# Aquiledine and Isoaquiledine, Novel Flavonoid Alkaloids from *Aquilegia ecalcarata*

Si-Bao Chen,*,† Guang-Yao Gao,† Hei-Wun Leung,‡ Hin-Wing Yeung,‡ Jun-Shan Yang,† and Pei-Gen Xiao†

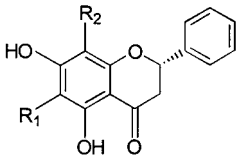
*Institute of Medicinal Plant Development, Peking Union Medical College & Chinese Academy of Medical Sciences, 100094 Beijing, People's Republic of China, and Department of Chemistry and the Institute for the Advancement of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong*

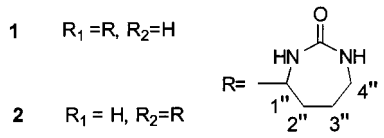
*Received May 24, 2000*

Two new flavonoid alkaloids, aquiledine (**1**) and isoaquiledine (**2**), were isolated from the whole herb of *Aquilegia ecalcarata*, and their structures were determined by detailed spectral analysis. Three known flavonoids were also isolated from this extract—apigenin, apigenin-7,4′-dimethyl ether, and luteolin—and have not been reported previously from *A. ecalcarata*.

*Aquilegia ecalcarata* Maxim. (Ranunculaceae) is a commonly used Chinese folk medicine widely distributed throughout the western regions of the People's Republic of China.[1] According to Chinese medical theory, the whole plant of *A. ecalcarata* has the effect of detoxifying the body and aiding tissue regeneration and is used for the treatment of various kinds of infections.[2]

There have been no previous literature reports on the constituents of *A. ecalcarata*. In our chemical investigation on the plant, two new flavonoid alkaloids, aquiledine (**1**) and isoaquiledine (**2**), have been isolated along with three known flavonoids. Flavonoid alkaloids belong to a very small class of natural products, which have been reported only rarely in the literature. The few reported include *N*-methylpyrrolidinoflavone and the pyrrolidinoflavone alkaloids ficine,[3] isoficine,[3] phyllospadine,[4] lilaline,[5] and vochysine.[6]




Aquiledine (**1**) was isolated as a white amorphous powder. The UV spectrum showed absorption maxima at 288 and 362 nm (sh), with an obvious bathochromic shift of the 288 nm absorption band to 328 nm after addition of sodium acetate, and to 310 nm after the addition of aluminum chloride with or without hydrochloric acid. These spectral observations suggested that **1** possesses a structure with hydroxyl moieties located in the C-5 and C-7 positions but without any *ortho*-coupled hydroxyl groups.[7] The IR spectrum displayed strong absorptions at 3400 and 1650 $cm^{-1}$, which indicated the presence of a hydroxyl group and a carbonyl group. The HREIMS displayed a molecular ion at *m/z* 368 (obsd 368.1371, calcd 368.1372 for $C_{20}H_{20}O_5N_2$), with significant or intense fragment ions at *m/z* 324 (base peak) (obsd 324.1226, calcd 324.1236 for $C_{19}H_{18}O_4N$) and at *m/z* 256 (obsd 256.0727, calcd 256.0736 for $C_{15}H_{12}O_4$). The EIMS gave a distinct peak for a flavanone fragment at *m/z* 256, and two typical daughter ions at *m/z* 152 ($[A_1]^+$) and 104 ($[B_1]^+$).[8] The $^1H$ NMR spectrum (DMSO-$d_6$) (Table 1) of **1** indicated the presence of hydroxyl signals at $\delta$ 12.73 and 11.11 for OH-5 and OH-7, respectively. A one-proton singlet at $\delta$ 5.98 was attributable to H-8. The five-proton multiplet signals at $\delta$ 7.45 were assigned to the five aromatic protons of the B ring. Signals at $\delta$ 3.25 (1H, dd, $J$ = 17, 12 Hz) and $\delta$ 2.75 (1H, dd, $J$ = 17, 3 Hz) were assigned to H-3. The one-proton double-doublet at $\delta$ 5.54 (1H, dd, $J$ = 12, 3 Hz) was assigned to H-2. The $^{13}C$ NMR spectrum (Table 1) indicated the presence of 15 distinct carbon resonances of the flavanone moiety, including one carbonyl carbon ($\delta$ 196.3, C-4) and two alicyclic carbons ($\delta$ 78.2, C-2 and $\delta$ 42.1, C-3). The aromatic region contained a total of 12 resonances according to the HMQC and DEPT spectra. These were six tertiary carbons ($\delta$ 94.9, C-8; $\delta$ 126.5, C-2′, C-6′; $\delta$ 128.5, C-3′, C-4′, C-5′) and six quaternary carbons ($\delta$ 160.9, 109.3,

**Table 1.** $^{13}C$ and $^1H$ NMR Data for Compounds **1** and **2**[a]

| | **1** | | **2** | |
|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 2 | 78.2 | 5.54 dd (12, 3) | 78.6 | 5.52 dd (12, 3) |
| 3 | 42.1 | 2.75 dd (17, 3) | 41.9 | 2.76 dd (17, 3) |
| | | 3.25 dd (17, 12) | | 3.08 dd (17, 12) |
| 4 | 196.3 | | 196.3 | |
| 5 | 160.9 | | 161.4 | |
| 6 | 109.3 | | 95.9 | 5.97 s |
| 7 | 164.4 | | 164.3 | |
| 8 | 94.9 | 5.98 s | 109.1 | |
| 9 | 160.8 | | 160.0 | |
| 10 | 101.6 | | 102.0 | |
| 1′ | 138.6 | | 138.5 | |
| 2′,6′ | 126.5 | 7.45 m | 126.5 | 7.42 m |
| 3′,4′,5′ | 128.5 | | 128.4 | |
| 1″ | 50.6 | 5.03 dd (7, 3.5) | 51.0 | 5.02 dd (7, 3.5) |
| 2″ | 31.6 | 2.05 m | 31.6 | 2.09 m |
| 3″ | 24.6 | 1.85 m | 24.6 | 1.69 m |
| 4″ | 46.5 | 3.42 m | 46.6 | 3.23 m |
| 5″ | 156.8 | | 157.2 | |
| OH-5 | | 12.73 s | | 12.18 s |
| OH-7 | | 11.11 s | | 11.13 s |
| NH | | 5.34 br s | | 5.29 br s |

[a] $\delta$ (ppm); 500 MHz; DMSO-$d_6$; $J$ values (Hz) in parentheses.

* To whom correspondence should be addressed. Present address: Institute for the Advancement of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong. Tel.: (852) 2339-5573. Fax: (852) 2339-5317. E-mail: chensibao@hotmail.com.
† Peking Union Medical College & Chinese Academy of Medical Sciences.
‡ Hong Kong Baptist University.

10.1021/np000256i CCC: $20.00 
Published on Web 11/28/2000

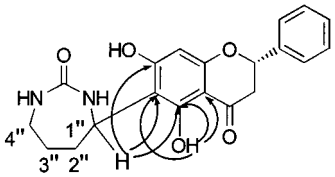



**Figure 1.** Principal HMBC correlations of **1**.

164.4, 160.8, 101.6, and 138.6 for C-5, C-6, C-7, C-9, C-10, and C-1′, respectively).

Residual resonances in the $^1$H and $^{13}$C NMR spectra of **1** were observed in the relatively upfield region. It was considered that the two broad singlets at $\delta$ 5.34 corresponded to the −NH unit of an ureido moiety due to their absence of cross-peaks in the HMQC spectrum. The $^1$H−$^1$H COSY and HMQC spectra revealed the presence of the system −CHR−$CH_2$−$CH_2$−$CH_2$− in the residual fragment and the signal at $\delta$ 5.03 was assigned to H-1″ (dd, $J$ = 7, 3.5 Hz), while the others at $\delta$ 2.05 (m, 2H), 1.85 (m, 2H), and 3.42 (m, 2H) were attributable to H-2″, H-3″, and H-4″, respectively. The $^{13}$C NMR spectrum showed five carbon resonances assigned, respectively, to $\delta$ 156.8 for the carbonyl carbon of the urea moiety and $\delta$ 50.6, 31.6, 24.6, and 46.5 due to the alicyclic carbons C-1″, C-2″, C-3″, and C-4″. All these observations indicated this moiety to be a butylene urea in **1**.

The DEPT spectrum of **1** showed that C-6 was a quaternary carbon, while C-8 was a methine carbon, which indicated that C-6 was substituted. Additionally, the signal of C-6 ($\delta$ 109.3) was observed in a more downfield region than C-8 ($\delta$ 94.9), which suggested that C-6 was substituted by the urea moiety.[9] The HMBC spectrum of **1** (Figure 1) permitted the placement of the flavanone group and the urea moiety. A long-range correlation between the signals at C-5 ($\delta$ 160.9) and H-1″ ($\delta$ 5.03) also supported the structure proposed for **1**. According to the HMBC spectrum, the signal for OH-5 ($\delta$ 12.73) should have a long-range correlation with C-6, but not with C-8. Thus, the cross-peaks between the signals at $\delta$ 12.73 and at $\delta$ 109.3 confirmed that the latter signal could be assigned to C-6, which was a quaternary carbon as determined from the DEPT and HMQC spectra. Therefore, by a process of elimination, the urea moiety was demonstrated to be linked with the flavanone group through C-1″ to C-6, so the planar structure of **1** was determined as 6-(1,4-ureylenebutyl)-5,7-dihydroxyflavanone.

The relative stereochemistry of **1** could be determined by the coupling constant of H-2−H-3. The coupling constant between H-2 and H-3 showed that $J_{aa}$ was 12.0 Hz and $J_{ae}$ was 3.0 Hz, which revealed that H-2 was sited in an axial position with α-stereochemistry.[10,11] The presence of the α-stereochemistry for H-2 is usual in the flavanones, but occurs in compounds such as scuteamoenoside[12] and scuteamoenin.[13] In comparison to these two flavanones, the $^1$H NMR chemical shifts and coupling constants of H-2 and H-3 in **1** were similar. Accordingly, **1** was assigned with the structure (2*S*)-6-(1,4-ureylenebutyl)-5,7-dihydroxyflavanone.

The NMR (Table 1) and other spectral data of **2** were very similar to those of **1**. However, the TLC $R_f$ values and melting point were different, which indicated that **2** was an isomer of **1**. From the DEPT and HMQC data of **2**, it was determined that C-6 was a methine carbon, and C-8 was a quaternary carbon, suggesting that the urea moiety must be at C-8. The HMBC spectra of **2** also supported this placement of the urea moiety, because there were coupling peaks between the signals at H″-1 ($\delta$ 5.03) and OH-7 ($\delta$ 11.13) to C-8 ($\delta$ 109.1). Thus, it could be deduced that compound **2** is the 8-isomer of **1**, (2*S*)-8-(1,4-ureylenebutyl)-5,7-dihydroxyflavanone.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected. The optical rotations were determined in $CH_3OH$ at 20 °C, using a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Philips Pye Unicam PU8800 spectrophotometer, while the IR spectra were determined on a Perkin-Elmer 983G spectrophotometer. The NMR spectra were recorded on a Bruker AM-500 spectrometer at 500 MHz, using DMSO-$d_6$ as solvent and TMS as internal standard. MS measurements were carried out on a ZABSPEC spectrometer. Column chromatography was carried out on Si gel (120−180 mesh; Merck, Darmstadt, Germany), and column fractions were monitored with TLC over precoated sheets of Si gel 60 $F_{254}$ (0.2-mm layer thickness) (E. Merck) under UV light (254 and 365 nm). Preparative TLC was accomplished using 20 × 20 cm glass plates precoated with 1.0 mm Si gel $GF_{254}$. For column chromatography, polyamide and Sephadex LH-20 (Pharmacia) were employed.

**Plant Material.** *A. ecalcarata* was collected in August 1997, from Kang-Ding County, Si-Chuan Province, People's Republic of China, and identified by Dr. Bao-Lin Guo, of the Institute of Medicinal Plant Development, Peking Union Medical College & Chinese Academy of Medical Sciences, where a voucher specimen (no. 97053) is maintained.

**Extraction and Isolation.** The air-dried, whole plant (9.0 kg) was extracted by percolation with petroleum ether, and then subsequently extracted with 95% EtOH. The EtOH extract residue (950 g) was partitioned between $CH_2Cl_2$ and $H_2O$. The $CH_2Cl_2$ residue (50 g) was chromatographed on a polyamide column with the use of mixtures of $H_2O$−MeOH (4:1, 3:2, 2:3, 1:4) in sequence as eluents. The fractions obtained with $H_2O$−MeOH (3:2, 2:3) were combined and subjected to Si gel vacuum−liquid chromatography, using a gradient system of cyclohexane and cyclohexane−EtOAc (9:1, 4:1, 7:3, and 1:1). The fractions obtained with cyclohexane−EtOAc (4:1) were subjected to preparative TLC, using the solvent system of $CHCl_3$− MeOH (95:5). This led to the purification of **1** (9 mg) and **2** (7 mg). From fractions obtained with cyclohexane−EtOAc (7:3), apigenin[14] (20 mg) and apigenin-7,4′-dimethyl ether[15] (13 mg) were isolated by Sephadex LH-20 chromatography, while luteolin[14] (15 mg) was obtained from the fractions obtained with cyclohexane−EtOAc (1:1).

**Aquiledine (1):** white amorphous powder ($CH_3OH$), mp 214−215 °C; $[\alpha]_D$ +21° (*c* 0.54, $CH_3OH$); UV $\lambda_{max}$: 288 (II), 368 (sh, I) nm (MeOH); 248 (sh), 326 (II) nm (NaOAc); 310 (II), 368 (sh, I) nm (MeOH + $AlCl_3$ and MeOH + $AlCl_3$ + HCl); IR (KBr) $\nu_{max}$ 3400, 3240 (broad, OH, NH), 1650 (C═O), 1600, 1500, 1460, 1350, 1320, 1190, 1100, 900 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS *m*/*z* [M]$^+$ 368 (8), [M − 18]$^+$ 350 (5), [M − $CONH_2$]$^+$ 324 (100), 325 (45), [M − $CONH_2$ − CNH]$^+$ 297 (40), 308 (20), 282 (35), 256 (15), 220 (55), 193 (16), 178 (13), 165 (13), 152 (10), 104 (14), 91 (5), 77 (10); HREIMS *m*/*z* 368.1371 (calcd 268.1372 for $C_{20}H_{20}O_5N_2$), 324.1226 (calcd 324.1236 for $C_{19}H_{18}O_4N$), 256.0727 (calcd 256.0736 for $C_{15}H_{12}O_4$); TLC, $R_f$, 0.62 ($CHCl_3$−MeOH, 95:5).

**Isoaquiledine (2):** white amorphous powder ($CH_3OH$), mp 232−233 °C; $[\alpha]_D$ +19° (*c* 0.43, $CH_3OH$); UV $\lambda_{max}$ 286 (II), 322 (sh, I) nm (MeOH); 286, 346 (sh) nm ($H_3BO_3$ + NaOAc); 306, 362 nm ($AlCl_3$ and $AlCl_3$ + HCl); IR (KBr) $\nu_{max}$ 3420, 3220, 1640, 1600, 1560, 1440, 1380, 1260, 1180, 980 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS *m*/*z* 368 (10), 325 (20), 324 (8), 297 (43), 256 (100), 220 (20), 179 (75), 165 (35), 152 (70), 124 (35), 104 (25), 91 (10), 77 (20), 69 (33); HREIMS *m*/*z* 368.1369 (calcd 368.1372 for $C_{20}H_{20}O_5N_2$), 324.1225 (calcd 324.1236 for $C_{19}H_{18}O_4N$), 256.0724 (calcd 256.0736 for $C_{15}H_{12}O_4$); TLC, $R_f$, 0.50 ($CHCl_3$−MeOH, 95:5).

**Acknowledgment.** This investigation was funded by the National Natural Science Foundation of the People's Republic of China no. 39570085.

## References and Notes

(1) Wang, W. C.; Xiao, P. G. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, 1979; Vol. 27, p 465.
(2) Wu, Z. Y.; Zhou, T. Y.; Xiao, P. G. *Xin Hua Compendium of Materia Medica*; Shanghai Science & Technology Press: Shanghai, 1998; Vol. 1, p 113.
(3) Johns, S. R.; Russel, J. H. *Tetrahedron Lett.* **1965**, *24*, 1987−1991.
(4) Baudouin, G.; Tillequin, F.; Koch, M.; Vuilhorgne, M.; Lallemand, J. Y.; Jacquemin, H. *J. Nat. Prod.* **1983**, *46*, 681−687.
(5) Takagi, M.; Funahashi, S.; Ohta, K.; Nakabayashi, T. *Agric. Biol. Chem.* **1980**, *44*, 3019−3020.
(6) Masterova, I.; Uhrin, D.; Tomko, J. *Phytochemistry* **1987**, *26*, 1844−1845.
(7) Shanghai Institute of Materia Medica, Chinese Academy of Sciences. *Handbook of Identification of Flavonoids*; Science Press: Beijing, 1981; p 399.
(8) Cong, P. Z. *Application of Mass Spectrometry in Natural Organic Chemistry*; Science Press: Beijing, 1987; pp 482−488.
(9) Wang, X. K. *Natural Medicine Chemistry*; People's Health Press: Beijing, 1986; p 307.
(10) Yu, D. Q.; Yang, J. S. *Handbook of Analytical Chemistry: Analysis of Nuclear Magnetic Resonance Spectra*; Chemical Industry Press: Beijing, 1999; Vol. 7, Chapter 3, pp 38−40.
(11) Liang, X. T. *Nuclear Magnetic Resonance Spectrometry*; Science Press: Beijing, 1982; pp 291−292.
(12) Hu, B. H.; Liu, Y. L. *Acta Pharm. Sin.* **1989**, *24*, 200−206.
(13) Hu, B. H.; Liu, Y. L.; Zhang, T.; Song, W. Z. *Acta Pharm. Sin.* **1990**, *25*, 302−306.
(14) Chen, S. B.; Wang, L. W.; Gao, G. Y.; Liao, M. C.; Xiao, P. G. *China J. Chin. Mater. Med.* **1999**, *24*, 158−160.
(15) Chen, C. C.; Huang, Y. L.; Sun, C. M.; Shen, C. C. *J. Nat. Prod.* **1996**, *59*, 412−414.

NP000256I