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PRUNIN-6″-*O*-*p*-COUMARATE, A NEW ACYLATED FLAVANONE GLYCOSIDE FROM *ANACARDIUM OCCIDENTALE*

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Key Word Index—*Anacardium occidentale*, Anacardiaceae; prunin-6″-*O*-*p*-coumarate, ^{13}C-NMR spectroscopy; acylated flavanone glycoside.

From the acetone extract of the defatted nut shells of *Anacardium occidentale* L. two phenolic compounds AO-1 and AO-2 could be isolated. Whereas AO-1 was shown to be identical with naringenin, the latter was a hitherto unknown glycoside mp 153–154°. Hydrolysis of AO-2 with 5% ethanolic HCl yielded naringenin, glucose and *p*-coumaric acid as hydrolysis products. Lack of characteristic shift of the UV maximum at 285 nm upon addition of NaOAc indicated the attachment of glucose to the C_7-OH group. After alkaline methanolysis naringenin-7-*O*-*β*-D-glucoside (prunin) and methyl *p*-coumarate were identified by TLC comparison with authentic samples. This showed that AO-2 was a *p*-coumarate of prunin as was also indicated by the presence of two carbonyl absorptions in the IR spectrum at 1675 and 1625 cm^{-1}. The peak at *m/e* 365 in the mass spectrum of the permethylation product of AO-2, corresponding to the tetra-*O*-methyl ether of the coumaryl-glucosyl ion, clearly demonstrated that the acyl residue was on the sugar moiety. The M^+ peak at *m/e* 678 and other fragments in the mass spectrum were in agreement with the formulation of the methylation product as prunin chalcone *p*-coumarate heptamethyl ether. A similar observation [1] has been made in the permethylation of the naturally occurring naringenin-7-*O*-(6″-*O*-galloyl)-*β*-D-glucoside. The ^{1}H-NMR spectra of AO-2 and its acetate were in agreement with the structure of naringenin-7-*O*-(coumaryl)-*β*-D-glucopyranoside.

The position of the *p*-coumaryl residue on the sugar was unequivocally determined by ^{13}C-NMR spectroscopy. The signals for the sugar carbon atoms $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$ of AO-2 appeared in the region 76.1 ppm to 63.3 ppm. Compared with the corresponding carbon resonances in the spectrum of prunin, the $C_{6''}$ signal in AO-2 was 2.7 ppm downfield and the $C_{5''}$ signal 3.3 ppm upfield. The assignment for $C_{6''}$ was confirmed by taking the off-resonance spectrum. Such changes in the chemical shifts of $C_{6''}$ and $C_{5''}$ can only be explained [2] if the primary hydroxyl group at $C_{6''}$ is esterified. This thus establishes the structure of AO-2 as naringenin-7-*O*-(6″-*O*-*p*-coumaryl)-*β*-D-glucoside.

EXPERIMENTAL

Mps are uncorr. The ^{1}H-NMR spectra were recorded on a Varian A-60A instrument; the ^{13}C-NMR spectra were recorded on a Jeol FX-100 NMR spectrometer. TLC was performed on Si gel plates with (A) C_6H_6–Py–HCO_2H (36:9:5); (B) Tol–EtOAc (2:1); (C) EtOAc–MeOH–H_2O (100:16.5:13.5); (D) $CHCl_3$–MeOH (3:1). Authentic naringenin-7-*O*-*β*-D-glucoside was prepared by partial hydrolysis of naringin [3].

Isolation of AO-1 and AO-2. The nut shells of *Anacardium occidentale* (1.5 kg), procured from Travancore, Kerala, India, were crushed and defatted with petrol. Subsequently they were extracted with boiling Me_2CO and the combined Me_2CO extracts concd under diminished pressure. The concentrate was successively digested with petrol, C_6H_6 and EtOAc The EtOAc soln was filtered and evapd to a brown mass (10 g). This residue gave the usual colour reactions for flavonoids and

was subjected to initial fractionation on a Si gel column. The flavonoid containing fraction (2.0 g) was eluted by 30% EtOAc–C_6H_6. TLC indicated the presence of two components R_f 0.7 (AO-1) and R_f 0.11 (AO-2) (Solvent A). Column chromatographic separation of the two components was effected on Si gel using EtOAc–C_6H_6 (10 and 20%) as eluent.

AO-1 (*naringenin*). Yield 0.5 g, pale yellow needles (EtOAc–C_6H_6), mp 255–257°. It was found to be identical with naringenin.

AO-2 (*naringenin-7-O-(6″-O-p-coumarryl)-β-D-glucoside*). Yield 0.5 g, pale yellow cubes, mp 153–154°. (EtOAc–C_6H_6) UV (MeOH) 212, 226, 285, 314 nm; (MeOH + NaOAc) 285, 320, 360 (*sh*) nm; (MeOH + NaOMe) 240 (*sh*), 288, 364 nm. IR (KBr): ν cm^{-1} = 3300 (OH), 1675 (CO_2R), 1625 (C=O), 815 (Ar) NMR: (^{1}H, DMSO-d_6 + TFA-d, TMS int.) δ = 7.59 ppm (*d*, 1H, *J* = 16 Hz, H-β), 7.55 (*d*, 2 H, *J* = 9 Hz, H-2‴, H-6‴), 7.35 (*d*, 2 H, *J* = 8.5 Hz, H-2′, H-6′), 6.83 (*d*, 4 H, *J* = 9 Hz, H-3′, H-3‴, H-5′, H-5‴), 6.63 (*d*, 1 H, *J* = 16 Hz, H-α), 6.22 (*s*, *br*, 2H, H-6, H-8) 5.50 (*d*, 1 H, *J* = 12 Hz, H-2), 5.13 (*d*, *br*, 1 H, *J* = 6 Hz, H-1″), 4.31 (*m*, 2 H, H-6″, H-6‴), 2.70–4.15 (*m*, 6 H, H-3, H-3, H-2″, H-3″, H-4″, H-5″). NMR: (^{13}C, DMSO-d_6, TMS int.) δ = 197.2 ppm (C-4), 166.4 (C-9‴), 165.0 (C-7), 163.0 (C-5), 162.6 (C-9), 159.8 (C-4‴), 157.7 (C-4′), 144.9 (C-8‴), 130.3 (C-2‴, C-6‴), 128.6 (C-1′), 128.4 (C-2′, C-6′), 125.0 (C-1‴), 115.7 (C-3‴, C-5‴), 115.1 (C-3′, C-5′), 113.9 (C-7‴), 103.3 (C-10), 99.2 (C-1″), 96.3 (C-6), 95.5 (C-8), 78.6 (C-2), 76.1 (C-3″), 73.8 (C-5″), 72.9 (C-2″), 69.8 (C-4″), 63.3 (C-6″), 42.0 (C-3).

Prunin-chalcone-6″-p-coumarate-PME. AO-2 (2 mg) was permethylated using NaH/MeI in DMF and worked up as usual [4]. MS $C_{37}H_{42}O_{12}$ (678.72) *m/e* 678 M$^+$ (21% rel. int.) 650 (12), 517 (8), 432 (17), 365 (84), 364 (58), 314 (100), 315 (36), 299 (71), 286 (90), 187 (54), 178 (77), 161 (500), 155 (85), 153 (92), 141 (65), 134 (130), 133 (92), 121 (86), 120 (45), 111 (30), 101 (110), 91 (61), 89 (65), 71 (95), 45 (78).

Hexa-acetate of AO-2. The acetylation was carried out with Py–Ac_2O for *ca* 18 hr at room temp. and worked up as usual and crystallized from $CHCl_3$, mp 115°. NMR (^{1}H, $CDCl_3$, TMS int.) δ = 7.72 (*d*, 1 H, *J* = 16 Hz, H-β), 7.57 (*d*, 2 H, *J* = 8.5 Hz, H-2‴, H-6‴), 7.46 (*d*, 2 H, *J* = 9 Hz, H-2′, H-6′), 7.19 (*d*, 4 H, *J* = 9 Hz, H-3′, H-5′, H-3‴, H-5‴), 6.60 (*d*, 1 H, *J* = 2.5 Hz, H-8), 6.43 (*d*, 1 H, *J* = 2.5 Hz, H-6), 6.41 (*d*, 1 H, *J* = 16 Hz, H-α), 5.36 (*q*, 2 H, *J* = 5 Hz and 11 Hz, H-2), 5.33 (*m*, 4 H, H-1″, H-2″, H-3″, H-4″), 4.41 (*m*, 2 H, H-6″, H-6‴), 4.08 (*m*, 1 H, H-5″), 2.57–3.25 (*m*, 2 H, H-3, H-3), 2.33 (*s*, 9 H, OAc-4′, 4‴, 5), 2.06 (*s*, 9 H, OAc-2″, 3″, 4″). MS: *m/e* 832 M$^+$ (rel. int. 1%), 790 (1), 748 (2), 477 (15), 435 (8), 356 (3), 331 (11), 315 (10), 314 (10), 272 (17), 271 (19), 229 (8), 189 (100), 169 (70), 164 (31), 147 (97), 127 (32), 120 (47), 109 (75), 95 (34), 70 (42), 43 (98).

Naringenin-7-O-β-D-glucoside (*prunin*). (^{13}C-NMR, DMSO-d_6, TMS int.) 197.2 ppm (C-4), 165.2 (C-7), 162.9 (C-5), 162.8 (C-9), 157.8 (C-4′), 128.8 (C-1′), 128.5 (C-2′, C-6′), 115.2 (C-3′, C-5′), 10.3.3 (C-10), 99.5 (C-1″), 96.5 (C-6), 95.4 (C-8), 78.7 (C-2), 77.1 (C-5″), 76.3 (C-3″), 73.1 (C-2″), 69.5 (C-4″), 60.6 (C-6″), 42.0 (C-3).

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AURMILLONE, A NEW ISOFLAVONE FROM THE SEEDS OF *MILLETTIA AURICULATA*

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Key Word Index—*Millettia auriculata*; Leguminosae; auriculatin; sumatrol; auriculasin; new isoflavone; aurmillone.

Past work on roots of *Millettia anriculata* has yielded auriculatin, sumatrol [1], auriculin, isoauriculatin [2], while the leaves contain auriculasin, isoauriculasin and isoauriculatin [3]. We now report the structure determination of a new isoflavone, aurmillone (**1**), isolated from the seeds of *Millettia auriculata* (supplied by the United Chemical and Allied Products, Calcutta) along with auriculatin, sumatrol and auriculasin.

Aurmillone (**1**) mp 157–158° was analysed for $C_{21}H_{20}O_6$ and M$^+$ 368. Its phenolic nature is indicated by its solubility in alkali and green ferric colour. Its UV data (λ_{max}^{MeOH} nm (log ε) 268 (4.56), 332 (3.90)) [4], its IR data (ν $C{=}O_{CHCl_3}^{max}$ 1650 cm^{-1}) and low field singlet at 8.52 δ in its PMR spectrum (recorded in DMSO-d_6) is indicative of its isoflavone nature [4]. Further the UV spectral shifts—bathochromic shift of 268 nm band by 10 nm and 14 nm upon addition of $AlCl_3$–HCl and NaOAc respectively, suggests the presence of 5,7-dihydroxyisoflavone skeleton [4]. Aurmillone (**1**) formed a diacetate (**2**) mp 84–85°, $C_{25}H_{24}O_8$ and M$^+$ 452 (PMR (60 MHz, $CDCl_3$) two OCO$\underline{CH_3}$ at 2.32 δ 3H, *s*; 2.35 δ, 3H, *s*) on treatment with AC_2O–Py and a dimethyl ether (**3**), mp 124–126°, $C_{23}H_{24}O_6$ and M$^+$ 396, on refluxing for 48 hr with $Me_2SO_4|K_2CO_3|Me_2CO$. Aurmillone (**1**) formed a monomethyl ether (**4**) mp 124°, $C_{22}H_{22}O_6$ and M$^+$ 382 (PMR ($CDCl_3$) two O$\underline{CH_3}$ at 3.91 δ, 3H, *s*; 3.88 δ, 3H, *s*) on treatment with CH_2N_2. The monomethyl ether (**4**) exhibits UV data λ_{max}^{MeOH} 265 nm (log ε 4.52) and it underwent bathochromic shift by 10 nm upon addition of $AlCl_3$–HCl and gave green ferric colour. Therefore, it is concluded that the C_5 hydroxyl is not methylated. Thus in aurmillone (**1**), the presence of two hydroxyls one of which is chelated is confirmed.

The PMR spectrum of aurmillone (**1**) revealed a set of peaks (4.55 δ, 2H, *d*, *J* = 7 Hz, —O—$\underline{CH_2}$—; 5.55 δ, 1 H, *m*, =C$\underline{H}$—, and 1.78 δ, 6H, *br s*, =C($\underline{CH_3}$)$_2$) characteristic of *O*-3-methylbut-2-enyl group [2, 5]. The spectrum also revealed four aromatic protons constituting