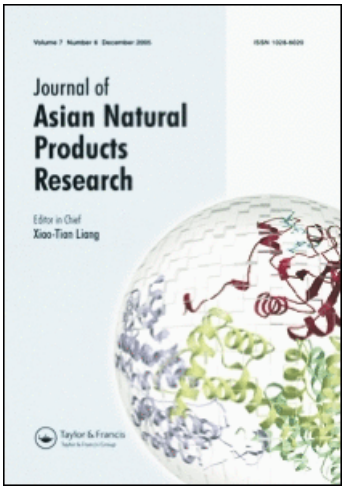

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C-GLUCOSIDE FLAVONOIDS FROM THE LEAVES OF *CRATAEGUS PINNATIFIDA* BGE. VAR. *MAJOR* N.E.BR.

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Two new acetyl C-glucoside flavonoids, 8-*C*-β-D-(2″-*O*-acetyl)glucofuranosylapigenin and 3″-*O*-acetylvitexin, along with four known C-glucoside flavonoids, vitexin, 6″-*O*-acetylvitexin, 2″-*O*-acetylvitexin, and 2″-*O*-rhamnosylvitexin were isolated from the leaves of *Crataegus pinnatifida* Bge. var. *major* N.E.Br. Their structures were elucidated by spectroscopic means and chemical evidence.

Keywords: *Crataegus pinnatifida* Bge. var. *major* N.E.Br; Rosaceae; Flavonoid C-glucosides; 8-*C*-β-D-(2″-*O*-Acetyl)glucofuranosylapigenin; 3″-*O*-acetylvitexin

INTRODUCTION

Crataegus pinnatifida Bge. var. *major* N.E.Br. (Rosaceae), widely distributed in the northeastern part of China, is used as medicinal plant to improve digestion, remove retention of food, promote blood circulation and resolve blood stasis both in traditional and folk medicine. Many phytochemical and pharmacological studies have been reported concerning various *Crataegus* species [1–4]. To date, more than fifty flavonoids have been isolated from *Crataegus* [5,6]. We recently reported the isolation and structures of six novel flavonoid ketohexosefuranosides, pinnatifinosides A–D [7], pinnatifin A, and pinnatifin B [8] from the leaves of this plant. In continuing efforts aimed at the discovery of new bioactive constituents from *C. pinnatifida* Bge. var. *major* N.E.Br., six C-glucoside flavonoids have been identified, including two new flavone C-glucosides, 8-*C*-β-D-(2″-*O*-acetyl)glucofuranosylapigenin (**1**) and 3″-*O*-acetylvitexin (**2**), as well as four known flavone C-glucosides, vitexin (**3**), 6″-*O*-acetylvitexin (**4**), 2″-*O*-acetylvitexin (**5**), and 2″-*O*-rhamnosylvitexin (**6**).

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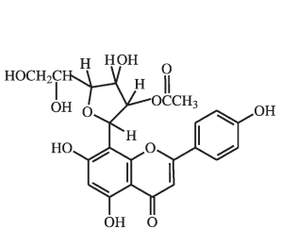
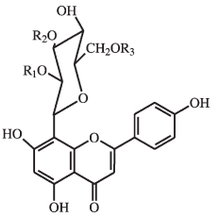


4 $R_1 = R_2 = H$, R_3 = Acetyl



1 **2** $R_1 = R_3 = H$, R_2 = Acetyl
3 $R_1 = R_2 = R_3 = H$

5 $R_2 = R_3 = H$, R_1 = Acetyl
6 $R_2 = R_3 = H$, R_1 = Rhamnosyl

RESULTS AND DISCUSSION

Compound **1** was obtained as yellow needles, mp 220–222°C, $[\alpha]_D^{25}$: +63.2 (*c* 0.10, MeOH) and exhibits a positive magnesium hydrochloric acid test. The HR-FAB-MS of **1** gave a molecular ion peak at *m/z* 474.1151, which is consistent with the molecular formula $C_{23}H_{22}O_{11}$. The absorption bands at ν_{max} 3370, 1710, 1650, 1606, and 1520 cm^{-1} in the IR spectrum are characteristic of hydroxyl, unconjugated carbonyl, hydrogen bonded carbonyl, and aromatic groups, respectively. The UV spectrum of **1** showed absorption maxima at 270 (Band II), 333 (Band I) nm in MeOH and bathochromic shifts of 47 nm with $A1C1_3$ and $A1C1_3$ + HC1 in Band I. These data revealed that **1** was a flavone without *ortho*-dihydroxyl and 3-hydroxyl groups in the structure. The bathochromic shifts of band II (6 nm) with NaOAc and band I (57 nm) with $NaOCH_3$ are indicative of the presence of two free hydroxy groups at C-7 and C-4′ in **1**. From the UV spectral data it is suggested that **1** has a 5,7,4′-trihydroxylflavone skeleton.

The ^1H NMR spectrum (Table I) of **1** indicated the presence of a hydrogen-bonded OH group (δ 13.81, 5-OH). Resonances for two set of doublets at δ 8.00 (2H, d, J = 8.8 Hz) and 7.27 (2H, d, J = 8.8 Hz) were assigned to H-2′,6′ and H-3′,5′, respectively, and two singlets at δ 6.91 and 6.72 were due to the protons at C-3 and C-6 in rings C and A, respectively. These data indicated that the aglycone moiety was analogous to apigenin except for the absence of one proton in ring A. Some 23 carbon signals in the ^{13}C NMR spectrum (Table I) exhibited the presence of a flavonoid nucleus, a saccharide moiety, and an acetyl group in **1**. The six carbon signals of the sugar moiety in the DEPT spectrum were at δ 85.4 (CH), 82.6 (CH), 79.2 (CH), 75.5 (CH), 70.1 (CH), and 65.3 (CH_2), suggesting that **1** was C-glucoside flavone. The site of the sugar linkage to the aglycone in **1** was considered to be at the C-8 position of 5,7,4′-trihydroxyapigenin, since the C-8 signal appeared at δ 105.0 in the ^{13}C NMR spectrum. It was further confirmed by the appearance of the cross peaks of the anomeric proton of the sugar at δ 6.03 (d, J = 3.1 Hz) with the carbons at δ 163.6 (C-7), 105.0 (C-8) and 155.5 (C-9) in the HMBC spectrum. Compared with the corresponding sugar signal of vitexin, either the proton signals or carbon signals in **1** were a lower field, and the J_{H-H} values in the ^1H NMR spectrum were smaller (about 3 Hz), which suggested that the sugar was in the furanose instead of pyranose form. Furthermore, the configuration of the sugar moiety could be determined by the $^1H-^1H$ COSY, NOESY (Table II) and HMBC

TABLE I NMR data of **1** and **2**

	1 (C_5D_5N)			**2** (DSMO-d_6)
Position	^{13}C	^1H	HMBC	^1H (Hz)
2	164.3			
3	104.0	6.91 s	C-2, C-4, C-10	6.77 s
4	182.9			
5	162.8	13.81(OH)		13.16 (OH)
6	101.1	6.72 s	C-5, C-7, C-8, C-10	6.27 s
7	163.6			10.95 s
8	105.0			
9	155.5			
10	103.5			
1′	122.4			
2′	129.0	8.00 d (8.7)	C-1′, C-4′, C-6′	8.02 d (8.8)
3′	117.0	7.27 d (8.7)	C-5′, C-4′	6.89 d (8.8)
4′	162.6			
5′	117.0	7.27 d (8.7)	C-4′, C-3′	6.89 d (8.8)
6′	129.0	8.00 d (8.7)	C-1′, C-4′, C-2′	8.02 d (8.8)
1″	79.2	6.03 d (3.1)	C-7, C-8, C-9, C-2″	4.78 d (9.7)
2″	85.4	5.76 d (3.1)	C-8, C-1″, C-4″, C-3″,–C═O	3.97 t (9.7)
3″	75.5	5.10 d (2.4)	C-1″, C-2″, C-4″	4.87 t (9.7)
4″	82.6	4.63 m	C-3″, C-1″	3.58 m
5″	70.1	4.93 m		3.58 m
6″	65.3	4.49–4.31 m	C-4″, C-5″	3.77 m, 3.58 m 4.74 brs
$-CH_3$	20.8	2.02 s	–C═O	1.96
–C═O	170.1			

(Table I) spectra. The proton signals at δ 6.03, 5.76, 5.10, 4.63, 4.93 and 4.49, 4.31 in the sugar moiety were assigned to H-1″, H-2″, H-3″, H-4″, H-5″ and H-6″, respectively, by $^1H-^1H$ COSY and HMQC. In the NOESY spectrum, the signals at δ 6.03 (H-1″) and δ 4.63 (H-4″) show correlation to H-3″ (δ 5.10), indicating that the sugar is β-D-glucofuranose. Finally, the position of the acetyl group was confirmed at C-2″ in the sugar moiety by the long-range correlation of the carbonyl signal at δ 170.1 and the proton at δ 5.76 (H-2″). Thus, the structure of **1** was 8-*C*-β-D-(2″-*O*-acetyl)glucofuranosylapigenin.

Compound **2** was obtained as yellow powder, mp 194–196°C, $[\alpha]_D^{25}$: −14.6 (*c* 0.12, MeOH). The HR-FAB-MS of **2** indicated a molecular ion peak at *m/z* 474.1188 corresponding to a molecular formula $C_{23}H_{22}O_{11}$. It was similar to **1** in the Mg–HCl color reaction, UV and IR spectra, suggesting that **2** has also a 5,7,4′-trihydroxyflavonoid skeleton.

TABLE II Scalar and spatial correlations of the protons of the sugar moieties of **1** (C_5D_5N)

H	δ_H (Hz)	$^1H-^1H$ COSY	NOESY
1″	6.03(d, 3.1)	2″-H	2′,6′-H, 4″-H, –COCH3
2″	5.76(d, 3.1)	1″-H	3″–H, –COCH3
3″	5.10(d, 2.4)	4″-H	4″-H
4″	4.63(m)	3″-H, 5″-H	1″-H, 3″-H, 5″-H
5″	4.93(m)	4″H, 6″-H	6″-H, 4″-H
6″	4.49–4.31(m)	5″-H	5″-H
$-COCH_3$	2.02(s)		6′-H, 5′-H, 1″-H, 2″-H,

The ^{1}H NMR of **2** (Table I) shows an aromatic hydroxyl signal at δ 13.16 (s), indicating the presence of 5-OH flavone moiety. The spectrum also shows the presence of a 4′-hydroxyphenyl group [δ 8.02, 6.89 (each 2H, d, $J = 8.8$ Hz)], and two aromatic proton signals at δ 6.27 (s, H-6) and δ 6.77 (s, H-3). The ^{13}C NMR spectrum (Table III) reveals 23 carbons, which suggested that the structure is a flavonoid containing a saccharide moiety and an acetyl group. Six carbon signals of the sugar moiety are at δ 81.4 (CH), 79.7 (CH), 73.4 (CH), 68.4 (CH), 67.9 (CH) and 60.4 (CH_2) in the DEPT spectrum suggesting that **2** is a flavone C-glucoside. The site of the sugar linkage to the aglycone in **2** is also considered to be at the C-8 position of 5,7,4′-trihydroxyapigenin. The hexose substituent at C-8 gave a pattern of ^{13}C NMR signals similar to that exhibited by the C-linked glucose in vitexin, but the signal of glucose C-3″ appeared at δ 79.7, which is shifted downfield by $\Delta = 1.0$ ppm compared with the corresponding signal of vitexin (δ 78.7). Meanwhile the signals of C-2″ (δ 68.4) and C-4″ (δ 67.9) of the glucose are shifted upfield by $\Delta = 2.5$ and $\Delta = 2.7$ ppm, respectively, compared with the corresponding signal (δ 70.9, 70.6) of vitexin. These results suggest that the acetyl group is attached to the C-3″ position of glucose. Furthermore, the sugar functionality was also identified as a β-D-glucopryranose by the ^{1}H NMR, ^{1}H–^{1}H, ^{13}C–^{1}H COSY spectra and J values. Thus, the structure of **2** was determined as 3″-*O*-acetylvitexin.

In addition, four known flavone C-glucosides, vitexin (**3**) [9], 6″-*O*-acetylvitexin (**4**), 2″-*O*-acetylvitexin (**5**) [10], and 2″-*O*-rhamnosylvitexin (**6**) were isolated. They were all identified by colour reactions, UV spectral analysis, chemical methods, and ^{1}H and ^{13}C NMR spectroscopy. ^{13}C NMR data of the four known C-glucosides flavone are given in Table III.

TABLE III ^{13}C NMR data of **1**–**6** (DMSO-d_6)

C	**1**	**2**	**3**	**4**	**5**	**6**
2	163.7	163.9	164.0	164.0	164.1	164.0
3	102.9	102.5	102.5	102.6	102.5	102.4
4	182.0	181.9	182.1	182.0	182.1	182.1
5	161.4	161.0	161.2	161.1	161.3	161.1
6	99.8	98.0	98.2	98.3	97.8	98.3
7	162.7	162.5	162.6	162.6	162.1	162.3
8	102.5	103.6	104.7	104.1	102.4	104.4
9	154.8	155.9	156.0	156.0	156.5	155.8
10	103.6	103.9	104.1	104.1	104.0	104.2
1′	121.3	121.6	121.7	121.5	121.6	121.6
2′,6′	128.7	128.9	129.0	128.6	129.1	129.0
3′,5′	116.1	115.8	115.8	115.8	115.9	115.8
4′	161.1	160.5	160.4	160.5	160.9	160.6
Glc-1″	77.5	73.4	73.4	73.6	71.0	71.6
2″	83.5	68.5	70.9	70.6	72.6	75.1
3″	74.4	79.7	78.7	78.2	75.7	79.9
4″	81.2	67.9	70.6	70.3	70.5	70.7
5″	68.5	81.4	81.9	78.3	82.1	81.8
6″	63.6	60.4	61.3	64.0	61.0	61.1
–$COCH_3$	20.8	20.4		20.6	20.5	
–C=O	169.6	169.8		170.4	169.3	
2″–O–Rha						
1‴						100.3
2‴						70.2
3‴						71.5
4‴						70.4
5‴						68.2
6‴						17.7

EXPERIMENTAL

General Procedures

Melting points were taken on an X4 micro-melting point apparatus and are uncorrected. UV spectra were determined with a Hitachi UV-2201 spectrophotometer and IR spectra with a Shimadzu (KBr) IR spectrophotometer. ^{1}H, ^{13}C and 2D NMR spectra were measured on a Bruker ARX at 300 MHz for ^{1}H and 75 MHz for ^{13}C with TMS as an internal standard, and coupling constants (J) are given in Hz. Mass spectra were recorded on a Finnigan ESIMS spectrometer. Optical rotations were measured on a Perkin–Elmer 241 digital polarimeter in CH_3OH. The HR-mass spectra were recorded on a VG-Autospec-3000 spectrometer.

Plant Material

The leaves of *Crataegus pinnatifida* Bge. var. *major* were collected from Liaoning Province of China, in June 1997. A voucher specimen (970706) was taxonomically identified by Professor Chunquan Xu and deposited in the Herbarium of the Department of Traditional Chinese Medicines, Shenyang Pharmaceutical University.

Extraction and Isolation

The leaves (20 kg) were first extracted 3×90 l with 80% EtOH under reflux. The extracts were combined and concentrated under reduced pressure. The concentrated extract was suspended in water and was subjected to macroporous resin column chromatography, eluting with 55% EtOH. The solvent was evaporated under reduced pressure to obtain a mixture (120 g). The mixture was chromatographed on a silica gel column, eluting with $CHCl_3$–CH_3OH (in gradient) to obtain 10 fractions (Fr 1–10). The first fraction (non-polar compounds) and the MeOH fraction were not investigated further. Fr 2–4 were investigated to obtain pinnatifinosides A–D [7] and pinnatifin A, B [8]. Fr 5 (10 g, $CHCl_3$–$CH_3OH = 10{:}1$) was rechromatographed on a silica gel column with $CHCl_3$–CH_3OH (in gradient) as eluent to yield two fractions, Fr 5-1 ($CHCl_3$–$CH_3OH = 20{:}1$–$10{:}1$) and Fr 5-2 ($CHCl_3$–$CH_3OH = 9{:}1$–$7{:}1$). Fr 5-1 was subjected to polyamide column chromatography with H_2O–EtOH (1:1) to yield **1** (8 mg). Fr 5-2 was subjected to polyamide column chromatography with H_2O–EtOH (3:2) to yield **2** (50 mg), **4** (200 mg), and **5** (80 mg). Fr **6** was subjected to polyamide column chromatography with H_2O–EtOH to yield **3** (30 mg) (H_2O–EtOH = 3:1), Fr 8 (11 g) was crystallized in $CHCl_3$–CH_3OH to give **6** (500 mg).

Compound **1**: yellow needles; mp 220–222°C; $[\alpha]_D^{25}$: + 63.2 (c 0.10, MeOH); HR-FAB-MS: $m/z = 474.1151$ (calcd for $C_{23}H_{22}O_{11}$: 474.1162); IR (KBr): $\nu_{max} = 3374$, 1650, 1712, 1608 and 1500 cm^{-1}; UV (MeOH): λ_{max} 333, 270 nm; λ_{max} (NaOMe) 390, 277 nm; λ_{max} (NaOAc) 376, 276 nm; λ_{max} ($AlCl_3$ and $AlCl_3$ + HCl) 380, 276 nm; ESI-MS: $m/z = 475$ $[M + H]^+$, 457 [475–18], 415 [475–60], 355 [475–120], 313 [355–42], 284 [313–29], 256 [284–28]; ^{1}H, ^{13}C NMR spectra (C_5D_5N) see Table I.

Compound **2**: light yellow needles; mp 194–196°C; $[\alpha]_D^{25}$: − 14.6 (c 0.12, MeOH); HR-FAB-MS: $m/z = 474.1188$ (calcd for $C_{23}H_{22}O_{11}$: 474.1162); UV (MeOH): λ_{max} 329, 269 nm; λ_{max} (NaOMe) 392, 279 nm; λ_{max} ($AlCl_3$) 380, 276 nm and λ_{max} ($AlCl_3$ + HCl) 381, 277 nm; IR (KBr): $\nu_{max} = 3371$, 1710, 1654, 1604 and 1520 cm^{-1}; ESI-MS: $m/z = 475$ $[M + H]^+$, 457 [475–18], 409 [457–48], 367 [409–42], 283 [367–84]; ^{1}H and ^{13}C NMR data see Tables I and III.

Compound **2** was heated with 0.1% KOH for 2 h at 80°C. The product of hydrolysis was identical with vitexin by polyamide TLC.

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