 this indicates the presence of two trisubstituted benzene rings in milletenone.

The most unusual feature of the NMR spectrum, however, is the presence of two sharp singlets integrating for 1/2 and 1/3 proton at 6·35 and 5·52 respectively the significance of which is discussed later.

The accurate mass spectrum of the compound also could not be interpreted in terms of any of the usual flavonoid structures and suggests strongly the existence of a dibenzoylmethane system.

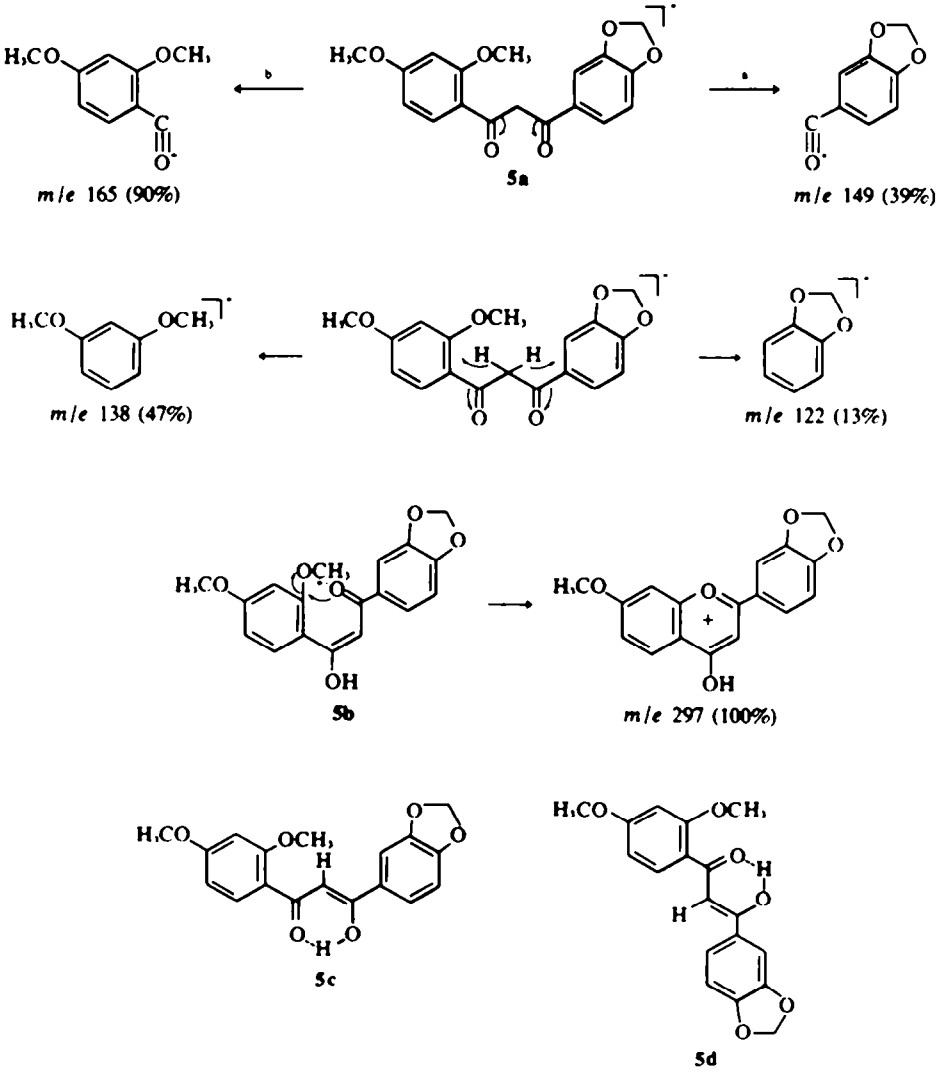
A very characteristic feature of the mass spectrum is the base peak at *m/e* 297 formed by

methoxy elimination from $M^{\cdot}$ to give a fragment $C_{17}H_{13}O_5$. Other fissions of the molecule lead to fragments having the elementary composition $C_{18}H_{14}O_5$ (18%), $C_9H_9O_3$ (90%), $C_8H_7O_3$ (39%) and $C_8H_{10}O_2$ (47%).

The formation of these ions is compatible only with structure **5a** for milletenone and the break down pattern can be rationalised as shown on the basis of this structure.

Among flavones OMe elimination is peculiar to compounds with OMe groups at C-2' and C-3 and is considered to be of diagnostic value for this type of substitution. Thus the $M^{\cdot}$-31 peak forms the base peak in the spectrum of 3, 5, 7, 2', 4'-pentamethoxy flavone.[11] The $M^{\cdot}$-OCH_3 ion here, however, can be derived directly from the enol **5b** by analogy with the behaviour of *ortho* OMe cinnamoyl compounds.[12]

The parent unsubstituted dibenzoylmethane has been shown by spectroscopic studies to exist exclusively in the enolic form[13] but that the situation here is more complicated is evident from the NMR spectrum of milletenone. The most unusual feature of this, as already stated, are the singlets at 6·35 and 5·52 both of which appear with reduced intensities after D_2O exchange in neutral medium and can therefore be assigned only to labile protons. The singlet at 6·35 can be attributed to the methylene protons of the dibenzoylmethane and its intensity corresponds to the presence of ~25% of the diketonic form. The singlet at 5·52 can be assigned with some certainty to the olefinic proton of the enolic form **5c** in which the ring carrying the OMe group is twisted out of the plane of the double bond so as to place the olefinic proton in the shielding zone of the benzene ring. Since this signal integ-



rates only for 1/3 proton the predominant enolic form must be represented by **5d**, the olefinic proton of which is deshielded by the aromatic rings on either sides and its signal is lost in the aromatic region of the spectrum. Further evidence in favour of these assignments is provided by the rise of the integral over the aromatic region which shows approximately seven protons in this region as against six actually present.

Structure **5a** for milletenone was confirmed by comparison with a synthetic sample obtained by condensation of 2,4-dimethoxy acetophenone with methyl piperonylate.

EXPERIMENTAL

The m.ps were taken on a Kofler block and are uncorrected. NMR spectra were determined with a Varian A-60 D instrument in $CDCl_3$ with TMS as internal standard. Analyses were carried out by the Australian Microanalytical service, Melbourne.

Isolation. Air dried leaves (1 kg) were defatted with light petroleum and then extracted in a soxhlet for 3 days with benzene. The extract was concentrated and left at room temp. The solid deposited gradually was collected after 2 days (0·8 g). It was dissolved in $CHCl_3$ (2 ml) and streaked on activated silica gel G plates (20 × 20 cm, 12 plates), which were developed with benzene–EtOAc (6:1) and the individual fluorescent zones scrapped off and worked up separately.

Milletenin A (1). Crystallised from light petroleum–benzene as colourless needles, m.p. 178–80° (0·5 g) (Found: C, 65·78; H, 4·82; $C_{18}H_{16}O_6$ requires: C, 65·85; H, 4·82%); M^+, *m/e* 328; $[\alpha]_D^{28} = -54°$ ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 278, 342 nm (log ϵ 4·18, 3·89); NMR (τ values)–OCH_3, (6H s, 6·05);–O–CH_2–O–(2H s, 3·92); C-3 H (2H mc, 7·08); C-2 H (1H q, 4·55); Ar*H* (1H each s, 2·58, 3·40); Ar*H* (3H m, 2·96).

Milletenin B (2). Crystallised from light petroleum–benzene as colourless needles, m.p. 185–88° (25 mg); M^+, *m/e* 338; $\lambda_{max}^{CHCl_3}$ 247, 278, 341 nm (log ϵ 4·3, 4·0, 3·6); NMR–OCH_3 (3h s, 6·0); –O–CH_2–O– (2H s, 4·0); C-3 H (2H mc, 6·98); C-2 H (1H q, 4·52); Ar*H* (1H s, 2·73); α and β furan H (2H d, 2·40, 3·08, J = 2 c/s); Ar*H* (3H m, 3·12).

Milletenin C (3). Owing to its lack of solubility in $CHCl_3$, benzene, EtOAc and acetone it could not be eluted completely from the adsorbent. Evaporation of the $CHCl_3$ eluate left a small amount of sticky residue which was homogeneous on TLC plate. Comparative TLC with the synthetic sample prepared as below showed the two to be identical.

A mixture of milletenin A (0·1 g), EtOH (25 ml), iodine (50 mg) and fused NaOAc (30 mg) was refluxed on a water bath for 6 h, diluted with water, extracted with $CHCl_3$, dried (Na_2SO_4) and evaporated. Crystallisation of the residue from dioxan–EtOH gave fine needles m.p. 252–53° (60 mg). TLC with natural milletenin C in acetone-benzene and EtOAc–benzene (1:3) showed the two to be identical.

Synthesis of milletenin A (1)

2-Hydroxy-4-methoxy acetophenone. A mixture of resacetophenone (9·8 g) dry acetone (100 ml) and freshly distilled Me_2SO_4 (6·2 ml) was refluxed over anhyd K_2CO_3 (10 g) for 6 h. The brown product obtained on work up was purified by chromatography on silica gel. Elution with benzene afforded an oil which was crystallised from light petroleum, colourless plates, m.p. 48° (8·7 g).

2,5-Dihydroxy-4-methoxy acetophenone. A stirred soln of 2-hydroxy-4-methoxy acetophenone (5 g) in pyridine (51·9 ml) and aqueous KOH (5·83 g in 81·2 ml) was treated with aqueous potassium persulphate (6·43 g in 243·6 ml) for 2 h. After keeping at room temp for 24 h the soln was acidified with conc HCl (pH 6) and the unreacted compound was removed by extraction with ether. The aqueous layer after extraction with ether was treated with Na_2SO_3 (8 g) and conc HCl (100 ml), kept on a boiling water bath for 30 min, cooled and extracted again with ether. The ether extract was washed thoroughly with water, dried (Na_2SO_4) and evaporated to give the title compound, m.p. 164° (1·2 g).

2-Hydroxy-4,5-dimethoxy acetophenone. 2,5-Dihydroxy-4-methoxy acetophenone (2 g) was dissolved in MeOH (50 ml), treated with an excess of ethereal diazomethane, kept over night in a refrigerator and evaporated. The residue crystallised from MeOH as colourless needles, m.p. 112–14° (2 g).

2'-Hydroxy-4',5'-dimethoxy-3,4-methylenedioxy chalcone. To a soln of 2-hydroxy-4,5-dimethoxy acetophenone (0·7 g) and piperonal (0·4 g) in EtOH (13 ml), KOH (1 g in 1·5 ml H_2O) was added dropwise with shaking and the soln kept at room temp for 48 h with occassional shaking. At the end of this period the orange coloured soln was diluted with excess water and extracted with ether. Work up of the ether extract afforded, after crystallisation from EtOH, orange needles m.p. 182° (0·52 g). (Found: C, 65·70; H, 5·18; $C_{18}H_{16}O_6$ requires: C, 65·85; H, 4·91%).

Isomerisation of chalcone to milletenin A (1). A soln of the above chalcone (50 mg) in EtOH (6 ml) was refluxed with conc H_2SO_4 (0·5 ml) for 48 h, diluted with water and extracted with ether. The residue obtained on evaporation of ether was found to be a mixture of the flavanone and chalcone. Resolution by preparative TLC and crystallisation from light petroleum–benzene gave colourless needles, m.p. 178–80° (15 mg), found identical with natural milletenin A by comparative TLC, mixture melting point and IR.

Bark extract. Air dried trunk bark (10 kg) was cut into small chips and extracted with $CHCl_3$ in a soxhlet. Removal of the solvent under reduced pressure gave a green oil which was extracted with ether in a liquid–liquid extractor. Evaporation of ether left a dark green sticky residue which was dissolved in benzene and adsorbed on a silica gel column. The column was run with light petroleum, benzene, EtOAc and their mixtures.

The light petroleum–benzene fractions with higher percentage of benzene contained **5a** along with varying amounts of lanceolatin B and ovalifolin (**4a**). Similarly the benzene-EtOAc fraction (9:1) afforded a mixture of lanceolatin B and ovalifolin. Rechromatography of the fractions containing milletenone over silica gel using light petroleum–benzene (1:3) gave almost pure milletenone. The fractions with benzene–EtOAc (9:1) were also combined and rechromatographed over neutral alumina (Woelm activity 1) using benzene–EtOAc (6:1) for elution. The earlier fractions from this chromatography afforded almost pure ovalifolin. After further purification by crystallisation the following products were obtained.

Ovalifolin (**4a**). Colourless needles from light petroleum–benzene, m.p. 134–35°, (0·15 g); (Found: C, 76·39; H, 5·42; $C_{20}H_{16}O_4$ requires: C, 76·28; H, 5·24%);

$M^+ - C_5H_8$ *m/e* 278; $\lambda_{max}^{CHCl_3}$ 266, 302 (log ε 4·54, 4·39); NMR side chain (1H m, 4·43, 2H d, 5·19, 6H s, 8·20); C-3 H (1H s, 3·04); ArH (6H m, 2·03–2·50); α and β furan H (d, 2·20, 2·80; J = 2 c/s).

Cleavage of the side chain: (*6-Hydroxy lanceolatin B*). The γγ-dimethyl-allyl ether (100 mg) was refluxed on a water bath with EtOH (2·5 ml) containing conc HCl (2·5 ml) for 4 h. The mixture was diluted, extracted with ether, dried and evaporated to give a colourless product (**4b**) which crystallised from EtOH as needles, m.p. 290°. λ_{max}^{EtOH} 265, 301 nm; $\lambda_{max}^{EtOH+NaOH\ vols}$ 280 nm.

Methylation of the phenol (**4b**). Methylation of the phenol (20 mg) with ethereal diazomethane gave colourless needles from MeOH, m.p. 191° (20 mg), (literature m.p. of kanjone 191°, IR superimposable).

Lanceolatin B. Colourless needles from light petroleum–benzene, m.p. 135–42° (0·8 g); (Found: C, 78·24; H, 4·21; $C_{17}H_{10}O_3$ requires: C, 77·85; H, 3·84%); M^+, *m/e* 262; $\lambda_{max}^{CHCl_3}$ 262, 295 nm (log ε 4·4, 4·25); NMR C-3 H (1H s, 3·13); ArH (6H m, 2·05–2·44, 1H d, 1·83 J = 8 c/s); α and β furan H (2H d, 2·24, 2·80, J = 2 c/s).

Milletenone (**5a**). Yellow needles from light petroleum–benzene m.p. 138° (0·45 g), (Found: C, 65·55; H, 5·07; $C_{18}H_{14}O_6$ requires: C, 65·85; H, 4·91%); M^+, *m/e* 328; $\lambda_{max}^{CHCl_3}$ 251, 273 nm; ν_{max}^{KBr} 1620 (>C=O); 1500, 1460, 815, 790 cm^{-1} (aromatic); NMR–OCH_3 (3H each s, 6·06, 6·14); –O–CH_2–O– (2H s, 3·97); ArH (1H s, 2·94; 1H each d, 2·08, 3·42, J = 8·6 c/s); (1H d, 3·15, J = 8·1 c/s; (1H q, 2·46, J = 8·1 and 1·5 c/s); (1/3H s, 6·35); (1/2H s, 5·52).

Synthesis of milletenone. A soln of 2,4-dimethoxy acetophenone (3 g) and methyl piperonylate (3 g) in dry ether (50 ml) was added to NaoEt, prepared from Na (1 g) and EtOH (~2 ml) in ether (50 ml) and refluxed for 4 h. Work up by addition of water and ether extraction gave product which was chromatographed over silica gel (50 g). The light petroleum–benzene (3:1) eluate gave (**5a** 350 mg), m.p. 138°, identical with the natural product (m.m.p., TLC, IR and NMR).

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