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Proanthocyanidins from *Prunus armeniaca* roots

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Abstract

Three isomeric A-type proanthocyanidins have been isolated from the root of *Prunus armeniaca*. They were identified as *ent*-epiafzelechin-(4α → 8; 2α → O → 7)-epiafzelechin (mahuannin A), *ent*-epiafzelechin-(4α → 8; 2α → O → 7)-(+)-afzelechin and *ent*-epiafzelechin-(4α → 8; 2α → O → 7)-(−)-afzelechin, by means of spectral studies.

Keywords: *Prunus armeniaca*; Rosaceae; A-type proanthocyanidins

1. Introduction

Prunus armeniaca L. is a moderate size tree, widely distributed in the northwestern Himalayas in India. *Prunus* species have been reported as an antipyretic, a refrigerant, a thirst quencher and for the treatment of leprosy and leucoderma (Kritikar & Basu, 1974; Chopra, Nayar, & Chopra, 1956). The plant growth inhibitory activity of aerial parts of *P. armeniaca* was previously reported. Proanthocyanidins possessing doubly linked structures (A-type) were shown to be the main active constituents (Rawat, Pant, Prasad, Joshi, & Pande). In our previous papers, we have reported on the isolation of ephedrannin A (Prasad, Joshi, Pant, & Rawat, 1997) and a novel A-type proanthocyanidin; *ent*-epiafzelechin-3-*O*-*p*-hydroxybenzoate-(4α → 8; 2α → *O* → 7)-epiafzelechin (Prasad et al., 1997) from the root of *P. armeniaca*. Further investigation of the remaining fraction of the ethyl acetate extract gave three isomeric A-type proanthocyanidins (**1**–**3**), which were characterized by extensive use of 1-D and 2-D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, NOESY) studies. It is interesting to isolate the three isomers from the same plant source. Compounds **2** and **3** seem to be the first reported isomers out of these three.

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2. Results and discussion

The EtOAc-soluble portion of the root of *P. armeniaca* was subjected to a combination of silica gel chromatography, Sephadex LH-20 separations and further by separations by HPLC to give three isomeric compounds (**1**–**3**). Compound **1** was identified as mahuannin A [*ent*-epiafzelechin-(4α → 8, 2α → *O* → 7)-epiafzelechin] by comparison of its spectral and physical data with those recorded in the literature (Hikino, Shimoyama, Kasahara, Takahashi, & Konno, 1982). Compound **1** was previously isolated from the root of *Ephedra* sp. by Hikino et al., and is reported to possess hypotensive properties (Hikino et al., 1982).

Compound **2** gave a positive vanillin–sulfuric acid test. Its negative FAB-mass spectrum showed a quasi-molecular ion peak $[M-H]^-$ at m/z 543. High resolution mass measurement established the molecular formula as $C_{30}H_{24}O_{10}$. The UV spectrum of compound **2** showed an absorption band at 280 nm typical of the phenolic chromophores of phloroglucinol rings. The ^{1}H NMR and ^{13}C NMR data (Table 1) of compound **2** resembled closely those of mahuannin A (**1**) (Hikino et al., 1982). A significant difference was observed in the chemical shifts and ^{1}H NMR coupling constants of the F-ring protons and carbons. The ^{13}C NMR spectrum of compound **1** displayed the presence of six aliphatic and 20 aromatic signals (four of double intensity) (Table 1). The assignments of all signals (protons

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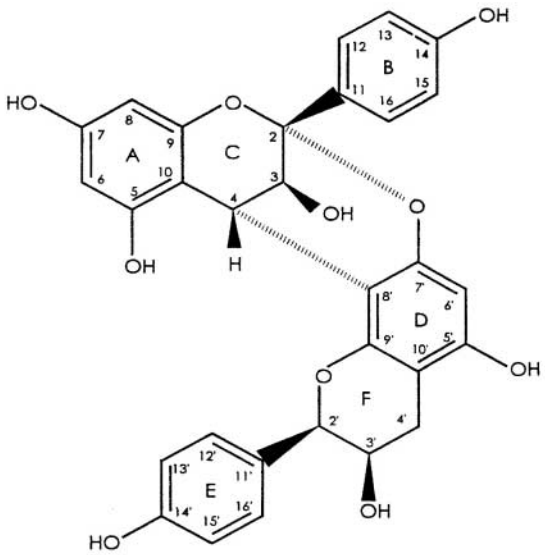

and carbons) were made by decoupling experiments i.e. ^{1}H–^{1}H and ^{1}H–^{13}C 2-D correlation spectroscopy.

The diagnostic feature of the ^{1}H NMR spectrum was the presence of an isolated AB system in the heterocyclic proton region [δ 4.17, H-3 and δ 4.24, H-4 ($J = 3.5$ Hz)], characteristic of the C-ring protons of A-type proanthocyanidins (Jacques, Haslam, Bedford, & Greatbanks, 1974). The other signals in the heterocyclic region of the ^{1}H NMR spectrum were observed at δ 3.06 (1H), 2.62 (1H) and 4.25 (1H) as an AA′X system, the latter being further coupled with a signal δ 4.79 (1H). The aromatic substitution pattern of compound **2** was evidenced by the presence of two *meta*-coupled doublets ($J = 2.4$) at δ 5.97 (H-6) and 6.09 (H-8), of ring A protons, a one proton singlet at δ 6.17 (H-6), and four A_2B_2 type doublet [δ 7.55, H-13,15, 6.87, H-12,16 ($J = 8.9$ Hz), and δ 7.38, H-13′,15′ and 6.89, H-12′,16′ ($J = 8.7$)] due to rings B and E. These data corroborated with the presence of a

Table 1
500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR data of compounds **1**–**3** (in acetone-d_6)

	1		**2**		**3**	
H/C	δC	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)
2	100.2s	–	100.1s	–	100.2s	–
3	67.3d	4.27, d (3.5)	67.3d	4.17, d (3.5)	67.1d	4.23, d (3.6)
4	28.9d	4.39, d (3.5)	28.9d	4.24, d (3.5)	28.9d	4.27, d (3.6)
5	156.6s	–	156.5s	–	156.5s	–
6	97.8d	5.89, d (2.4)	97.9d	5.97, d (2.4)	97.8d	5.93, d (2.4)
7	158.0s	–	158.0s	–	157.9s	–
8	96.2d	6.08, d (2.4)	96.3d	6.09, d (2.4)	96.2d	6.08, d (2.4)
9	154.1s	–	154.1s	–	153.9s	–
10	103.9s	–	103.8s	–	103.8s	–
11	131.6s	–	131.5s	–	131.5s	–
12	129.5d	7.57, d (8.9)	129.4d	7.55, d (8.9)	129.5d	7.55, d (8.9)
13	115.3d	6.88, d (8.9)	115.3d	6.87, d (8.9)	115.2d	6.87, d (8.9)
14	158.6s	–	158.5s	–	158.5s	–
15	115.3d	6.88, d (8.9)	115.3d	6.87, d (8.9)	115.2d	6.87, d (8.9)
16	129.5d	7.57, d (8.9)	129.4d	7.55, d (8.9)	129.5d	7.55, d (8.9)
2′	80.7d	5.17, brs	84.5d	4.79, d (8.6)	83.7d	4.84, d (8.3)
3′	66.6d	4.29, ddd (1.4, 3.0, 4.2)	67.5d	4.25, ddd (8.6, 5.9, 8.9)	67.9d	4.14, ddd (8.5, 5.5, 9.1)
4′	29.3t	2.91, ddd (0.8, 4.2, 16.9) 2.61, ddd (1.0, 3.0, 16.9)	29.8t	3.06, dd (5.9, 16.4) 2.62, dd (8.9, 16.4)	29.5t	3.05, dd (5.5, 16.3) 2.61, dd (9.1, 16.3)
5′	156.1s	–	155.6s	–	155.5s	–
6′	96.5d	6.15, s	96.7d	6.17, s	96.5d	6.15, s
7′	151.0s	–	151.2s	–	151.9s	–
8′	106.6s	–	106.7s	–	106.4s	–
9′	151.0s	–	151.2s	–	150.7s	–
10′	101.8s	–	102.7s	–	102.7s	–
11′	130.5s	–	129.4s	–	130.0s	–
12′	129.2d	7.52, d (8.7)	130.4d	7.38, d (8.7)	129.9d	7.41, d (8.7)
13′	115.9d	6.91, d (8.7)	116.1d	6.89, d (8.7)	116.1d	6.91, d (8.7)
14′	158.2s	–	158.7s	–	158.5s	–
15′	115.9d	6.91, d (8.7)	116.1d	6.89, d (8.7)	116.1d	6.91, d (8.7)
16′	129.2d	7.52, d (8.7)	130.4d	7.38, d (8.7)	129.9d	7.41, d (8.7)

The multiplicity of the signals were determined by DEPT spectrum.

Table 2
^{1}H–^{13}C long range coupling (HMBC) of compounds **1**–**3**

C	1	2	3
2	H-2, H-12, H-16	H-4, H-12, H-16	H-4, H-12, H-16
3	H-4	H-4	H-4
4	H-3	H-3	–
5	H-4, H-6	H-4, H-6	H-4, H-6
6	H-8	–	H-8
7	H-6, H-8	H-6, H-8	H-6, H-8
8	H-6	H-6	H-6
9	H-4, H-8	H-4, H-8	H-4, H-8
10	H-3, H-4, H-6, H-8	H-3, H-4, H-6, H-8	H-3, H-4, H-6, H-8
11	H-13, H-15	H-13, H-15	H-13, H-15
12	H-16	H-16	H-16
13	H-15	H-15	H-15
14	H-12, H-13, H-15, H-16	H-12, H-13, H-15, H-16	H-12, H-13, H-15, H-16
15	H-13	H-13	H-13
16	H-12	H-12	H-12
2′	H-4′a, H-4′b, H-12′, H-16′	H-4′a, H-12′, H-16′	H-4′a, H-12′, H-16′
3′	H-4′a, H-4′b	H-4′a, H-4′b, H-2′	H-4′a, H-4′b, H-2′
4′	H-3′,	–	H-3′,
5′	H-4′a, H-4′b, H-6′	H-6′	H-4′b, H-6′
6′	–	–	–
7′	H-4, H-6′	H-4, H-6′	H-4, H-6′
8′	H-4, H-6′	H-4, H-6′	H-4, H-6′
9′	H-4, H-4′a, H-4′b	H-4, H-4′a, H-4′b	H-4, H-4′a, H-4′b, H-2′
10′	H-6′, H-4′a, H-4′b, H-3′	H-6′, H-4′a, H-4′b	H-6′, H-3′, H-4′a, H-4′b
11′	H-13′, H-15′, H-2′	H-13′, H-15′	H-13′, H-15′, H-12′, H-16′, H-2′
12′	H-16′, H-2′	H-16′, H-2′	H-16′, H-2′
13′	H-15′	H-15′	H-15′
14′	H-12′, H-13′, H-15′, H-16′	H-12′, H-13′, H-15′, H-16′	H-12′, H-13′, H-15′, H-16′
15′	H-13′	H-13′	H-13′
16′	H-12′, H-2′	H-12′, H-2′	H-12′, H-2′

tetra- and a penta-substituted benzene ring having oxygen functions at the 1,3,5-positions and two *p*-hydroxyphenyl systems. The latter being further confirmed by the ^{13}C NMR chemical shifts of the carbon signals at δ 129.5 (C-12,16), 115.3 (C-13,15), 129.2 (C-12′,16′) and 115.9 (C-13′,15′) which corresponded with those of hydrogen carrying carbons of *p*-cresol (δ 130.2, 115.3) (Pouchart & Campbell, 1974).

The ^{13}C NMR resonances at δ 100.1 and 28.9 indicated that the two flavan-3-ol units are joined via a C-4 carbon–carbon and a C-2 ether linkage. The ^{13}C NMR resonances of the three oxygen-carrying carbons (C-5′, C-7′ and C-9′) of ring-D of the lower flavanyl unit were consistent with the allocation of the other end of the C–O–C linkage at the C-7′-position. The C_4–$C_{8'}$ carbon–carbon linkage was evident by the chemical shift of the hydrogen-carrying carbon of ring-D at δ 96.7 and the proton at δ 6.17, which was compatible with that of C-6′ in the spectra of related proanthocyanidins (Hikino et al., 1982; Nonaka, Morimoto, Kinjo, Nohara, & Nishioka, 1987; Kolodziej, Sakar, Burger, Engelshowe, & Ferreira, 1991; Pant, Nautiyal, Rawat, Sutherland, & Morris, 1992).

The long-range coupling information obtained from the HMBC experiment allowed the various fragments to be connected (Table 2). The proton at δ 4.24 (H4) showed a $^{2}J_{CH}$ interaction with C-8′, C-3 and C-10, and a $^{3}J_{CH}$ interaction with C-2, C-5, C-9, C-7′ and C-9′, while the proton at δ 6.17 showed a $^{2}J_{CH}$ interaction with C-5′ and C-7′ and $^{3}J_{CH}$ interaction with C-10′ and C-8′, indicating the attachment of C-4 with C-8 and C-10. Moreover, in the NOESY spectrum the cross peaks between H-4 and H-12′,16′ provided strong evidence for the β-orientation of ring-E of the lower flavanyl unit as well as C_2–O–$C_{7'}$ and C_4–$C_{8'}$ interflavanyl linkages.

The 2,3-*trans* configuration of ring-F of the lower flavanyl unit was evidenced by the appearance of a doublet at δ 4.79 (J = 8.6) due to H-2′. Comparison of the ^{13}C NMR data with compound **1** suggests that compound **2** share the same stereochemistry at the 3,4-position of the heterocyclic ring-C. The 2α,4α absolute configuration of ring-C was determined by the high amplitude negative Cotton effect in the diagnostic wavelength region (Barrett et al., 1979; Botha, Ferreira, & Roux, 1978) of the CD spectrum of compound **2**.

The ^1H and ^{13}C NMR spectra of compound **3** were almost indistinguishable from those of **2**. The appearance of a doublet ($J = 8.3$ Hz) at δ 4.84 due to H-2′ suggested the presence of a flavan-3-ol moiety with 2,3-*trans* stereochemistry. However, the absence of NOESY cross peak between H-4 and H-12′,16′ indicated the α-orientation of phenyl ring-E hence the substituents at C-3′ had to be β-orientated. From these observations, compound **3** was deduced to be a diastereomer of **2**.

3. Experimental

3.1. General

Mps: uncorr.; ^{13}C and ^1H NMR: 125 and 500 MHz, respectively, CD_3COCD_3 with TMS as int. standard. Chemical shifts are given in δ (ppm) from TMS. IR: KBr; UV: MeOH; FABMS: glycerol matrix; TLC silica gel (Merck).

3.1.1. Plant material, extraction and isolation

The roots (2 kg) of *P. armeniaca* were collected in May 1996, from Musoli, Uttar Pradesh India; specimens were identified by an authority of the Department of Botany, HNB Garhwal University and are preserved in its herbarium (No. 8751). The air dried, coarsely powdered roots were defatted with petrol (b.p. 60–80°C). The defatted material was exhaustively extracted with 90% EtOH and concentrated under reduced pressure. The extract was fractionated by successive partition with $CHCl_3$ and EtOAc. The EtOAc fraction was purified by repeated CC on silicagel and Sephadex LH-20 with $CHCl_3$, $CHCl_3$–MeOH with increasing MeOH content and further purification by HPLC on ODS–C_{18} using MeOH–H_2O (2:1, 6:4), UV 254 nm, at 40°C using 6 ml/min flow rate afforded compound **1**–**3**.

*3.1.1.1. ent-Epiafzelechin-(2α → O → 7, 4α → 8)-epiafzelechin (**1**).* Amorphous powder, mp > 300°C. Negative HRFAB-MS: calc. for $C_{30}H_{23}O_{10}$, m/z 543.1242 $[M–H]^-$; UV (MeOH) λ_{max} (log ϵ) 280 (4.06) nm; IR (KBr) ν_{max} cm^{-1}: 3400–3200, 1600, 1420, 1300, 1240, 1120; CD (MeOH) $[\theta]_{230} - 1.1 \times 10^4$, $[\theta]_{277} + 835$; negative-ion FAB-MS: m/z 543 $[M–H]^-$, 527, 446, 428, 407; ^1H and ^{13}C NMR: see Table 1.

*3.1.1.2. ent-Epiafzelechin-(2α → O → 7, 4α → 8)-(+)-afzelechin (**2**).* Amorphous powder, mp > 300°C. Negative HRFAB-MS: calc. for $C_{30}H_{23}O_{10}$, m/z 543.1241 $[M–H]^-$; UV λ_{max} nm (log ϵ): 279 (4.35); IR (KBr) ν_{max} cm^{-1}: 3340–3220, 1615, 1500, 1400; negative ion FAB MS m/z: 543 $[M–H]^-$, 367, 275, 194, 165, 151; CD (MeOH) $[\theta]_{230} - 1.21 \times 10^4$, $[\theta]_{205} + 2.11 \times 10^3$; ^1H and ^{13}C NMR: see Table 1.

*3.1.1.3. ent-Epiafzelechin-(2α → O → 7, 4α → 8)-(−)-afzelechin (**3**).* Amorphous powder, mp > 300°C. Negative HRFAB-MS: calc. for $C_{30}H_{23}O_{10}$, m/z 543.1242 $[M–H]^-$; UV λ_{max} nm (log ϵ): 278 (4.08); IR (KBr) ν_{max} cm^{-1}; 3500–3300, 1610, 1500, 1400; Negative ion FAB MS m/z: 543 $[M–H]^-$, 446, 407, 194, 165, 151; CD (MeOH) $[\theta]_{235} - 8.9 \times 10^3$, $[\theta]_{279} + 8.4 \times 10^2$; ^1H and ^{13}C NMR: see Table 1.

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