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ISOFLAVONOIDS FROM *Sophora tonkinensis*

Rui-Yun Yang,[1*] Yan-Su Lan,[1] Zhong-Jing Huang,[2]
Chang-Lun Shao,[3] Hong Liang,[1*]
Zheng-Feng Chen,[1] and Jun Li[4]

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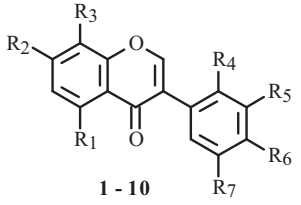
The genus *Sophora* belongs to the family Leguminosae, containing about 21 species, 14 varieties, and two forms that are widely distributed in Southwest China, South China, and East China [1]. Among them, *Sophora tonkinensis* is an important traditional Chinese medicinal plant, known as Guang-Dou-Gen in Chinese, distributed chiefly in South China. Its stems and roots are commonly used as a traditional Chinese drug to treat acute pharyngolaryngeal infections and sore throats [2]. Previous investigations on the constituents of *S. tonkinensis* have revealed the presence of flavonoids, alkaloids, triterpenoids, phenols, etc. [3–11]. To explore the plant further and make use of Chinese herbal sources, part of the EtOAc fraction was subjected to a series of chromatographic techniques, yielding ten isoflavonoids. We report herein the isolation and structural elucidation of these compounds.

Dried and powdered stems and roots of S. *tonkinensis* (20.0 kg) were extracted with 95% EtOH three times at room temperature. Evaporation of EtOH under reduced pressure gave a brown residue (3.0 kg). The residue was suspended in H_2O and fractionated with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (1.0 kg) was applied to a silica gel column using gradient mixtures of petroleum ether–EtOAc (100:0–0:100) to afford five fractions (Fr. A–E). Fraction C (50.0 g) was isolated on silica gel eluted with petroleum ether–EtOAc step gradients (60:40–40:60) to give five subfractions (Fr. C1–C5). Fraction C1 (11.5 g) was subjected to silica gel column chromatography and eluted with petroleum ether–$(CH_3)_2CO$ (90:10–70:30), and then chromatographed repeatedly on Sephadex LH-20 eluted with MeOH to afford compounds **1** (12.0 mg), **2** (10.0 mg), and **3** (2.5 mg). Fraction C2 (5.5 g) was further separated on silica gel column chromatography eluted with CH_2Cl_2–$(CH_3)_2CO$ (75:25–60:40) and Sephadex LH-20 column chromatography to yield compounds **4** (3.2 mg), **5** (9.0 mg), **6** (15.0 mg), and **7** (15.0 mg). Fraction C3 (5.2 g) was purified by column chromatography on silica gel with CH_2Cl_2–$(CH_3)_2CO$ (95:5–80:20) to give four subfractions (Fr. C3-1–C3-4). Fraction C3-2 (1.5 g) was first isolated on silica gel with CH_2Cl_2–$(CH_3)_2CO$ (100:0–90:10), and then on Sephadex LH-20 eluted with MeOH to give compounds **8** (8.5 mg) and **9** (30 mg). Fraction C3-4 (2.2 g) was subjected to silica gel column chromatography with CH_2Cl_2–$(CH_3)_2CO$ (95:5–80:20) and applied to an RP-C_{18} column using H_2O–MeOH (50:50), and finally purified on a Sephadex LH-20 column to obtain compounds **10** (5.0 mg).



1 - 10

1: $R_1 = R_4 = R_5 = R_7 = H$, $R_2 = OCH_3$, $R_3 = R_6 = OH$; **2:** $R_1 = R_2 = R_4 = R_6 = OH$, $R_3 = R_5 = R_7 = H$
3: $R_1 = R_4 = R_5 = R_7 = H$, $R_2 = OH$, $R_3 = R_6 = OCH_3$; **4:** $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = R_7 = OH$, $R_6 = OCH_3$
5: $R_1 = R_3 = R_4 = R_6 = H$, $R_2 = R_7 = OH$, $R_5 = OCH_3$; **6:** $R_1 = R_3 = R_4 = R_7 = H$, $R_2 = R_6 = OH$, $R_5 = OCH_3$
7: $R_1 = R_3 = R_4 = R_5 = R_7 = H$, $R_2 = R_6 = OH$; **8:** $R_1 = R_4 = R_7 = H$, $R_2 = R_5 = OH$, $R_3 = R_6 = OCH_3$
9: $R_1 = R_4 = R_5 = R_7 = H$, $R_2 = R_3 = OH$, $R_6 = OCH_3$; **10:** $R_1 = R_3 = R_4 = R_7 = H$, $R_2 = R_5 = R_6 = OH$

1) Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, Ministry of Education, School of Chemistry and Chemical Engineering, Guangxi Normal University, 541004, Guilin, P. R. China, fax: 86 773 2120958, e-mail: yang_rui_yun@163.com; 2) School of Chemistry and Ecological Engineering, Guangxi University for Nationalities, 530006, Nanning, P. R. China; 3) School of Medicine and Pharmacy, Ocean University of China, 266003, Qingdao, P. R. China; 4) Key Laboratory of Ecology of Rare and Endangered Species and Environmental Conservation, Ministry of Education, College of Environment and Resources, Guangxi Normal University, 541004, Guilin, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 603–605. Original article submitted May 4, 2011.

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Their structures were elucidated as 8,4′-dihydroxy-7-methoxyisoflavone (**1**) [12], 5,7,2′,4′-tetrahydroxyisoflavone (**2**) [13], 8-*O*-methylretusin (**3**) [14], calycosin (**4**) [15], 7,3′-dihydroxy-5'-methoxyisoflavone (**5**) [16], 7,4′-dihydroxy-3′-methoxyisoflavone (**6**) [17], daidzein (**7**) [18], 7,3′-dihydroxy-8,4′-dimethoxyisoflavone (**8**) [19], 7,8-dihydroxy-4′-methoxyisoflavone (**9**) [17], and 7,3′,4′-trihydroxyisoflavone (**10**) [17], on the basis of their spectral data and by comparison of their data with the literature. To the best of our knowledge, compounds **1**, **2**, **5**, **8**, and **9** were first characterized from the genus *Sophora*, and compounds **4**, **6**, and **10** were reported from *S. tonkinensis* for the first time.

8,4′-Dihydroxy-7-methoxyisoflavone (1). Pale yellow crystals, mp 240–242°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.37 (1H, s, H-2), 7.51 (1H, d, J = 9.0, H-5), 7.50 (2H, d, J = 8.5, H-2′, 6′), 7.00 (1H, d, J = 8.5, H-6), 6.97 (2H, d, J = 8.5, H-3′, 5′), 3.79 (3H, s, OCH_3-7). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 174.6 (C-4), 158.3 (C-4′), 152.3 (C-2), 149.4 (C-8), 146.1 (C-7), 132.3 (C-9), 129.5 (C-2′, 6′), 123.7 (C-1′), 122.0 (C-3), 116.8 (C-10), 115.1 (C-6), 113.6 (C-5), 113.0 (C-3′, 5′), 54.4 (OCH_3-7).

5,7,2′,4′-Tetrahydroxyisoflavone (2). White needles. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.03 (1H, s, H-2), 7.06 (1H, d, J = 8.5, H-6′), 6.42 (1H, d, J = 2.5, H-8), 6.40 (1H, d, J = 2.5, H-3′), 6.38 (1H, dd, J = 2.5, 9.0, H-5′), 6.25 (1H, d, J = 2.0, H-6). ^{13}C NMR (125 MHz, CD_3OD, δ): 180.7 (C-4), 164.0 (C-7), 161.7 (C-5), 158.3 (C-4′), 158.0 (C-9), 155.8 (C-2′), 154.8 (C-2), 131.2 (C-6′), 120.7 (C-3), 108.0 (C-1′), 106.3 (C-5′), 104.3 (C-10), 102.4 (C-3′), 98.3 (C-8), 94.9 (C-6).

8-*O*-Methylretusin (3). White needles, mp 230–231°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.40 (1H, s, H-2), 7.65 (1H, d, J = 8.5, H-5), 7.50 (2H, d, J = 8.0, H-2′, 6′), 7.18 (1H, d, J = 8.5, H-6), 6.97 (2H, d, J = 8.5, H-3′, 5′), 3.91 (3H, s, OMe-8), 3.76 (3H, s, OMe-4′). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 176.8 (C-4), 160.5 (C-4′), 155.2 (C-7), 152.7 (C-2), 147.5 (C-9), 136.2 (C-8), 131.7 (C-2′, 6′), 125.8 (C-1′), 124.4 (C-3), 122.0 (C-5), 120.2 (C-10), 116.0 (C-6), 114.9 (C-3′, 5′), 57.8 (OMe-8), 56.4 (OMe-4′).

Calycosin (4). Colorless needles, mp 241–243°C. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.18 (1H, s, H-2), 8.07 (1H, d, J = 9.0, H-5), 7.18 (1H, d, J = 2.0, H-2′), 6.98 (1H, dd, J = 8.5, 2.0, H-6), 6.97 (1H, d, J = 8.5, H-5′), 6.88 (1H, dd, J = 8.5, 2.0, H-6′), 6.87 (1H, d, J = 2.0, H-8), 3.90 (3H, s, OMe-4′). ^{13}C NMR (125 MHz, CD_3OD, δ): 178.0 (C-4), 163.8 (C-7), 159.0 (C-9), 154.0 (C-2), 148.4 (C-4′), 146.7 (C-3′), 128.6 (C-5), 126.3 (C-1′), 125.0 (C-3), 120.9 (C-6′), 118.3 (C-10), 116.7 (C-2′), 115.7 (C-6), 112.0 (C-5′), 103.3 (C-8), 56.5 (OMe-4′).

7,3′-Dihydroxy-5′-methoxyisoflavone (5). Pale yellow needles, mp 210–211°C. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.12 (1H, s, H-2), 8.04 (1H, d, J = 8.5, H-5), 7.04 (1H, s, H-4′), 6.97 (2H, s, H-2′, 6′), 6.94 (1H, dd, J = 8.8, 2.5, H-6), 6.85 (1H, d, J = 2.5, H-8), 3.88 (3H, s, OCH_3-5′). ^{13}C NMR (125 MHz, CD_3OD, δ): 178.1 (C-4), 164.7 (C-7), 160.0 (C-9), 154.9 (C-2), 149.2 (C-5′), 147.5 (C-3′), 128.6 (C-5), 126.3 (C-3), 125.9 (C-1′), 121.7 (C- 6′), 118.3 (C-10), 117.5 (C-4′), 116.5 (C-6), 112.8 (C-2′), 103.3 (C-8), 56.6 (OMe-5′).

7,4′-Dihydroxy-3′-methoxyisoflavone (6). White amorphous powder, mp 240–242°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.32 (1H, s, H-2), 7.98 (1H, d, J = 8.5, H-5), 7.17 (1H, s, H-2′), 7.00 (1H, d, J = 8.5, H-6′), 6.94 (1H, d, J = 8.5, H-6), 6.87 (1H, s, H-8), 6.82 (1H, d, J = 8.5, H-5′), 3.79 (3H, s, OMe-3′). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 176.0 (C-4), 163.8 (C-7), 158.7 (C-9), 154.3 (C-2), 148.5 (C-4′), 147.8 (C-3′), 128.6 (C-5), 124.8 (C-1′), 124.3 (C-3), 122.8 (C-6′), 118.0 (C-10), 116.9 (C-2′), 116.5 (C-6), 114.6 (C-5′), 103.4 (C-8), 56.5 (OMe-3′).

Daidzein (7). White powder, mp 294–296°C. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 9.54 (1H, br.s, OH-4′), 8.28 (1H, s, H-2), 7.96 (1H, d, J = 9.0, H-5), 7.37 (2H, d, J = 7.0, H-2′, 6′), 6.93 (1H, d, J = 8.5, H-6), 6.84 (1H, br.s, H-8), 6.80 (2H, d, J = 7.5, H-3′, 5′). ^{13}C NMR (125 MHz, CD_3OD, δ): 174.6 (C-4), 162.5 (C-7), 157.3 (C-9), 157.0 (C-4′), 152.6 (C-2), 129.9 (C-2′, 6′), 127.1 (C-5), 123.4 (C-1′), 122.5 (C-3), 116.5 (C-10), 115.0 (C-6), 114.8 (C-3′, 5′), 102.0 (C-8).

7,3′-Dihydroxy-8,4′-dimethoxyisoflavone (8). Yellow crystals, mp 207–210°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 10.7 (1H, br.s, OH-7), 9.06 (1H, br.s, OH-3′), 8.37 (1H, s, H-2), 7.71 (1H, d, J = 9.0, H-5), 7.04 (1H, d, J = 2.0, H-2′), 7.03 (1H, d, J = 8.5, H-6), 7.00 (1H, d, J = 8.5, H-5′), 6.95 (1H, dd, J = 2.0, 8.5, H-6′), 3.86 (3H, s, OCH_3-8), 3.79 (3H, s, OCH_3-4′). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 175.2 (C-4), 156.2 (C-7), 153.4 (C-2), 151.1 (C-9), 148.0 (C-4′), 146.5 (C-3′), 135.2 (C-8), 125.1 (C-1′), 123.6 (C-3), 121.2 (C-5), 120.0 (C-6′), 117.9 (C-10), 116.0 (C-6), 115.7 (C-5′), 112.5 (C-2′), 61.3 (OCH_3-8), 56.3 (OCH_3-4′).

7,8-Dihydroxy-4′-methoxyisoflavone (9). Pale yellow crystals, mp 204–206°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.37 (1H, s, H-2), 7.53 (2H, d, J = 8.5, H-2′, 6′), 7.49 (1H, d, J = 9.0, H-5), 6.98 (2H, d, J = 8.5, H-3′, 5′), 6.96 (1H, d, J = 8.5, H-6), 3.78 (3H, s, OCH_3-4′). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 175.1 (C-4), 158.9 (C-4′), 153.0 (C-2), 150.0 (C-7), 146.5 (C-9), 132.9 (C-8), 130.1 (C-2′, 6′), 124.4 (C-1′), 122.6 (C-3), 117.4 (C-10), 115.6 (C-5), 114.2 (C-6), 113.6 (C-3′, 5′), 55.1 (OCH_3-4′).

7,3′,4′-Trihydroxyisoflavone (10). Colorless needles, mp 276–279°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.20 (1H, s, H-2), 7.92 (1H, d, J = 9.0, H-5), 7.00 (1H, d, J = 2.0, H-2′), 6.90 (1H, dd, J = 1.5, 8.5, H-6), 6.80 (1H, d, J = 2.0, H-8), 6.79 (1H, d, J = 2.0, 8.5, H-6′), 6.75 (1H, d, J = 8.0, H-5′). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 174.7 (C-4), 163.6 (C-7), 157.5 (C-9), 152.5 (C-2), 145.3 (C-4′), 144.8 (C-3′), 127.2 (C-5), 123.6 (C-3), 123.1 (C-1′), 119.8 (C-6′), 116.7 (C-2′), 116.1 (C-10), 115.8 (C-5′), 115.3 (C-6), 102.2 (C-8).

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REFERENCES

1. Chinese Academy of Sciences, *Flora of China*, Science Press, Beijing, Vol. **40**, 1994, 65 pp.
2. China Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China*, Chemical Industry Press, Beijing, Vol. **1**, 2005, 19 pp.
3. T. Takeshita, K. Yokoyama, Y. Ding, J. Kinjo, and T. Nohara, *Chem. Pharm. Bull.*, **39**, 1908 (1991).
4. Y. Ding, T. Takeshita, K. Yokoyama, J. Kinjo, and T. Nohara, *Chem. Pharm. Bull.*, **40**, 139 (1992).
5. Y. Ding, R. H. Tian, T. Takeshita, J. Kinjo, and T. Nohara, *Chem. Pharm. Bull.*, **40**, 1831 (1992).
6. S. Sakamoto, M. Kuroyanagi, A. Ueno, and S. Sekita, *Phytochemistry*, **31**, 1339 (1992).
7. P. L. Ding and D. F. Chen, *Helv. Chim. Acta.*, **89**, 103 (2006).
8. P. L. Ding and D. F. Chen, *Helv. Chim. Acta.*, **90**, 2236 (2007).
9. X. N. Li, Z. Q. Lu, G. T. Chen, H. X. Yan, N. Sha, S. H. Guan, M. Yang, H. M. Hua, and D. A. Guo, *Magn. Reson. Chem.*, **46**, 898 (2008).
10. X. N. Li, Z. Q. Lu, S. Qin, H. X. Yan, M. Yang, S. H. Guan, X. Liu, H. M. Hua, L. J. Wu, and D. A. Guo, *Tetrahedron Lett.*, **49**, 3797 (2008).
11. X. N. Li, N. Sha, H. X. Yan, X. Y. Pang, S. H. Guan, M. Yang, H. M. Hua, L. J. Wu, and D. A. Guo, *Phytochem. Lett.,* **1**, 163 (2008).
12. W. Z. Huang, J. A. Duan, and Z. L. Li, *Zhongguo Zhongyao Zazhi*, **26**, 403 (2001).
13. T. Yin, H. Liu, L. Wang, G. Z. Tu, H. Liang, and Y. Y. Zhao, *Yaoxue Xuebao*, **43**, 67 (2008).
14. Y. Shirataki, S. Matsuoka, M. Komatsu, M. Ohyama, T. Tanaka, and M. Iinuma, *Phytochemistry*, **50**, 695 (1999).
15. P. Kamnaing, S. N. Free, A. E. Nkengfack, G. Folefoc, and Z. T. Fomum, *Phytochemistry*, **51**, 829 (1999).
16. X. M. Ma, L. S. Wang, Y. J. Gou, and S. R. Guo, *Zhongguo Zhongyao Zazhi*, **30**, 1159 (2005).
17. H. Goto, Y. Terao, and S. Akai, *Chem. Pharm. Bull.,* **57**, 346 (2009).
18. W. Z. Huang, J. A. Duan, and Z. L. Li, *Zhongguo Yaoke Daxue Xuebao*, **31**, 8 (2000).
19. G. J. Zhang, N. Li, Y. J. Xiong, X. Li, Y. Li, and X. G. Jia, *Zhongguo Yaoxue Zazhi*, **44**, 897 (2009).