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EXTRACTIVES OF *MILLETIA AURICULATA*—III

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Abstract—Two new pyranoisoflavones, auriculasin and isoauriculasin were isolated from *M. auriculata* and assigned structures 1 and 3 respectively. The structure assigned earlier to isoauriculatin has been revised to 4.

The occurrence of the rotenoid summatrol and the pyranoisoflavones auriculatin, auriculin and isoauriculatin in *M. auriculata* roots was reported earlier.[1,2] From the benzene extract of the leaves of this plant two new isoflavones, designated as auriculasin and isoauriculasin have now been isolated and assigned structures **1** and **3** on the basis of data presented. Further, a reinvestigation of isoauriculatin during the course of this work makes it necessary to revise its structure to **4**.

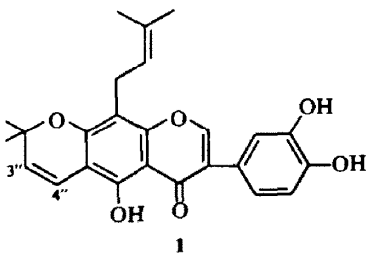
Elementary analysis and mass spectrum of **1** agree with the molecular formula $C_{25}H_{24}O_6$. The similarity of this compound with auriculatin is apparent from its spectral characteristics; UV absorption at 295 nm, IR bands at 3250 and 1650 cm^{-1} (chelated –OH, $\rangle C{=}O$) and NMR signals at 8·54, 8·33, 8·21 ($-\overset{|}{\underset{|}{C}}-CH_3$ of the chromene ring and the prenyl side chain) and 2·15τ (isoflavone proton). Though the aromatic region of the spectrum differs from that of auriculatin the remaining protons of the chromene ring and the side chain resonate at about the same values and have the multiplicities required by structure **1**. The chelated OH gives a sharp singlet at −4·0τ but the resonances of the two remaining OH protons merge with the signals of the aromatic protons. The three phenolic OH groups are evident from the signals of three Me groups in the NMR spectra of the acetate and methyl ether.

In order to distinguish between the linear or angular fusion of the pyran ring the NMR spectra of auriculasin and its triacetate were compared. The comparison revealed shifts of +0·21 and −0·15τ in the positions of the doublets of protons 4″ and 3″ as required by structure **1**. The presence of an ion *m/e* (215) arising from the fragment **2** confirms that the side chain is attached to ring A, and hence must be located at the only remaining site C-8.

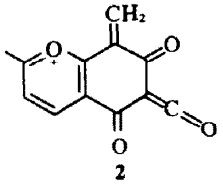
The substitution in ring B can not be inferred from the complicated pattern stretching from 2·58 to 2·70τ in the NMR spectrum of the triacetate and integrating for three protons. A 2′,4′-substitution pattern is ruled out as it would make the compound identical with auriculatin. Evidence for a vicinal dihydroxy system was obtained by conversion to the diphenyl methylenedioxy derivative with diphenyl dichloromethane. On this basis, the most likely substitution pattern 3′,4′, was confirmed by oxidation of the trimethyl ether to veratric acid.

The mass spectrum of isoauriculasin gave the molecular formula $C_{25}H_{24}O_6$. The spectrum differs from that of auriculasin most notably in that the peak due to the molecular ion is barely discernible and is followed by a strong $M^{+\cdot}$–68 peak indicative of the loss of a C_5H_8 fragment, a characteristic feature of the mass spectra of $\gamma\gamma$-dimethyl allyl ethers of phenols.[3] The presence of an O-allyl grouping was further substantiated by the NMR spectrum which showed the methylene protons as a doublet at 5·34τ. Isoauriculasin thus has the same spectral features as isoauriculatin. Since like the latter it failed to give a homogeneous acetate, the NMR spectrum did not distinguish between the linear and angular pyranoisoflavone structures. In order to establish the position of the side chain as well as to get conclusive evidence regarding the nature of the pyranoisoflavone nucleus, the 2′-monomethyl ether (**3a**) was subjected to allylic rearrangement under conditions employed by Murray *et al.*[4] This gave, after hydrolysis of the butyrate, an oily product which though homogeneous on TLC showed in its NMR spectrum contamination with traces of butyric acid.

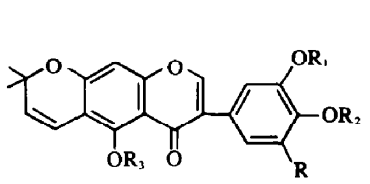
As required by structure **3b** for this compound the NMR spectrum showed the aromatic protons of ring B as a broad singlet at 2·94τ and the vinylic protons of the $\alpha\alpha$-dimethyl allyl group as quartet and multiplet of an ABX system. The meta relationship of the B ring protons in the rearranged product is clearer in the spectrum of the derived diacetate (**3c**) in which the broad singlet is resolved into two doublets at 3·06 and 2·97τ (J = 2 Hz). Comparison of the positions of the 3″,4″ protons of the chromene ring in **3b** and **3c** showed shifts of +0·21 and −0·19τ respectively thus confirming structure **3** for isoauriculasin.



1



2



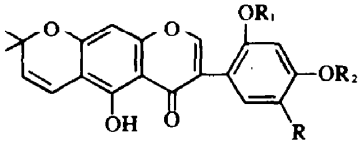
3: R = R_1 = R_3 = H, R_2 =

a: R = R_3 = H, R_1 = CH_3, R_2 =

b: R_1 = CH_3, R_2 = R_3 = H, R =

c: R_1 = CH_3, R_2 = R_3 = Ac, R =

Since Claisen rearrangement under the conditions employed gave fairly good yields, it was decided to apply it to isoauriculatin. The structure assigned initially to this compound was based on the evidence of the Gibb's test which is of a controversial nature. Rearrangement of isoauriculatin monomethyl ether **4a** gave a mixture of two components which on resolution by preparative TLC, afforded the major component as a crystalline product, m.p. 120°. The minor component, representing about 20% of the product ratio, could not be sufficiently purified for a m.p. The NMR spectrum of the product, m.p. 120°, showed three singlets of aromatic protons confirming the substitution pattern of ring B depicted in **4b**. Acetylation of this gave a homogeneous acetate though again in amounts insufficient for crystallisation. Comparison of the NMR spectra of **4b** and its acetate revealed unexpectedly shifts in the position of the chromene protons the direction and magnitude of which is not in accord with the angular structure assigned earlier, and this must therefore be replaced by **4**.



4: R = R_1 = H, R_2 =

a: R = H, R_1 = CH_3, R_2 =

b: R_2 = H, R_1 = CH_3, R =

EXPERIMENTAL

The m.ps were taken on a Kofler block and are uncorrected. NMR spectra were determined with HA-100 instrument in $CDCl_3$ with TMS as internal standard. Analyses were carried out by the Australian Microanalytical Service, Melbourne.

Isolation. The benzene extract of air dried *M. auriculata* leaves (2 kg) was passed through a silica gel column (400 g), using light petroleum, benzene, EtOAc and their mixtures for elution. Isoauriculasin along with traces of isoauriculatin was eluted by light petroleum-benzene, and purified by preparative TLC which gave isoauriculasin (75 mg). Auriculasin (8 g) was obtained from benzene and benzene–EtOAc fractions and purified by repeated crystallisations.

Auriculasin-1. Yellow needles (EtOH) m.p. 176–178°. (Found: C, 71·74; H, 5·74: $C_{25}H_{24}O_6$ requires: C, 71·41; H, 5·74%) M^+, *m/e* 420; λ_{max}^{MeOH} 225 (inf), 295 nm; ν_{max} (Nujol), 3420, 3250, 1650, 1620, 1385, 1370 cm^{-1}; NMR: 2·15 (1Hs, 2-H); −4·0 (1Hs, 5–OH); chromene ring (3·31, 1Hd, 4″–H, 4·42, 1Hd, 3″–H, J = 10 Hz; 8·54, 6Hs); side chain (8·33, 3Hs, 8·21, 3Hs, 6·63, 2Hd, 4·86, 1Hm); ArH (3·08–3·26, 3Hm).

Trimethyl auriculasin. Prepared by refluxing auriculasin (1g), freshly distilled Me_2SO_4 (3 ml), dry acetone (50 ml) and anhyd K_2CO_3 (2 g) for 60 hr. Crystallisation from EtOH afforded trimethyl auriculasin (0·8 g) m.p. 120–21°. (Found: C, 72·68; H, 6·57; $C_{28}H_{30}O_6$ requires; C, 72·71; H, 6·54%). NMR: 2·18 (1Hs, 2–H); chromene ring (3·28, 1Hd, 4″–H, 4·33, 1Hd, 3″–H, J = 10 Hz, 8·54, 6Hs); side chain (8·32, 3Hs, 8·18, 3Hs, 6·56, 2Hd, 4·82, 1Hm); (6·17, 6·15, 6·13, 3′,4′,5,–OCH_3).

Triacetyl auriculasin. Auriculasin (0·5 g), Ac_2O (20 ml) and fused AcONa (3 g) refluxed for 3 hr and worked up. Crystallisation from EtOH gave needles (0·55 g) m.p. 174–76°. (Found: C, 67·92; H, 5·46; $C_{31}H_{30}O_9$ requires: C, 68·12; H, 5·53%). NMR: 2·14 (1Hs, 2–H); chromene ring (3·52, 1Hd, 4″–H, 4·27, 1Hd, 3″–H, J = 10 Hz, 8·52, 6Hs); side chain (8·3, 3Hs, 8·10, 3Hs, 6·53, 2Hd, 5·0, 1Hm); (7·58, 3Hs, 7·74, 6Hs, 5,3′,4′–OAc).

Diphenyl methylenedioxy auriculasin. Auriculasin (100 mg) dichlorodiphenyl methane (0·05 ml) were heated on a metal bath at 185° for 5 min. The mixture in benzene was passed through a small column of silica gel and the product crystallised from EtOH, needles (80 mg), m.p. 180°. (Found: C, 77·56; H, 5·61. $C_{37}H_{32}O_6$ requires: C, 77·60; H, 5·63%).

$KMnO_4$ *oxidation of trimethyl auriculasin.* Auriculasin trimethyl ether (400 mg) in acetone (50 ml) was refluxed with a small amount of powdered $KMnO_4$. After decolourisation further small amounts of $KMnO_4$ were added till the colour persisted. The solvent was removed under reduced pressure, the residue taken up in boiling water, filtered and acidified. Repeated crystallisation from water afforded veratric acid (20 mg) m.p. 176–78°.

Isoauriculasin 3 crystallised from EtOH as colourless needles m.p. 134–35°; λ_{max}^{MeOH} 225 (inf), 295 nm; ν_{max}^{Nujol}, 3400, 3250, 1640, 1620, 1380, 1360 cm^{-1}; M^+, *m/e* 420 (2%), $C_{25}H_{24}O_6$; NMR: (60 mc); 2·14 (1Hs, 2–H); −2·37 (1Hs, 5–OH); chromene ring (3·22, 1Hd, 4″–H, 4·34, 1Hd, 3″–H, J = 10 Hz, 8·48, 6Hs); side chain (8·16, 6H broad singlet, 5·34, 2Hd, 4·46, 1Hm); ArH (3·6, 1Hs, 8–H, 2·84–2·97, 3Hm).

Allylic rearrangement of **3a**. Isoauriculasin (60 mg) was treated with CH_2N_2 to give the monomethyl eher (40 mg) m.p. 105°. A mixture of the monomethyl ether, butyric anhydride (0·5 ml) and N,N-diethylaniline (0·5 ml) was heated under N_2 on an oil bath for 8 hr while the temp was maintained at 190 ± 5°. The mixture was poured into water and worked up. The butyrate was taken up in EtOH and treated with 1% alc NaOH on a water bath for 1 min, neutralised with HCl, extracted with ether. TLC of the resulting oily product showed a single spot under UV which gave a positive ferric colouration. NMR: 2·15 (1Hs, 2–H); −4·0 (1Hs, 5–OH) chromene ring (3·35, 1Hd, 4″–H, 4·45, 1Hd, 3″–H, J = 10 Hz, 8·55, 6Hs), side chain (8·30, 6Hs, 5·0, 2Hm, 4·1, 1Hq, J = 10 and 16·5 Hz); ArH (3·7, 1Hs, 8–H, 2·94 broad singlet); 6·18 (3Hs, 3′–OMe).

Acetate of **3b** was prepared as in case of auriculasin. NMR: 2·23 (1Hs, 2–H); chromene ring (3·56, 1Hd, 4″–H, 4·26, 1Hd, 3″–H, J = 10 Hz, 8·59, 6Hs); side chain (8·5, 6Hs, 5·0, 2Hm, 4·03, 1Hq, J = 10 and 16·5 Hz); ArH (3·3, 1Hs, 8–H, 3·06, 1Hd, 2·97, 1Hd, J = 2 Hz); 6·21 (3Hs, 3′–OMe); 7·56 (6Hs, 5,4′–OAc).

Allylic rearrangement of **4a**. Monomethyl isoauriculatin (50 mg) was rearranged by the same procedure, crystallised from EtOH (15 mg) m.p. 120°, M^+, 434; NMR: (60 mc) 2·2 (1Hs, 2–H); −3·34 (1Hs, 5–OH) chromene ring (3·28, 1Hd, 4″–H, 4·40, 1Hd, 3″–H,

J = 10 Hz, 8·60, 6Hs); side chain (8·56, 6Hs, 5·04, 2Hm, 4·1, 1Hq); ArH (3·64, 1Hs, 8–H, 2·7, 1Hs, 6'–H, 3·38, 3'–H); 6·26 (3Hs, 2'–OMe).

Acetate of **4b**. NMR (60 mc) 2·24 (1Hs, 2–H); chromene ring (3·50, 1Hd, 4"–H, 4·26, 1Hd, 3"–H, J = 10 Hz, 8·6, 6Hs); side chain (8·56, 6Hs, 5·08, 2Hm, 4·04, 1Hq); 6·28 (3Hs, 2'–OMe); 7·60 (6Hs, 5,4'–OAc); ArH (3·43, 1Hs, 8–H, 3·30, 1Hs, 3'–H, 2·76, 1Hs, 6'–H).

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