An A-Type Proanthocyanidin from *Prunus armeniaca*

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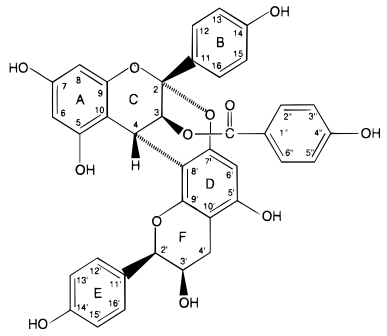
Roots of *Prunus armeniaca* yielded a new A-type proanthocyanidin whose structure was assigned as *ent*-epiafzelechin-3-*O*-*p*-hydroxybenzoate-(4α→8,2α→*O*→7)-epiafzelechin (**1**). The structure of **1** was determined through extensive 1D and 2D NMR studies.

Prunus armeniaca L. (Rosaceae) is a moderate-sized tree widely distributed in the northern western Himalayas in India. *Prunus* species have been used in indigenous medicine for a variety of purposes.[1] Several A-type proanthocyanidins have been reported from *Prunus* species.[2−4] The present study has led to the isolation of a new A-type proanthocyanidin (**1**) from an EtOAc soluble extract of the roots of *P. armeniaca*. The structure of **1** has been elucidated through extensive 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) studies as *ent*-epiafzelechin-3-*O*-*p*-hydroxybenzoate-(4α→8,2α→*O*→7)-epiafzelechin (**1**).



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Compound **1** was found to have a molecular weight of 664 as deduced by the presence of the molecular ion peak $[M + H]^+$ at *m*/*z* 665 in the positive-ion FABMS. Elemental analysis of compound **1** corresponded to the molecular formula $C_{37}H_{28}O_{12}$, showing 24 double-bond equivalents in the molecule. The UV spectrum exhibited absorption of phenolic chromophore(s) at λ_{max} 280 nm. The IR absorption band at 3400−3200 and 1705 cm^{-1} indicated the presence of phenolic and carbonyl functions in the molecule. The ^{13}C NMR spectrum of **1** indicated the presence of 37 carbon atoms (Table 1), which included six aliphatics, 24 aromatics (with six of double intensity), and one carbonyl. The DEPT spectrum showed the absence of CH_3 groups, with the presence of one methylene, 13 methines (six of double intensity), and 17 quaternary carbon atoms (of which 10 were oxygenated). The methine and methylene carbon atoms were readily assigned by the HMQC NMR spectrum.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Compound 1[a]

position	δ_C	multiplicity (DEPT)	δ_H (*J* in Hz)	^{1}H−^{13}C connectivities (HMBC) $^2J_{CH}$	$^3J_{CH}$
2	98.9	C			
3	69.0	CH	5.58, d (3.7)		C=O
4	27.0	CH	4.72, dd (3.7, 0.5)	C-10, C-8′	C-2, C-5, C-9, C-7′
5	156.3	C			
6	98.1	CH	5.89, d (2.4)	C-5, C-7	C-8, C-10
7	158.4	C			
8	96.1	CH	6.18, dd (2.4, 0.5)	C-7, C-9	C-6, C-10
9	154.1	C			
10	103.4	C			
11	130.5	C			
12	129.2	CH	7.59, d (8.9)		C-2, C-16
13	115.5	CH	6.79, d (8.9)	C-14	C-11, C-15
14	159.0	C			
15	115.5	CH	6.79, d (8.9)	C-14	C-11, C-13
16	129.2	CH	7.59, d (8.9)		C-2, C-12
2′	80.8	CH	5.13, br s		C-12′, C16′
3′	66.6	CH	4.28, ddd (1.3, 4.2, 3.0)		C-10′
4′a	29.4	CH_2	2.93, ddd (0.7, 4.2, 16.9)	C-3′, C-10′	C-2′, C-5′, C-9′
b			2.88, ddd (1.0, 3.0, 16.9)	C-3′, C-10′	C-2′, C-5′, C-9′
5′	156.7	C			
6′	96.4	CH	6.17, d (0.5)	C-5′, C-7′	C-8′
7′	151.6	C			
8′	105.5	C			
9′	151.5	C			
10′	102.4	C			
11′	130.3	C			
12′	129.1	CH	7.49, d (8.9)		C-2′, C-16′
13′	115.8	CH	6.84, d (8.9)	C-14′	C-11′, C-15′
14′	158.2	C			
15′	115.8	CH	6.84, d (8.9)	C-14′	C-11′, C-13′
16′	129.1	CH	7.49, d (8.9)		C-2′, C-12′
1″	121.3	C			
2″	132.7	CH	7.50, d (9.0)		C=O, C-6″
3″	116.0	CH	6.73, d (9.0)	C-4″	C-1″, C-5″
4″	163.3	C			
5″	116.0	CH	6.73, d (9.0)	C-4″	C-1″, 3″
6″	132.7	CH	7.50, d (9.0)		C=O, C-2″
C=O	165.5	C			

[a] Measured in Me_2CO-d_6 and MeOH-d_4 (2:1); data are expressed in δ units relative to TMS.

The ^{1}H NMR spectrum of **1** (Table 1) showed characteristic signals for H-3 and H-4 protons of an A-type proanthocyanidin[5] centered at δ 5.58 (d) and 4.72 (dt), respectively. The other heterocyclic protons appeared at δ 5.13 (br s, 1H, H-2′), 4.28 (ddd, 1H, H-3′), 2.93 (ddd, 1H, H-4′a), and 2.88 (ddd, 1H, H-4′b). The aromatic region of the ^{1}H NMR spectrum of **1** showed the presence of two *meta*-coupled doublets (J = 2.4 Hz) at

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δ 5.89 and 6.18 and a 1H singlet at δ 6.17, which indicated the presence of a tetra- and a penta-substituted phenyl system having oxygen functionalities at the C-1, C-3, and C-5 positions.[6] Six A_2B_2-type doublets (J = 8.9 Hz) demonstrated the presence of three *p*-substituted phenyl moieties. These were further confirmed to be *p*-hydroxyphenyl systems from the ^{13}C NMR chemical shifts of the carbon signals at δ 129.2 (C-12,16), 115.5 (C-13,15), 129.1 (C-12′,16′), 115.8 (C-13″,15″), 132.7 (C-2″, C-6″), and 116.0 (C-3″,C-5″), which corresponded with analogous data of the aromatic carbons of *p*-cresol (δ 115.3, 130.2).[7] The ^{13}C NMR spectrum (Table 1) showed the presence of a carboxyl group and a *p*-hydroxyphenyl ring in addition to 30 carbon resonances assignable to an A-type proanthocyanidin. The downfield chemical shift of the H-3 proton was consistent with the allocation of the *p*-hydroxybenzoate moiety at the C-3 position.[8] The presence of the *p*-hydroxybenzoate unit at C-3 was further confirmed by the analysis of the HMBC spectrum (Table 1), which displayed $^3J_{CH}$ interactions between H-3 and the carbonyl carbon and between the latter and the H-2″ 6″ protons. The above-mentioned NMR studies led to the conclusion that compound **1** is constituted by two flavan moieties, with the upper flavan moiety substituted at the C-3 position by a *p*-hydroxybenzoate function.

The two flavan moieties in **1** were linked through C−O−C and C−C groups attached to C-2 and C-4 of the upper flavan moiety. The ^{13}C NMR chemical shift of the quaternary carbon (C-2, δ 98.9) demonstrated that the terminus of the C−O−C linkage must be located at C-2, and hence the C−C linkage was attached to the C-4 position. The other end of the C−O−C linkage was attached to C-6′, C-7′, or C-8′. The ^{13}C NMR chemical shifts of the three oxygen-carrying carbons (C-5′, C-7′, and C-9′) of ring-D of the lower flavan moiety, which appeared at lower field than δ 150, were consistent with the allocation of the other end of C−O−C unit being linked at the C-7′ position.[6,9] On the other hand, if the C−O−C linkage were located at C-6′ or C-8′, then the three oxygen-bearing carbons should have appeared in the range δ 140−150.[9] Consequently, the other end of the C−C linkage must be at either C-6′ or C-8′, and it was found to be at C-8′ by the chemical shift of the proton-carrying carbon of the D-ring of the lower flavan moiety at δ 96.4 and the hydrogen at δ 6.17, which was compatible with those of C-6′ in the spectra of related proanthocyanidins.[2,6,9] These assignments were further confirmed by the HMBC spectrum (Table 1), which showed long-range coupling of the H-4 proton with C-7′and C-8′ of the lower flavan moiety and with C-10, C-9, C-5, and C-2 of the upper flavan moiety. Other long-range correlations observed in HMBC spectrum are shown in Table 1. Moreover, the NOESY correlation between H-4 and the H-12′,H-16′ protons provided strong evidence for the C-4/C-8′ and C-2−O−C-7′ interflavanoid linkages.

The appearance of a broad singlet at δ 5.13 due to H-2′ suggested the presence in the lower flavan-3-ol moiety of 2′,3′-cis (epiafzelechin-type) stereochemistry. This was supported by the ^{13}C NMR chemical shift (δ 80.8) at C-2, which is consistent with that observed in the epiafzelechin moiety (δ 80.8) of mahuannin A[6] and the epicatechin moiety [δ 81.04 (MeOH-d_4)] of 13′-hydroxymahuannin A.[2] The NOESY cross peak between H-4 and H-12′,H-16′ and the coupling constant ($J_{2',3'}$ = 1.3 Hz) of H-2′ and H-3′ confirmed the stereochemistry of lower flavan moiety as shown in structure **1**. The magnitude of the mutual coupling constant (J = 3.7 Hz) of the H-3 and H-4 protons did not provide unambiguous information concerning the 3,4-stereochemistry; however, the ^{13}C NMR chemical shifts of **1** closely resembled those of mahuannin A[6] and 13′-hydroxymahuannin A.[2] The absolute stereochemistry at C-4 for compound **1** was established from the sign of the Cotton effect near 230 nm.[10] The CD measurement in MeOH, showed a negative Cotton effect at 230 nm ($[\theta]$ -4.1×10^3), indicating (4*S*)-configuration as found in mahuannin A and 13′-hydroxymahuannin A. On the basis of all these observations, compound **1** was assigned as *ent*-epiafzelechin-3-*O*-*p*-hydroxybenzoate-(4α→8,2α→*O*→7)-epiafzelechin.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Beckman DU-64 UV−vis spectrophotometer in MeOH. IR spectra were recorded on a Pye Unicam SP 3−200 IR spectrophotometer as KBr pellets. The CD data were recorded in MeOH on a JASCO DIP-4 spectropolarimeter. The FABMS was recorded on JEOL DX-300 instrument, ^1H (500 MHz, $(CD_3)_2CO-CD_3OD$, 2:1), ^{13}C NMR (125 MHz), and 2D NMR spectra were recorded on a JEOL GSX-500 spectrophotometer, using TMS as internal standard. The chemical shifts are reported in parts per million, and the coupling constants are in Hertz. Column chromatography was carried out on Si gel (Merck). Spots on TLC were visualized by spraying with 7% w/w H_2SO_4 followed by heating.

Plant Material. The roots (2 kg) of *P. armeniaca* were collected in May 1996, from Musoli, Uttar Pradesh, India. The specimen was identified by Dr. L. R. Dangwal, Department of Botany, H. N. B. Garhwal University, and is preserved in that herbarium (no. 8751).

Extraction and Isolation. The air-dried, coarsely powdered roots were defatted with light petroleum ether (bp 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 on repeated column chromatography on Si gel, eluted with $CHCl_3$ and a mixture of $CHCl_3$−MeOH of increasing MeOH content. Further purification of fraction by HPLC on ODS−C_{18} using MeOH−H_2O (6:4), UV detector at 254 nm, a temperature of 40 °C, and a 6-mL/min flow rate, afforded pure compound **1** [*ent*-epiafzelechin-3-*O*-*p*-hydroxybenzoate-(4α→8,2α→O→7)-epiafzelechin, 11 mg), as an amorphous solid: UV (MeOH) λ_{max} (log ϵ) 280 (4.06) nm; IR (KBr) ν_{max} 3400−3200, 1705, 1600, 1585, 1420, 1300, 1240, 1120 cm^{-1}; CD (MeOH) $[\theta]_{230}$ -4.18×10^3, $[\theta]_{287}$ $+8.35 \times 10^{-1}$; NMR data, see Table 1; positive-ion FABMS *m/z* $[M + H]^+$ 665, 542, 299, 267, 166; *anal.* C 66.64%, H 4.17, calcd for $C_{37}H_{28}O_{12}$, C 66.87%, H 4.22%.

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