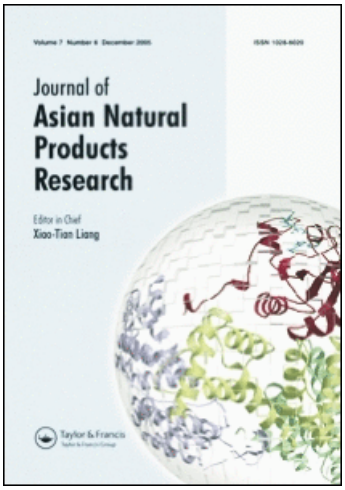

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Two Novel Flavonoid Glycosides from *Crataegus Pinnatifida*

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TWO NOVEL FLAVONOID GLYCOSIDES FROM *CRATAEGUS PINNATIFIDA* BGE.VAR. *MAJOR* N.E.BR.

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Two novel natural products, namely pinnatifida C, pinnatifida D, were isolated from the leaves of *Crataegus pinnatifida* Bge.var.*major* N.E.Br. Their structures were elucidated by the spectroscopic analysis and chemical evidence.

Keywords: *Crataegus pinnatifida* Bge.var.*major* N.E.Br.; *Rosaceae*; Flavonoid glycosides; Pinnatifin C; Pinnatifin D

INTRODUCTION

Crataegus pinnatifida Bge.var.*major* N.E.Br. has been taxonomically consigned to *Rosaceae*, which is widely distributed in the northeast part of China. It is used as medicine plant to improve digestion, remove retention of food, promote blood circulation and resolve blood stasis both in official and traditional folk medicine [1]. At the same time, it shows numerous mild, but well documented pharmacological activities and few side effects. Preparations of *Crataegus pinnatifida* Bge.var.*major* (leaves or fruit) improve the heart function, and their indications are cases of declining cardiac performance, deficiency of the coronary blood supply and mild forms of arrhythmia [2–4]. There were about fifty flavonoids isolated from *Crataegus* [5,6]. In our work, the chemical investigation of the leaves of *Crataegus*

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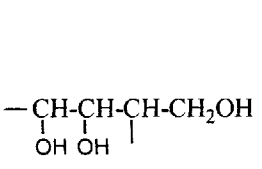
pinnatifida Bge.var.*major* N.E.Br. has led to isolation of two new compounds, which were named as pinnatifin C(**1**), pinnatifin D(**2**).

RESULTS AND DISCUSSION

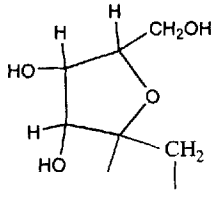
1 was obtained as yellow needles, and positive to Mg–HCl color reaction. The absorption bands at 3370, 1650 and 1600, 1508 cm^{-1} in IR spectrum were typical of hydroxyl, chelated carbonyl and aromatic groups, respectively. UV spectra of compound **1** exhibited absorption maxima at λ_{max} 325 (Band I) and 271 (Band II) (in MeOH), bathochromic shifts of 66, 41, 66 and 66 nm with NaOMe, NaOAC, $AlCl_3$ and $AlCl_3$ + HCl in Band I respectively, as well as bathochromic shifts of 8 nm with $AlCl_3$ and $AlCl_3$ + HCl in Band II. These data suggested that compound **1** had two free hydroxy groups at C-5, 4′ and no free hydroxyl group at C-7 in the flavone skeleton. In the ^{1}H-NMR spectrum, a downfield proton at δ 13.87 confirmed the presence of 5-OH, two set of doublets at δ 7.87 (2H, d, $J = 8.4$) and 7.29 (2H, d, $J = 8.4$) assigned to H-2′,6′ and H-3′,5′ respectively and two high field proton singlets at δ 6.92 and 6.47 were due to the protons in A and C rings which indicated aglycone moiety was analogous to apigenin except for the absence of one proton in A ring. In the ^{13}C-NMR spectrum (see Tab. I), 15 carbon signals from aglycone moiety were similar to those of vitexin [7] and 6 carbon signals from sugar moiety were supplied. In addition, ESI-MS exhibited a quasimolecular ion peak at *m/z*: 415 $(M + H)^+$. Thus, a molecular formula of $C_{21}H_{18}O_9$ for **1** was deduced. It was essential to clarify the type and configuration of sugar moiety. In the DEPT spectrum, carbon signals from sugar moiety were at δ 118.0 (C), 83.7 (CH), 79.6 (CH), 73.5 (CH), 63.2 (CH_2) and 32.7 (CH_2). Furthermore, carbon signal at δ 32.7 was directly attached to protons at δ 3.50 (1H, d, $J = 16.5$) and 3.34 (1H, d, $J = 16.5$). Consequently, the sugar was a ketohexose. In the ^{1}H–^{1}H COSY spectrum, proton at δ 3.87 (1H, t) correlated with protons at δ 3.98 (1H, d) and 3.80 (1H, m) and proton at δ 3.80 (1H, m) correlated with protons at $\delta \sim 3.75$ (2H, m). Combining with HMQC spectrum, it confirmed the presence of fragment A (Scheme 1). Furthermore, long range correlations between carbon signal at δ 118.0 and protons at δ 3.98 (1H, d), 3.50 (1H, d), 3.34 (1H, d), and 3.80 (1H, m) in the HMBC spectrum (Tab. I) assured the presence of a furan-ring fragment B consisted of a quarternary carbon and fragment A (Scheme 1). Cross peaks between protons at δ 3.98 (1H, d) and 3.80 (1H, m), at δ 3.87 (1H, t) and $\sim$3.50 (2H, m), and at δ 3.50 (1H, d) and 3.98 (1H, d) in the NOESY spectrum suggested the

TABLE I NMR data of **1** and **2** (DMSO-d_6)

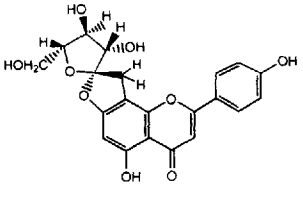
	1			**2**	
Position	δ_C	δ_H (*Hz*)	*HMBC*1	δ_C	δ_H (*Hz*)
2	164.1		2′-H, 6′-H, 3-H	163.9	
3	102.8	6.83s		102.8	6.83s
4	181.8		3-H	181.9	
5	161.7	13.20s (OH)	6-H, 5-OH	161.8	13.21s (OH)
6	93.8	6.31s	5-OH	93.7	6.33s
7	163.3		6-H, 1″-H	163.4	
8	102.2		6-H, 1″-H	102.1	
9	151.3		1″-H	151.4	
10	104.0		3-H, 6-H, 5-OH	104.1	
1′	120.9		3-H	120.9	
2′	128.4	7.95d (8.4)	6′-H	128.4	7.94d (8.4)
3′	115.9	6.94d (8.4)	5′-H	115.9	6.95d (8.4)
4′	161.2	10.40s (OH)	2′,6′-H, 3′,5′-H	161.2	10.40s (OH)
5′	115.9	6.94d (8.4)	3′-H	115.9	6.93d (8.4)
6	128.4	7.95d (8.4)	2′-H	128.4	7.95d (8.4)
1″	32.7	3.50d (16.5), 3.34d (16.5)		32.5	3.49d (16.5), 3.34d (16.5)
2″	118.0		1″-H, 3″-H, 5″-H	117.8	
3″	79.6	3.98t (6.6)	1″-H, 4″-H	78.9	~4.00 m
4″	73.5	3.87 m	3″-H, 5″-H, 6″-H	73.3	~4.00 m
5″	83.7	3.80 m	4″-H, 6″-H	80.0	4.00 m
6″	63.2	~3.50 m	4″-H	64.5	4.37d (9.3), ~4.00
$-CH_3$				20.6	2.03
$-C=O$				170.3	



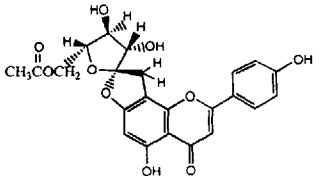
A



B



C (**1**)



D(**2**)

SCHEME 1 Structures of fragment A, B, compounds **1** and **2**.

configuration of sugar moiety as that of β-D-frucofuranose [8]. The connectivity of aglycone and sugar moiety was established on the basis of the following evidence. A downfield quarternary carbon (δ 118.0) of fructosyl C-2 should be linked to position C-7 due to the absence of free 7-OH in UV and ^{1}H-NMR spectra. Carbon signal (δ 32.7) of fructosyl C-1 was linked to C-8, instead of C-6, of flavone skeleton, which was confirmed by the HMBC spectrum: carbon signal at δ 151.3 (C-9) was correlated with the protons at δ 3.50, 3.34 (H-1″). Thus, the structure of the compound was elucidated as C (Scheme 1). It is a rare flavonoid in nature, here named as pinnatifin C.

Compound **2** was obtained as light yellow needles. It was identical with compound **1** in Mg–HCl color reaction, UV spectrum and IR spectrum except for absorption band at 1710 cm^{-1}. ^{1}H-NMR, ^{13}C-NMR spectra of compound **2** were also analogous to those of **1** (Tab. I). But a methyl singlet at δ 2.03 (s) in the ^{1}H-NMR spectrum and two carbon signals at δ 20.6, 170.1 in the ^{13}C-NMR spectrum of **2** indicated the presence of an acetyl group. Absence of a free hydroxyl group at δ 4.85 of sugar moiety in ^{1}H-NMR spectrum, typical downfield shift of C-6″ at δ 64.5 (Δ δ 1.3) and highfield shift of C-5″ at δ 80.0 (Δ δ 3.7) in the ^{13}C-NMR spectrum, when compared with those of **1**, showed the acylation of C6″-OH. ESI-MS gave an expected quasimolecular ion peak at *m/z*: 457 (M + 1), corresponding a molecular formula: $C_{23}H_{20}O_{10}$. Alkaline hydrolysis of **2** with 0.1% KOH afforded **1**. Thus, **2** was established as D (Scheme 1). As far as we know, it is also a new compound, named as pinnatifin D.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were taken on an X4 micro-melting point apparatus and are uncorrected. UV spectra were determined with Hitachi UV-2201 spectrophotometer and IR spectra with a Shimadzu (KBr) IR spectrophotometer. ^{1}H-, ^{13}C-NMR and 2D-NMR spectra were measured on Bruker ARX 300 with TMS as an internal standard and coupling constant (J) in Hz. Mass spectra were recorded on Finnigan ESI-MS 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 Prof. Xu Chunquan and deposited in the Herbarium of Department of Chinese Traditional Medicines, Shenyang Pharmaceutical University.

Extraction and Isolation

The leaves (20 kg) were first extracted with 80% EtOH under reflux for three times. The extract solutions 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. The obtained mixture 120 g was chromatographed on silica gel column eluting with $CHCl_3$–CH_3OH (in gradient) to get Fr3 and Fr4. Fr3 (3g, $CHCl_3$–$CH_3OH = 20:1$) was subjected to polyamide column chromatography with H_2O–EtOH (1 : 1) to yield **2**. Fr4 ($CHCl_3$–$CH_3OH = 10:1$) was rechromatographed on silica gel column with $CHCl_3$–CH_3OH (in gradient) as eluent, then the combined parts ($CHCl_3$–$CH_3OH = 15:1 \sim 9:1$) were subjected to polyamide column chromatography with H_2O–EtOH (1 : 1) to yield **1**.

1 yellow needles; m.p. 263–265°C; IR (KBr) cm^{-1} 3400–3300, 1650, 1606 and 1502; UV (MeOH) λ_{max} 325, 271 nm; λ_{max} (MeONa) 391, 271 nm; λ_{max} (NaAC) 366, 271 nm; λ_{max} (AlCl3) 391, 279 nm and λ_{max} ($AlCl_3$ + HCl) 391, 279 nm; ESI-MS *m/z*: 415, 397, 367 and 283; ^{1}H-,^{13}C-NMR and HMBC spectra see Table I.

2 light yellow needles; m.p. 251–253°C; IR (KBr) cm^{-1} 3400–3300, 1710, 1650, 1606 and 1520; UV (MeOH) λ_{max} 324, 271 nm; λ_{max} (MeONa) 392, 271 nm; λ_{max} ($AlCl_3$) 391, 280 nm and λ_{max} ($AlCl_3$ + HCl) 391, 280 nm; ESI-MS *m/z*: 457, 439, 379, 283 351, 323 and 295; ^{1}H-,^{13}C-NMR data see Table I.

Alkaline Hydrolysis of **2**: **2** was heated with 0.1% KOH for 0.5 h at 60°C. The hydrolysate was neutralized with 0.1 N HCl and extracted by EtOAC. EtOAC extract was evaporated, and the residue was subjected to polyamide TLC using $CHCl_3$: MeOH : MeCOEt (4 : 1 : 0.5) and EtOH : H_2O (1 : 1) as solvent, respectively. The product of hydrolysis was identical with pinnatifin C by their Rf values.

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