Chemistry of Natural Compounds, Vol. 46, No. 4, 2010

FLAVONOIDS FROM *Anemone tomentosa* ROOTS

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UDC 547.972

The genus *Anemone* (Ranunculaceae) comprises about 150 species, of which 52 are distributed in northern China. As triterpenoid saponins are the main effective components of the genus *Anemone*, it has extensive biological activities, such as antitumor, antibacterial, and insect antifeeding properties. *Anemone tomentosa* (Maxim.) Pei is a perennial herb that is commonly called "Da Huo Cao" in China [1]. Its roots have been used as traditional Chinese medicine for the treatment of dysentery, malaria, infantile malnutrition, carbuncles, etc. [2]. Previous phytochemical studies of *A. tomentosa* have led to the isolation of coumarins [3], triterpenoids, and sterols [4, 5], but there are no records of the flavonoid constituents. Herein we communicate the additional isolation and structural elucidation of eight known flavonoids **1–8**, of which **1**, **4**, and **6** were isolated from plants of the genus *Anemone* for the first time. Flavonoids were found first in *A. tomentosa*.

Roots of *A. tomentosa* were collected in September 2007 from Ziwuling Mountain areas of Gansu Province (China) and identified by Prof. Xiaoqiang Guo, Department of Life Science, Longdong University.

Ground air-dried roots (5 kg) of *A. tomentosa* were extracted at room temperature with 80% aq. EtOH (2 × 25 L). The residue (448 g) was partitioned between H_2O and petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The EtOAc fraction (103 g) was chromatographed over silica gel with gradient elution by *n*-hexane:EtOAc (25:1→15:1) and $CHCl_3$:MeOH (10:1→2:1) and monitoring by TLC, in order to produce thirteen subfractions (E1-E13). Subfractions E2 and E8 were applied to a Sephadex LH-20 column with elution by MeOH, and recrystallized to give compounds **1** (30 mg) and **4** (12 mg). The *n*-BuOH extract (92 g) was subjected to repeated silica gel column chromatography by elution with MeOH:$CHCl_3$ (3:10→2:1) and monitoring by TLC, in order to produce sixteen subfractions (B1-B16). Subfractions B2, B4, B7, B8, B10, and B12 were repeatedly subjected to silica gel or Sephadex LH-20 column chromatography and preparative TLC, and recrystallized to afford compounds **2** (45 mg), **3** (19 mg), **5** (8 mg), **6** (21 mg), **7** (32 mg), and **8** (13 mg).

Compound 1, $C_{15}H_{10}O_7$, yellow powder (CH_3COCH_3), mp 310–312°C, positive response to Mg–HCl, $AlCl_3$, and $FeCl_3$–$K_3Fe(CN)_6$ tests, negative response to Molisch test. UV spectrum (MeOH, λ_{max}, nm): 256, 302, 376. IR (KBr, ν, cm^{-1}): 3409 (OH), 1663 (C=O), 1612, 1563, 1520 (Ar). Spectral data (EI-MS, PMR, and ^{13}C NMR) for **1** agreed with those reported in the literature for quercetin [6].

Compound 2, $C_{21}H_{20}O_{12}$, yellow needles (MeOH), mp 221–223°C, positive response to Mg–HCl and Molisch tests. UV spectrum (MeOH, λ_{max}, nm): 207, 252, 301, 354. IR (KBr, ν, cm^{-1}): 3398 (OH), 1656 (C=O), 1603, 1560, 1493 (Ar). MS (FAB, *m/z*): 465 $[M + H]^+$, 303 $[M + H - Glc]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.61 (1H, s, HO-5), 11.24 (1H, s, HO-7), 9.71 (1H, s, HO-4′), 9.21 (1H, s, HO-3′), 7.53 (1H, d, J = 2.0, H-2′), 7.41 (1H, dd, J = 2.0, 8.4, H-6′), 6.91 (1H, d, J = 8.4, H-5′), 6.37 (1H, d, J = 2.0, H-8), 6.24 (1H, d, J = 2.0, H-6), 5.31 (1H, d, J = 7.5, H-1″), 3.99 (1H, dd, J = 2.1, 12.0, H-6″a), 3.76 (1H, dd, J = 6.1, 12.0, H-6″b), 3.61–3.35 (4H, m, H-2″-5″). ^{13}C NMR spectra data agreed with those reported for quercetin-3-*O*-β-D-glucopyranoside [7].

Compound 3, $C_{21}H_{20}O_{12}$, yellow crystals (MeOH), mp 245–247°C, positive response to Mg–HCl and Molisch tests. MS (FAB, *m/z*): 465 $[M + H]^+$, 303 $[M + H - Glc]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.50 (1H, s, HO-5), 7.48 (1H, d, J = 2.1, H-2′), 7.40 (1H, dd, J = 2.2, 8.5, H-6′), 6.90 (1H, d, J = 8.6, H-5′), 6.56 (1H, d, J = 2.0, H-8), 6.30 (1H, d, J = 2.0, H-6), 5.34 (1H, d, J = 7.4, H-1″), 3.85 (1H, dd, J = 2.0, 11.9, H-6″a), 3.76 (1H, dd, J = 6.1, 11.9, H-6″b), 3.65–3.31 (4H, m, H-2″-5″). ^{13}C NMR spectral data agreed with those reported in the literature for quercetin-7-*O*-β-D-glucopyranoside [8].

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Compound 4, $C_{15}H_{10}O_6$, light yellow crystals (CH_3COCH_3), mp 277–279°C, positive reaction with Mg–HCl, $AlCl_3$ and $FeCl_3$ reagent. UV (MeOH, λ_{max}, nm): 204, 264, 325, 365. IR (KBr, ν, cm^{-1}): 3402 (OH), 1658 (C=O), 1608, 1578, 1501 (Ar). MS (EI, *m/z*, %): 286 $[M]^+$ (100), 258 (12), 229 (11), 153 (15), 93 (10), 73 (12), 60 (32). The spectral data (PMR and ^{13}C NMR) for **4** agreed with those reported in the literature for kaempferol [9].

Compound 5, $C_{21}H_{20}O_{11}$, yellow crystals (MeOH), mp 211–213°C (MeOH), positive response to Mg–HCl and Molisch tests. UV spectrum (MeOH, λ_{max}, nm): 267, 296, 349. IR (KBr, ν, cm^{-1}): 3347 (OH), 1654 (C=O), 1602, 1501, 1456 (Ar). MS (FAB, *m/z*): 449 $[M + H]^+$, 287 $[M + H – Glc]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.59 (1H, s, HO-5), 8.03 (2H, dd, J = 2.8, 11.8, H-2′, 6′), 6.88 (2H, dd, J = 2.8, 11.8, H-3′, 5′), 6.42 (1H, d, J = 2.0, H-8), 6.23 (1H, d, J = 2.0, H-6), 5.36 (1H, d, J = 7.6, H-1″), 4.01 (1H, dd, J = 2.2, 12.1, H-6″a), 3.76 (1H, dd, J = 6.1, 12.1, H-6″b), 3.65–3.39 (4H, m, H-2″-5″). ^{13}C NMR spectra data agreed with those reported for kaempferol-3-*O*-β-D-glucopyranoside [8].

Compound 6, $C_{23}H_{24}O_{12}$, yellow powder (MeOH), mp 212–214°C, positive response to Mg–HCl and Molisch tests. UV (MeOH, λ_{max}, nm): 247, 352. IR (KBr, ν, cm^{-1}): 3451 (OH), 1650 (C=O), 1603, 1498, 1456 (Ar). MS (FAB, *m/z*): 493 $[M + H]^+$, 331 $[M + H – Glc]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.76 (1H, s, HO-5), 7.36 (2H, s, H-2′, 6′), 7.07 (1H, s, H-3), 6.94 (1H, d, J = 2.0, H-8), 6.47 (1H, d, J = 2.0, H-6), 5.06 (1H, d, J = 7.5, H-1″), 3.89 (6H, s, CH_3O-3′, 5′), 3.84–3.28 (5H, m, H-2″-6″). ^{13}C NMR spectral data for **6** agreed with those reported for tricin 7-*O*-β-D-glucopyranoside [10, 11].

Compound 7, $C_{21}H_{20}O_{11}$, yellow crystals (MeOH), mp 263–265°C (MeOH), positive response to Zn–HCl and Molisch tests. IR (KBr, ν_{max}, cm^{-1}): 3420 (OH), 1660 (C=O), 1608, 1510 (Ar). MS (FAB, *m/z*): 449 $[M + H]^+$, 303 $[M + H – Rha]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.52 (1H, br.s, HO-5), 7.75 (1H, d, J = 2.1, H-2′), 7.61 (1H, dd, J = 8.5, 2.1, H-6′), 6.92 (1H, d, J = 8.5, H-5′), 6.72 (1H, d, J = 1.9, H-8), 6.42 (1H, d, J = 1.9, H-6), 5.38 (1H, d, J = 1.7, H-1″), 4.32–3.38 (4H, m, H-2″-5″), 0.97 (3H, d, J = 6.1, H-6″). ^{13}C NMR spectral data agreed with those reported for quercetin-7-*O*-α-L-rhamnopyranoside [12].

Compound 8, $C_{27}H_{30}O_{16}$, pale yellow crystals (MeOH), mp 221–223°C, positive response to Zn–HCl and Molisch tests. UV spectrum (MeOH, λ_{max}, nm): 362, 257, 208. IR (KBr, ν, cm^{-1}): 3329 (OH), 1658 (C=O), 1605, 1559, 1510 (Ar). MS (FAB, *m/z*): 611 $[M + H]^+$, 465 $[M + H – Rha]^+$, 449 $[M + H – Gal]^+$, 303 $[M + H – Gal – Rha]^+$. PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 12.58 (1H, br.s, HO-5), 7.69 (1H, dd, J = 8.5, 2.0, H-6′), 7.59 (1H, d, J = 2.0, H-2′), 6.85 (1H, d, J = 8.5, H-5′), 6.70 (1H, d, J = 1.9, H-8), 6.43 (1H, d, J = 1.9, H-6), 5.49 (1H, d, J = 7.5, H-1‴), 5.31 (1H, d, J = 2.0, H-1″), 4.69–4.01 (5H, m, H-2‴-6‴), 4.25–3.36 (5H, m, H-2″-6″), 1.03 (3H, d, J = 6.1, CH_3).

^{13}C NMR spectral data for **8** agreed with those reported in the literature for quercetin-3-*O*-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside [13].

ACKNOWLEDGMENT

The authors are grateful to Prof. Xiaoqiang Guo for identifying the plant sample. The work was supported financially by the Science Research Project of Longdong University (XYZK0709).

REFERENCES

1. *Flora Reipublicae Popularis Sinicae*, Chinese Flora, Science Press, Beijing, 1980, Vol. **28**, 29 pp.
2. *Northwest Institute of Botany, Flora of Qinling*, Chinese Academy of Sciences, Science Press, Beijing, 1974, 278 pp.
3. H. R. Wang, S. L. Peng, M. K. Wang, J. T. Feng, and L. S. Ding, *Acta Bot. Sin*., **41**, 107 (1999).
4. J. R. Wang, J. Q. Ma, S. L. Peng, J. T. Feng, and L. S. Ding, *Acta Bot. Boreal. Occident. Sin*., **18**, 643 (1998).
5. X. Liao, S. L. Peng, B. G. Li, Y. Z. Chen, and L. S. Ding, *Chin. Chem. Lett*., **10**, 1035 (1999).
6. M.-H. Yang and L.-Y. Kong, *Chem. Nat. Comp*., **44**, 98 (2008).
7. R. M. Yu, X. Li, H. J. Zhang, L. J. Wu, T. R. Zhu, W. Li, L. Y. Liu, and G. Zhang, *Acta Bot. Sin*., **34**, 369 (1992).
8. K. R. Markham, B. Ternal, R. Stanley, H. Geiger, and T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
9. Y. J. Lee and T. D. Wu, *J. Chin. Chem. Soc*., **48**, 201 (2001).
10. K. D. Yoon, C. Y. Kim, and H. Huh, *Korean J. Pharmacogn*., **31**, 224 (2000).
11. Y. Hirai, S. Sanada, Y. Ida, and J. Shoji, *Chem. Pharm. Bull*., **34**, 82 (1986).
12. Z. J. Jia, G. J. Zhu, and W. H. Wang, *J. Lanzhou Univ*. (Nat. Sci. Edition), **27**, 102 (1991).
13. K. R. Markham, R. W. Hammett, and D. J. Ofman, *Phytochemistry*, **31**, 549 (1992).