Tetrahedron. Vol. 26, pp. 5041 to 5044 Pergamon Press 1970. Printed in Great Britain

STRUCTURE OF ISOAURICULATIN AND AURICULIN, EXTRACTIVES OF *MILLETIA AURICULATA*—II

M. Shabbir and A. Zaman*

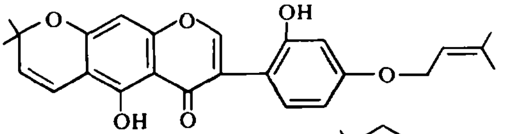
Department of Research in Unani Medicine, Tibbiya College and Department of Chemistry,
Aligarh Muslim University, Aligarh, India

(*Received in the UK* 18 *May* 1970; *Accepted for publication* 10 *June* 1970)

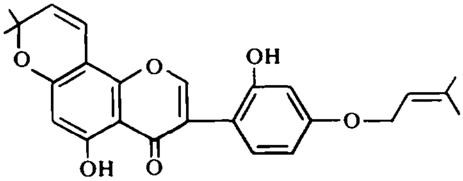
Abstract— Isoauriculatin and auriculin, the remaining isoflavones of *M. auriculata* have been shown to have structures II and VIII respectively.

In an earlier communication, isolation of sumatrol and auriculatin was reported.[1] Elemental analysis of isoauriculatin gave the molecular formula $C_{25}H_{24}O_6$, in agreement with the molecular ion peak at *m/e* 420 in its mass spectrum, showing it to be isomeric with auriculatin. The UV and IR spectra of isoauriculatin resemble those of auriculatin and show respectively a single maximum at 285 mμ (log ε 4·8) and IR bands at 1660 cm^{-1}, 1380 and 1360 cm^{-1} indicating the presence of a chromeno-isoflavone nucleus. Positive ferric colouration and formation of derivatives with diazomethane, dimethyl sulphate and ethyl iodide established the presence of two free hydroxyls of which one is located in the 5 position. Hydrogenation of isoauriculatin over Adam's catalyst gave a crystalline product m.p. 128–130° which was not identical with tetrahydro auriculatin m.p. 210°, obtained by similar reduction of auriculatin. This ruled out the possibility of isoauriculatin being a double bond isomer of auriculatin. Further confirmation of the identity of side chains in the two was provided by ozonolysis which again gave acetone as the sole cleavage product.

The NMR spectrum confirmed the chromeno-isoflavone nature of isoauriculatin by signals at τ 2·09 (1H), 8·52 (6H), 4·34 (1H), 3·1 (1H) (doublets, $J = 10$ c/s), and shows the presence of hydroxyls at 5 and 2′ by peaks at τ − 3 and 1·6 (1H). There is, however, a significant difference from the NMR spectrum of auriculatin in the position of signals for the allylic side chain which now shows a methylene group at τ 5·5 (2H, doublet) and an olefinic proton at τ 4·6 (1H, multiplet) whereas the signals for these protons in the NMR spectrum of auriculatin appear at τ 6·6 (2H, doublet) and τ 4·75 (1H, multiplet). These values are in agreement with the position of the methylene protons of an allylic side chain attached to oxygen as in durlettone.[2] Since spectroscopic evidence points to the presence of hydroxyls at both the 5 and 2′ positions the allylic side chain must be attached to the 4′ oxygen. In agreement with this, alkali degradation of diethyl isoauriculatin gave 4-hydroxy-2-ethoxy-phenylacetic acid with elimination of the allylic side chain. Treatment with alkali under milder conditions, afforded the deoxybenzoin (III) with an intact allyloxy group. Isoauriculatin can, thus be represented by either I or II.



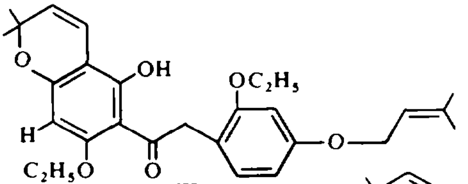
I



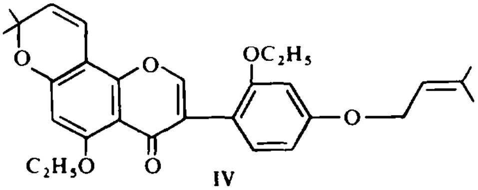
II

A choice between I and II could not be made by a comparison of the chemical shifts of the olefinic protons of the chromene ring in isoauriculatin and its acetate as in spite of repeated efforts a crystalline diacetate could not be obtained from isoauriculatin. A decision in favour of II was finally reached by the Gibb's test as modified by King *et al.*[3]

Isoauriculatin monomethyl ether showed the absence of a free position *para* to a hydroxyl by a negative Gibb's test but since negative tests are seldom reliable, the test was repeated with the deoxybenzoin (III) obtained from isoauriculatin diethyl ether (IV). A positive test indicated conclusively that isoauriculatin is represented by the formula II.



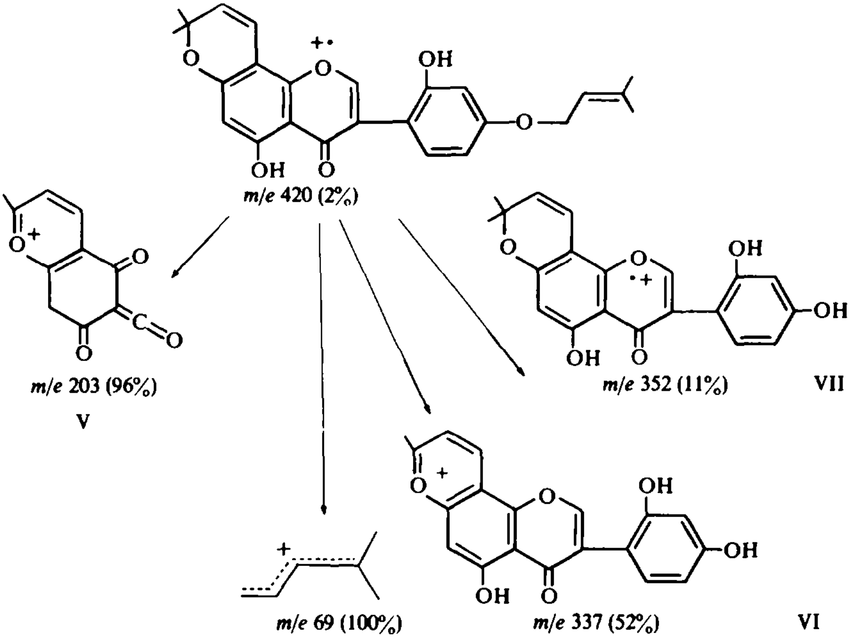
III

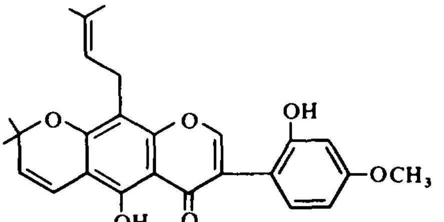


IV

The mass spectrum of isoauriculatin is consistent with the structure assigned to it. A major difference here from the spectrum of auriculatin is the low intensity of the parent peak, a normal feature of compounds having an allyloxy side chain.[4] Cleavage of the side chain gives rise to the base peak at *m/e* 69. Further prominent peaks occur at *m/e* 203 (96%), 337 (52%), 352 (11%) representing ions V, VI and VII respectively.

Auriculin, $C_{26}H_{26}O_6$, the third isoflavone constituent of the plant was shown to be 4-methyl auriculatin (VIII) by methylation with diazomethane to auriculatin dimethyl ether and oxidation of diethyl auriculin to 4-methoxy-2-ethoxybenzoic acid with alkaline H_2O_2.





VIII

EXPERIMENTAL

Isolation. The ether extract of the roots of *M. Auriculata* (1 kg) after separation of sumatrol, was chromatographed several times over silica gel. The benzene eluate gave mainly auriculatin while the light petroleum-benzene eluate furnished a mixture of two products as revealed by TLC. Separation of the mixture was effected by fractional crystallisation from EtOH when 100 mg of isoauriculatin crystallised out first while auriculin (50 mg) was obtained from the mother liquor.

Isoauriculatin (II). Isoauriculatin pale yellow plates m.p. 132–134° λ_{max}^{MeOH}222 mμ (infl). 285 mμ (log ε 4·8) (Found: C, 71·85; H, 5·82; $C_{25}H_{24}O_6$ requires: C, 71·4; H, 5·75%).

Monomethyl isoauriculatin. Isoauriculatin (200 mg) was methylated with diazomethane to give pale yellow needles m.p. 157–158° (150 mg). λ_{max}^{MeOH} 223 mμ (infl), 280 mμ (log ε 4·54). (Found: C, 71·79; H, 6·06; OMe, 7·67; $C_{26}H_{26}O_6$ requires: C, 71·87; H, 6·03; OMe, 7·14%).

Dimethyl isoauriculatin. Isoauriculatin (400 mg), dimethyl sulphate (2 ml), dry acetone (50 ml) and anhyd K_2CO_3 (1 gm), were heated under reflux for 48 hr. The mixture was filtered and the filtrate evaporated to dryness. The residue crystallised from MeOH as colourless needles m.p. 192–194° (300 mg) (Found: C, 71·97; H, 6·26; OMe, 13·68; $C_{27}H_{28}O_6$ requires: C, 72·3; H, 6·29; Ome, 13·84%).

Diethyl isoauriculatin (IV). Isoauriculatin (500 mg) was refluxed with EtI (6 ml) in dry acetone (80 ml) over anhyd K_2CO_3 (1·5 g) till it gave no ferric colouration and was worked up to give 300 mg of isoauriculatin diethyl ether from MeOH as colourless needles m.p. 158–160°. (Found: C, 72·67; H, 6·75; OEt, 17·5; $C_{29}H_{32}O_6$ requires: C, 73·09; H, 6·77; OEt, 19·2%).

Deoxybenzoin (III) *from diethyl isoauriculatin.* Diethyl isoauriculatin (200 mg) was refluxed for 3 hr with 5% ethanolic KOH. The soln was then cooled, diluted with water, acidified and extracted with ether. The residue on evaporation of the solvent crystallised from MeOH as pale yellow needles (100 mg), m.p. 75°. (Found: C, 71·5; H, 7·44; $C_{28}H_{34}O_6$ requires: C, 72·08; H, 7·35%).

Hydrogenation of isoauriculatin. Isoauriculatin (200 mg) was hydrogenated for 6 hr over Adam's catalyst. The product crystallised from EtOH as pale yellow plates m.p. 128–130° (150 mg); λ_{max}^{MeOH} 263 mμ (log ε 4·55). (Found: C, 69·98; H, 6·44; $C_{25}H_{28}O_6$ requires: C, 70·74; H, 6·65%).

Ozonolysis of isoauriculatin. A soln of isoauriculatin (250 mg) in glacial AcOH (20 ml) was cooled and a stream of O_3 was passed for 2 hr. The ozonide was decomposed by addition of water and Zn dust. Acetone, formed in the reaction, was steam distilled and identified as its 2,4-dinitrophenyl hydrazone.

Alkali cleavage of diethyl isoauriculatin. Diethyl isoauriculatin (1 g) was dissolved in abs EtOH (40 ml) containing KOH (12 gm) and the soln was refluxed under N_2 for 48 hr, cooled, diluted with water, acidified and extracted with ether. The ether extract was washed with $NaHCO_3$ aq. The washings were worked up in the usual way to give 4-dihydroxy-2-ethoxyphenylacetic acid, m.p. 125°. (Found: C, 61·19; H, 6·18; calc for $C_{10}H_{12}O_4$: C, 61·21; H, 6·17%). The acid was methylated with diazomethane and hydrolysed to give 4-methoxy-2-ethoxyphenylacetic acid m.p. 112°, identical with a synthetic sample by mixed m.p. and superimposable IR spectra.

Auriculin (VIII). Yellow needles m.p. 124–125°; λ_{max}^{MeOH} 222 mμ (inf), 290 mμ (log ε 4·83). (Found: C, 71·96; H, 6·12; OMe, 6·37; $C_{26}H_{26}O_6$ requires: C, 71·87; H, 6·03; OMe 7·14%); M + 434; NMR in $CDCl_3$ (varian A-60 spectrometer) τ 2·01 singlet (2-H), τ −2·5 and 1·48 (5 and 2′ hydroxyls); τ 6·21 (3H) (methoxyl group); τ 8·55 (6H, gem dimethyl); τ 4·35 (1H) (doublets, olefinic $J = 10$ c/s); τ 8·32 (3H), τ 8·2 (3H), τ 6·5 (2H, doublet), τ 4·7 (1H triplet) for γγ dimethyl allyl group.

Dimethyl auriculin. Auriculin (60 mg) was methylated with ethereal diazomethane. The product crystallised from EtOH as pale yellow plates m.p. 162–163° (30 mg); λ_{max}^{MeOH} 224 mμ (inf), 285 mμ (log ε 4·62). (Found: C, 72·13; H, 6·23; OMe 13·9; $C_{27}H_{28}O_6$ requires: C, 72·3; H, 6·29; OMe, 13·84%). The mixed m.p. with dimethyl auriculatin was found to be undepressed and IR spectra were superimposable.

Alkaline H_2O_2 *oxidation of diethyl auriculin.* Auriculin (500 mg) was refluxed with EtI in dry acetone (100 ml) over K_2CO_3 (2 gm) until it gave no colour with $FeCl_3$. The product obtained was directly oxidised with an alkaline H_2O_2 as adopted by Wolform *et al.*[5]. On work up it gave from light petroleum-benzene 4-methoxy-2-ethoxybenzoic acid (30 mg) m.p. 113° (lit.[6] 114–115°). Mixed m.p. was undepressed with a synthetic specimen.

Acknowledgement—The authors wish to thank the Council of Scientific and Industrial Research (India) for the award of a Senior Research Fellowship (to M.S.).

REFERENCES

[1] M. Shabbir, A. Zaman, L. Crombie, B. Tuck and D. A. Whiting, *J. Chem. Soc.* (C), 1899 (1968)

[2] W. D. Ollis, C. A. Rhodes and I. O. Sutherland, *Tetrahedron* **23**, 4741 (1967)

[3] F. E. King, T. J. King and L. C. Manning, *J. Chem. Soc.* 563 (1957)

[4] E. Ritchie, W. C. Taylor and J. S. Shanon, *Tetrahedron Letters* No. 23, 1437 (1964)

[5] M. L. Wolform and A. S. Gregory *J. Am. Chem. Soc.* **62**, 651 (1940)

[6] F. E. King and K. G. Neill *J. Chem. Soc.* 4752 (1952)