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AURICULOSIDE, A NEW FLAVAN GLYCOSIDE FROM *ACACIA AURICULIFORMIS**

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Key Word Index—*Acacia auriculiformis*; Leguminosae; auriculoside; 7,3',5'-trihydroxy-4'-methoxyflavan 3'-glucoside; α-spinasterol.

Abstract—The structural elucidation of auriculoside, a new flavan glucoside from *Acacia auriculiformis*, is described. This is the third report of a flavan glycoside unsubstituted in the heterocyclic ring.

INTRODUCTION

Acacia auriculiformis A. Cunn. (Leguminosae) was found to have CNS depressant activity [1] (barbiturate potentiation test in mice), which was found to be in the BuOH fraction of the plant extract. This prompted a detailed investigation, resulting in the isolation of the active constituent, a flavan glycoside named auriculoside (**I**). We believe this to be the third report [2, 3] of a flavan glycoside lacking oxygen substitution in the heterocyclic ring and the first report of a flavan from the Leguminosae. Apart from these three glycosides, all other flavans unsubstituted in the pyran ring have been found in the Liliaceae [4]. These include a racemic 5,7,4'-trimethoxyflavan from the methylated, resinous exudate of Xanthorrhoea preissii [5] and the first reported optically-active flavans, (−)-4'-hydroxy-7-methoxyflavan and its 8-methyl analogue, which were isolated from *Stypandra grandis* and *Dianella revoluta* [6], respectively. A 4'-methoxy flavan has also been identified from the scent glands of the Canadian beaver [7] (castor fiber).

The heartwood of *Acacia auriculiformis* has been shown to contain a number of different constituents: three isomeric flavan-3,4-diols ((−)-teracacidin, (−)-isoteracacidin and its (+)-2,3-*trans*-3,4-*cis* isomer) and a dihydroflavonol, flavanone, flavonol and chalcone based on 7,8,4'-trihydroxylation. Polymeric polyphenols consisting of prodelphinidins and procyanidins were found mainly in the bark [8].

RESULTS AND DISCUSSION

The alcoholic plant extract was successively partitioned with hexane, $CHCl_3$, *n*-BuOH and H_2O. The hexane fraction was repeatedly chromatographed over Si gel and alumina to yield substance A, $C_{29}H_{48}O$, M^+412, which yielded a monoacetate, mp 185°, and was identified as α-spinasterol.

The BuOH fraction showed CNS depressant activity and was further resolved into EtOAc-soluble and EtOAc-insoluble fractions. The EtOAc-soluble portion, in which the activity was confirmed, contained one major constituent, auriculoside (**I**). **I**, $C_{22}H_{26}O_{10}$, exhibited 80% CNS depressant activity and gave positive reactions with $FeCl_3$ and Fiegel's test, suggesting that it is a phenolic glycoside. The UV maxima of auriculoside at 221, 278 and 286 (sh) nm suggested an unconjugated aromatic system [6]. The absence of a carbonyl group in the IR and the presence of two methylene multiplets centred at δ 1.96 and 2.6 assignable to C-3 and C-4, respectively, six glycosyl protons (δ 3.0–3.65), and the overlapping signals of a anomeric and a methine proton of the benzyl ether system (δ 4.60–5.0) in the ^{1}H NMR spectrum clearly established that it is a flavan glycoside [9]. Auriculoside formed a dimethyl ether on selective methylation and a hexaacetyl derivative. The ^{1}H NMR study indicated that the aromatic region integrated for five protons along with a phenoxymethyl group and the substitution pattern was evident by the presence of two *ortho*-coupled protons (H-5 and H-6) at δ 6.75 and 6.2 (J = 8 Hz), respectively. The latter proton was further *meta*-coupled (J = 2.5 Hz) with H-8 at δ 6.13 which itself appeared as a singlet [10]; two *meta*-coupled proton doublets at δ 6.57 and 6.50 (J = 1.5 Hz) are assignable to H-2' and H-6'.

Acid hydrolysis of auriculoside gave an aglycone (auriculin, **IV**) and glucose. The ^{1}H NMR of auriculin exhibited a singlet due to φ-OMe and 3,4-CH_2 protons of the heterocyclic ring, but the methine proton of the benzyl ether system appeared as a quartet at σ 4.71 (J = 8, 4 Hz), indicating the disposition of an aromatic B-ring at C-2 as α (*eq*). The remaining five aromatic protons appeared in conformity with the assignments already indicated above except that the H-8 now appeared as a doublet (δ 6.23, J = 2 Hz) and the H-2' and H-6' protons appeared as a singlet at δ 6.31.

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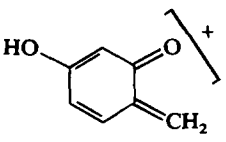
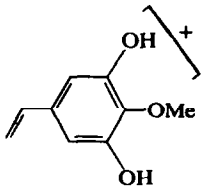
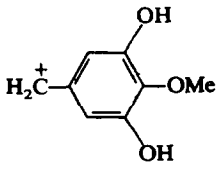
In its NMR spectrum, the TMS derivative of auriculin showed two multiplets centred at δ 2.01 and 2,65 assignable to methylenes at C-3 and C-4, and a quartet at δ 4.76 ($J = 8$, 4 Hz) due to the methine of the benzyl ether system which were confirmed by DNMR experiments. This established that the heterocyclic ring was unsubstituted. The MS of auriculin was also consistent with that reported for flavans with an unsubstituted heterocyclic ring [11]. The fragment ions *m/e* 123 and 166 arose by retro-Diels–Alder cleavage and *m/e* 153 due to retro-Diels–Alder with H transfer. The fragment ion *m/e* 151 resulted by the loss of Me from the *m/e* 166 ion.



m/e 123

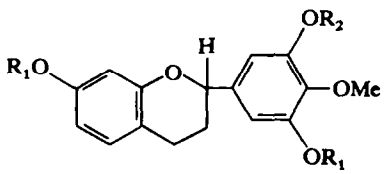


m/e 166



m/e 153

Auriculoside gave a hexaacetate with two phenolic acetoxymethyl signals at δ 2.25 and 2.3 and showed acetylation-induced downfield shifts of the doublets of H-8 and H-2′ to δ 6.83 and 7.0 ($J = 2$ Hz), respectively. Similarly, on acetylation, auriculin yielded a triacetyl derivative whose ^{1}H NMR indicated three acetoxymethyls at δ 2.16 (C-7) and 2.23 (C-3′,5′) and the H-8 and H-2′,6′ protons were shifted downfield to δ 6.5 and 6.9, respectively. The presence of two phenolic OH groups and the attachment of a glucosyl



I $R_1 = H$; $R_2 = Glc$
II $R_1 = Ac$; $R_2 = Glc(OAc)_4$
III $R_1 = Me$; $R_2 = Glc$
IV $R_1 = R_2 = H$
V $R_1 = R_2 = Ac$

residue to a third phenolic OH in auriculoside was thus evident and the acetate-induced shifts fixed the glucoside linkage at C-3′ and a phenolic OH at C-5′. That the signal of H-2′,6′ appeared as a singlet in the ^{1}H NMR of auriculin and its derivatives lends further support to the placement of OMe at C-4′.

The absence of substitution in the auriculoside heterocyclic ring was also confirmed by ^{13}C NMR signals at 24.9 (t, $J_{C\text{-}3/H\text{-}3} = 128.7$ Hz) and 30.9 (t, $J_{C\text{-}4/H\text{-}4} = 128.2$ Hz) due to C-3 and C-4, respectively. In addition, the ^{13}C NMR showed that the C-3′ signal of **I** at 152.0 ppm was shifted upfield [12] by 0.4 ppm in **IV**, and that the C-2′ signal (109.1 ppm) of **I** appeared 2.5 ppm upfield in **IV** thus suggesting that the glucose was attached at C-3′.

Auriculoside was easily hydrolysed with emulsin in acetate buffer (pH 5.5) which confirmed a β-glucosidic linkage in the molecule. Auriculoside is, therefore, 7,3′,5′-trihydroxy-4′-methoxyflavan 3′-*O*-β-D-glucopyranoside (**I**).

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded on 60 and 90 MHz instruments in $CDCl_3$ (TMS as int. standard), unless otherwise specified. Si gel was used for TLC and spots were visualized either with I_2 vapour or diazotized sulphanilic acid. The plant material was collected by Dr. K. K. Singh of this Institute from Lakhimpur-Kheri, U.P., India and a voucher specimen is preserved in herbarium of CDRI, Lucknow.

Isolation of constituents. The powdered aerial part of the plant (1.9 kg) was extracted with 90% EtOH and concd to a

Table 1. ^{13}C NMR of auriculoside (**I**) and auriculin (**IV**)*

Substance	C-2	C-3	C-4	C-5	C-6	C-7†	C-8	C-9†
I	78.6	24.9	30.9	130.9	109.1	156.8	104.0	157.5
IV	78.7	25.1	31.3	130.9	109.1	156.9	104.0	157.5

Substance	C-10	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	-OMe
	114.3	137.7	109.1	152.0	139.9	151.7	106.6	61.5
IV	114.3	136.1	106.6	151.6	139.4	151.6	106.6	60.8

Substance	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
I	102.4	74.9	78.1	71.8	78.6	62.3

* Chemical shifts are given in ppm solvent (CD_3OD).
† The assignments could be reversed. C-1″–C-6″ represent glucosyl carbons.

green semi-solid (215 g), diluted with H_2O and repeatedly partitioned to give hexane (22.5 g), $CHCl_3$ (22.5 g) and *n*-BuOH (70 g) fractions. The hexane fraction was chromatographed on Si gel using C_6H_6 with increasing amounts of MeOH, and eluates containing 2–5% MeOH were combined together. The residue was rechromatographed on Al_2O_3 with $CHCl_3$–EtOAc as eluant to give α-spinasterol as colourless needles, mp 165° (hexane–$CHCl_3$).

The BuOH-soluble residue (70 g), dissolved in EtOH, was partially pptd with Et_2O repeatedly to give a brown solid (30 g), which was macerated with warm EtOAc to give a soluble fraction (12.4 g) which showed a major spot (auriculoside, **I**) with R_f 0.4 in $CHCl_3$–MeOH–H_2O (35:8:2). After further chromatography and decolourization of the relevant eluant fraction with charcoal, **I** crystallized from dil EtOH as colourless crystals, mp 140° (218 mg).

α-Spinasterol. Mp 165°, $[\alpha]_D$ +2° (*c* 1, $CHCl_3$). IR(KBr) cm^{-1}: 3380, 2930, 1640, 1440, 1365, 1080, 965, 840. ^{1}H NMR: δ 0.53 (3H, *s*, Me), 0.75–1.17 (18H, 6×Me), 3.68 (1H, *m*, –CHOH), 5.10 (3H, *m*, vinyl H). MS *m/e*: 412 (M^+), 397, 369, 314, 300, 271, 255, 231, 213. The acetate cryst. from MeOH, mp 185°, $[\alpha]_D$ –5.7° (*c* 1, $CHCl_3$). ^{1}H NMR: δ 2.03 (3H, *s*, –OAc), 4.7 (1H, *m*, –CHOAc).

Auriculoside (**I**). Mp 140°, $C_{22}H_{26}O_{10}$, $[\alpha]_D$ –77.0° (*c* 1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 221 (5.67), 278 (4.53), 286 (sh) (4.42). ^{1}H NMR(DMSO-d_6): δ 1.96 (2H, *m*, C-3 H_2), 2.6 (2H, *m*, C-4 H_2), 3.0–3.65 (6H, *m*, glucose 2,3,4,5,6-H), 3.67 (3H, *s*, OMe), 4.60–5.0 (2H, *m*, anomeric H of glucose, H-2), 6.13 (1H, *s*, H-8), 6.20 (1H, *dd*, *J* = 8, 2.5 Hz, H-6), 6.5 (1H, *d*, *J* = 1.5 Hz, H-6'), 6.57 (1H, *d*, *J* = 1.5 Hz, H-2'), 6.75 (1H, *d*, *J* = 8 Hz, H-5). (Found: C, 58.72; H, 5.65. $C_{22}H_{26}O_{10}$ requires: C, 58.66; H, 5.77%.)

Auriculoside hexaacetate (**II**). **I**, on treatment with Ac_2O–Py, gave a hexaacetyl derivative as a viscous mass. ^{1}H NMR: δ 1.95, 2.05 (3H each, *s*, glucose OAc×2), 2.0 (6H, *s*, glucose OAc×2), 1.92–2.10 (2H, *m*, C-3 H_2), 2.25, 2.30 (3H each, *s*, C-3', 7-OCOMe), 2.8 (2H, *m*, C-4 H_2), 3.5 (1H, *m*, glucose 5-H), 3.78 (3H, *s*, OMe), 3.86–4.36 (3H, *m*, glucose 2,6-H), 4.76–5.5 (4H, *m*, H-2, glucose 1,3,4-H), 6.58 (1-H, *dd*, *J* ≈ 8.5, 2.0 Hz, H-6), 6.67 (1H, *d*, *J* = 2 Hz, H-2'), 6.83 (1H, *d*, *J* = 2 Hz, H-8), 7.01 (1H, *d*, *J* = 2 Hz, H-6'), 7.04 (1H, *d*, *J* = 8.5 Hz, H-5).

Auriculoside dimethyl ether (**III**). **I** (50 mg) in dry Me_2CO (30 ml) was stirred with MeI (4 mol) and dry K_2CO_3 (250 mg) for 6 hr in N_2 at room temp. The reaction mixture was worked up and the product purified by Si gel chromatography to give a dimethyl ether (20 mg) as an amorphous powder. ^{1}H NMR: δ 2.0 (2H, *m* C-3 H_2), 2.70 (2H, *m*, C-4 H_2), 3.1–4.0 (6H, *m*, glucose 2,3,4,5,6-H), 3.64 (3H, *s*, OMe), 3.73 (6H, *s*, 2× OMe), 4.75 (2H, *m*, aromeric H of glucose, H-2), 6.35 (2H, H-6,8), 6.4, 6.46 (1H each, *d*, *J* = 1.5 Hz, H-2',6'), 6.83 (1H, *d*, *J* = 9 Hz, H-5).

Acid hydrolysis. **I** (150 mg) was refluxed in dil alcholic HCl (4%, 15 ml) for 2 hr in N_2, neutralized with Ag_2CO_3 and the reaction mixture extracted with EtOAc. Glucose was identified from the aq. phase (co-PC in BAW). The aglycone **IV** was purified on a Si gel column and cryst. from $CHCl_3$–MeOH as needles, mp 210°. λ_{max}^{MeOH} nm (log ε): 222 (5.68), 278 (5.08), 286 (sh) (4.91). $[\alpha]_D$ –9.23° (*c* 1.3, MeOH). ^{1}H NMR(DMSO-d_6): δ 1.91 (2H, *m*, C-3 H_2), 2.7 (2H, *m*, C-4H_2), 3.71 (3H, *s*, OMe), 4.71 (1H, *q*, *J* = 8,4 Hz H-2), 6.23 (1H, *dd*, *J* = 9, 2 Hz, H-6), 6.23 (1H, *s*, H-8), 6.31 (2H, *s*, H-2', 6'), 6.7 (1H, *d*, *J* = 9 Hz, H-5). MS (rel.int.): *m/e*: 288 (M^+, 95.2), 255 (16.6), 178 (100), 166 (64.6), 153 (35.5), 151 (83.5), 147 (34.8), 131 (17.5), 128 (17.3), 123 (60.9), 97 (21.9).

Auriculin triacetate (**V**). Auriculin (**IV**) on acetylation with Ac_2O–Py gave a triacetate as viscous oil. ^{1}H NMR: δ 2.16 (3H, *s*, C-7 OAc), 2.23 (6H, *s*, C-3',5' 2×OAc), 1.9–2.15 (2H, *m*, C-3 H_2), 2.74 (2H, *m*, C-4 H_2), 3.7 (3H, *s*, OMe), 4.87 (1H, *q*, *J* = 10, 3 Hz, H-2), 6.44 (1H, *dd*, *J* = 8, 2 Hz, H-6), 6.5 (1H, *s*, H-8), 6.9 (2H, *s*, H-2',6'), 6.92 (1H, *d*, *J* = 8 Hz, H-5). MS *m/e*: 414 (M^+), 372, 354, 330, 299, 288, 270, 255, 170, 166, 153, 151, 123, 97.

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