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PUDDUMIN-A, A NEW FLAVANONE GLUCOSIDE FROM *PRUNUS CERASOIDES*

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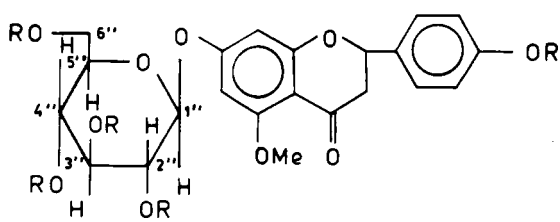
Prunus cerasoides D. Don. (syn. *Prunus puddum*, Rosaceae) is a tree distributed in the temperate Himalayas to an altitude of 1700 m (1,2). The stems are reported to be antipyretic, refrigerant, and useful in vomiting, thirst, asthma, leprosy, and leucoderma (1,2). The kernels and branches are used for the treatment of kidney stones and gravel (2). The stem bark has been investigated and is found to contain sakuranetin, prunetin, genkwanin, and genistein (3). In a previous paper (4) we reported the isolation of some known flavonoids and flavonoid-glycosides from the EtOH extract of the stem sapwood.

In the current work a final fraction was obtained by eluting the column with $CHCl_3$/MeOH. This fraction (300 mg) was then separated into three compounds by preparative tlc. Based on the ^{1}H-nmr and eims spectra, the structure of the first compound was determined to be prunetin; this was confirmed by comparison with an authentic specimen (co-tlc, co-ir, mmp). From similar spectral data, the second compound was interpreted to be genistein. This was confirmed by comparison with an authentic sample (mmp, co-ir, co-tlc).

The third compound [**1**] was purified by acetylation (pyridine/Ac_2O) to **2** (6). The acetylated compound **2** was subsequently deacetylated (10% KOH) to yield **1** which was purified by preparative tlc.

Enzymatic hydrolysis of **1** gave glucose (co-pc, co-tlc) and an aglycone which gave a positive Shinoda's test for flavanones (12). The presence of one methoxy group was determined with the help of Zeisel's method (13). The aglycone was treated with HI to afford naringenin, the identification of which along with its acetate was established with the help of spectral studies (6).

The ^{1}H-nmr spectra of **1** and **2** substantiated the presence of the 4′,5,7-trihydroxy flavanone (naringenin) skeleton (8). The presence of one aromatic (δ 2.30 ppm) and four alcoholic (δ 2.05, 2.06, 2.10, 2.11) acetoxyls and an aromatic methoxyl group (δ 3.81) was revealed by the ^{1}H-nmr spectrum of **2**. Therefore, **1** should be a mono-*O*-glycosylated, mono-*O*-methylated naringenin. The chemical shifts for the protons of the B and C rings of **2** were in good agreement with those of triacetylnaringenin; whereas, H-6 and H-8 of **1** were observed to be shifted upfield. Accordingly, the -OH group at C-4′ must



1 R=H
2 R=Ac

be unsubstituted. The existence of a methoxyl group on the A-ring was confirmed by the presence of ions at *m/z* 286, 167, and 120 in the eims of **1** (6). The eims of **2** exhibited ions typical of 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (*m/z* 331, 271, 169, and 109) (9). The chemical shifts of the sugar moiety in the ^{13}C-nmr spectra of **1** and **2** supported the identification of the attached sugar as glucose (10,11). The position of the methoxy group was determined as follows: The C-10 (δ 106.5 ppm) scarcely shifted on acetylation (δ 107.0) in the ^{13}C-nmr spectrum of **2**. On the other hand, both C-6 and C-8 (δ 93.7 and 95.1) shifted upfield (δ 95.8 and 98.8). Moreover, the chemical shift of C-10 was consistent with that of 5-methylated flavonoids. Likewise, the shift of C-1″ was consistent with 7-*O*-glucosides (10). Thus, the structure of the aglycone was elucidated as 5-*O*-methyl naringenin and that of **1**, a new glycoside, 7-*O*-(β-D-glucopyranosyl)-5-*O*-methyl naringenin, which we have named puddumin-A.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting points are uncorrected. Ir spectra were recorded on KBr discs (JASCO-IR-810 spectrometer). The ^{1}H-nmr spectra were run at 100 MHz and ^{13}C-nmr spectra at 25.1 MHz (JEOL JNM-MH 200 and JNM-FX 100 spectrometers) using TMS as an internal standard. Mass spectra (70 eV JEOL JMS-DX 300 spectrometers) were taken with a direct inlet.

PLANT MATERIAL.—The plant material was collected from Kirora (Dist. Tehri Garhwal) UP, India. The authentication of the plant material was made at the Botany Department, Garhwal University, Srinagar, UP. Voucher specimens are available in the Herbarium of the Botany Department (no. B-77) and from us.

EXTRACTION AND ISOLATION.—The plant material was defatted with light petroleum (60-80°) followed by exhaustive extraction with EtOH. The extract was concentrated under reduced pressure to afford a light yellow solid mass. This solid mass was subjected to column chromatography (Si gel, $CHCl_3$/MeOH). Several known flavonoids were isolated and identified (4). The last fraction which was obtained by eluting the column with $CHCl_3$-MeOH (80:20) afforded one band (300 mg) which was further separated by preparative tlc (Si gel, $CHCl_3$-MeOH, 17:3) into three compounds: prunetin (14 mg), genistein (11 mg), and **1** (99 mg).

IDENTIFICATION OF PRUNETIN AND GENISTEIN.—Prunetin gave mp 231-234° (MeOH), lit. mp 236° (5). The ir and ^{1}H- and ^{13}C-nmr data are in agreement with published results for prunetin (6). The identity of this compound was confirmed by comparison with an authentic sample. Genistein gave mp 296-297° (MeOH), lit. mp 298° (5). The ir and ^{1}H- and ^{13}C-nmr data are in agreement with published results for genistein (6); hreims *m/z* 270.0520 (M^+). Final identification was made by comparison with an authentic sample.

CHARACTERIZATION OF COMPOUND **1**.—Compound **1** (99 mg) was purified by acetylation (Ac_2O/pyridine). The acetate [**2**], mp 197-199° (MeOH), 10 mg] was refluxed for 10 min with 1 ml of 10% KOH in MeOH (2 ml). The reaction mixture was concentrated in vacuo and submitted to preparative tlc ($CHCl_3$-MeOH, 17:3) to recover the deacetylated compound **1** (5 mg); mp (MeOH) 210°; Found: C, 58.85; H, 5.30%. Calcd. for $C_{22}H_{24}O_{10}$, C, 58.92; H, 5.35%; eims *m/z* 448 (M^+), 286 (M^+ of aglycone, calcd. for $C_{16}H_{14}O_5$); ir ν max cm^{-1} 3420, 2950, 1685, 1610, 1350, 1210; ^{1}H nmr (d_6-DMSO) δ 2.70 (s, 1H, 3-Heq), 3.30 (d, *J*=2 Hz, 3-Hax), 3.80 (s, 1H, OCH_3), 5.60 (s, 1H, OH), 6.15 (s, 1H, H-2), 6.40 (d, 1H, *J*=2 Hz, H-6), 6.78 (d, 1H, *J*=2 Hz, H-8), 6.83 (d, 2H, *J*=9 Hz, H-3′ and 5′), 7.65 (d, 2H, *J*=9 Hz, H-2′ and 6′); ^{13}C nmr (d_6-DMSO) see Table 1.

COMPOUND **2** (ACETATE OF **1**).—Found: C, 58.28; H, 5.14%. Calcd. for $C_{32}H_{34}O_{15}$: C, 58.35; H, 5.20%; eims *m/z* 658 (M^+); ir ν max cm^{-1} 1745, 1680, 1610, 1215; ^{1}H nmr ($CDCl_3$) δ 2.05, 2.06, 2.10, 2.11, 2.30 (each s, 3H, acetoxyls), 2.8-2.9 (s, 1H, 3-Heq), 3.81 (s, 3H, OCH_3), 4.24 (2H, br-d, *J*=4 Hz, 3-Hax), 5.44 (s, 1H, H-2), 6.26 (d, 1H, *J*=2 Hz, H-6), 6.40 (d, 1H, *J*=2 Hz, H-8), 7.15 (d, 2H, *J*=9 Hz, H-3′ and 5′), 7.47 (d, 2H, *J*=9 Hz, H-2′ and 6′); ^{13}C nmr ($CDCl_3$) see Table 1.

The spectral data of the aglycone, naringenin, and triacetylnaringenin are in agreement with published results (6,8,10). The structures of these three compounds were confirmed by comparison with authentic samples.

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TABLE 1. ^{13}C-nmr Assignments in δ ppm

Carbon no.	Compounds (solvent)			
	naringenin (in d_6-DMSO)	triacetyl-naringenin (in $CDCl_3$)	**1** (in d_6-DMSO)	**2** (in $CDCl_3$)
C-2	78.4, d	79.0, d	78.3, d	78.7, d
C-3	42.0, t	45.1, t	44.5, t	45.5, t
C-4	196.2, s	188.9, s	192.4, s	187.4, s
C-5	163.6, s	151.2, s	159.9, s	158.8, s
C-6	95.9, d	110.6, d	93.7, d	95.8, d
C-7	166.,7, s	156.0, s	165.6, s	164.5, s
C-8	95.0, d	109.1, d	95.1, d	98.8, d
C-9	162.9, s	163.1, s	165.1, s	165.4, s
C-10	101.8, s	111.8, s	106.5, s	107.0, s
C-1′	128.9, s	135.6, s	126.0, s	136.1, s
C-2′	128.2, d×2	127.4, d×2	130.8, d×2	127.3, d×2
C-3′	115.2, d×2	122.0, d×2	115.9, d×2	121.9, d×2
C-4′	157.8, s	151.0, s	159.6, s	150.8, s
C-5′	115.2, d×2	122.0 d×2	115.9, d×2	121.9, d×2
C-6′	128.2, d×2	127.4, d×2	130.8, d×2	127.3, d×2
O-CH_3	—	—	55.5, q	55.7, q
C-1″	—	—	100.3, d	99.5, d
C-2″	—	—	73.3, d	70.3, d
C-3″	—	—	76.8, d	72.6, d
C-4″	—	—	69.6, d	68.5, d
C-5″	—	—	77.5, d	72.3, d
C-6″	—	—	60.6, t	62.1, t
Others	—	169.2×2, s; 168.0, s; 21.1×3, q	—	170.5, s; 170.2, s; 169.6, s; 169.4, s; 169.3, s; 21.1, q 20.8, q 20.6×3, q×3

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