CHEMICAL RESEARCH OF *Caragana microphylla* Seeds

Yan Huo,[1,2,3] Cheng Guo,[2] Shan Lu,[3] Qiao-Yan Zhang,[1] and Lu-Ping Qin[1]*

UDC 547.972

Caragana microphylla Lam. (*Leguminosae*) is widely distributed in the desert of the northwest part of China. Its roots and seeds have been used to heal swollen and painful throat in folk medicine [1]. Some studies on this plant showed that it had some bioactivities [2]. Recently, our research showed that the crude extract of seeds of the plant exhibited analgesic activities *in vivo*. Its bioactivities prompted us to continue to investigate its chemical components. In the present work, the chemical investigation of the chloroform extract of the seeds led to the isolation of seven compounds.

Machaeric acid (1) [3]: white needles (MeOH), $C_{30}H_{46}O_4$. EI-MS *m/z* 469.27 (M-1), 285.02, 256.26. ^{1}H NMR ($CDCl_3$): 0.72 (1H, s, 5-H), 0.89 (2H, m, 7-H), 1.11 (3H, m, 27-H), 1.20 (3H, s, 29-CH_3), 1.28 (2H, m, 1-H), 1.51(1H, m, 9-H), 1.85 (2H, m, 11-H), 2.35 (1H, dd, 18-H), 3.05(1H, dd, 3-H), 5.32(1H, s, 12-H), 0.60~0.73 (3H, d, 24-CH_3), 0.75~0.95 (12H, m, 23,25,26,30-H), 1.00~1.125 (3H, s, 27-CH_3), 1.11, 1.71 (each 1H, m, 16-H), 1.11, 2.10 (each 1H, m, 2-H), 1.375~1.61 (4H, m, 6, 15-H), 1.51, 2.42 (2H, m, d, 19-H), 2.10, 2.95, (2H, m, d, 22-H).

^{13}C NMR ($CDCl_3$): 15.14(C-23), 15.87(C-24), 16.43(C-26), 17.88(C-6), 20.27(C-25), 21.00(C-27), 22.95(C-11), 24.46(C-16), 24.93(C-29), 26.25(C-2), 26.87(C-15), 28.09(C-30), 32.11(C-1), 36.42(C-10), 38.10(C-7), 38.29(C-8), 39.17(C-4), 40.29(C-19), 41.27(C-14), 43.10(C-17), 45.19(C-22), 45.74(C-18), 46.86(C-9), 47.10(C-20), 54.63(C-5), 76.72(C-3), 123.73(C-12), 140.75(C-13), 177.07(C-28), 214.10(C-21).

7-Hydroxyl-4′-methoxyflavone (Pratol) (4) [4]: white needles (MeOH), mp 263~264°C. $C_{16}H_{12}O_4$. ESI-MS (*m/z*): 269.26(M+1), 253.15, 225.12, 197.06, 118.09. ^{1}H NMR (DMSO-d_6, J/Hz): 6.05 (1H, s, H-3), 7.97 (1H, d, J = 8.7, H-5), 6.94 (1H, dd, J = 2.1, 8.7, H-6), 6.86 (1H, d, J = 2.1, H-8), 7.72 (2H, d, J = 8.7, H-2′, H-6′), 6.99 (2H, d, J = 8.7, H-3′, H-5′), 10.81 (s, OH), 3.77 (s, OCH_3).

^{13}C NMR (DMSO-d_6): 162.50 (C-2), 107.94 (C-3), 174.50 (C-4), 125.67(C-5), 115.06(C-6), 158.89(C-7), 102.02(C-8), 157.35(C-9), 116.56(C-10), 129.94(C-1′), 127.19(C-2′), 113.52 (C-3′), 162.45(C-4′), 113.52(C-5′), 127.19(C-6′), 55.05 (4′-OCH_3).

Dehydrothalictrifoline (5) [5]: yellow powder (MeOH/EtOAc), $C_{21}H_{20}NO_4^+$. ESI-MS (*m/z*): 350.27 (M^+), 338.21, 334.22, 320.17, 306.22, 277.24. The positive reaction with Dragendorff's reagent was characteristic of alkaloids. ^{1}H NMR (DMSO-d_6, J/Hz): 2.95 (3H, s, 13-CH_3), 3.15 (2H, t, J = 6, 6, H-5), 3.93 (3H, s, 2-OCH_3), 3.85 (3H, s, 3-OCH_3), 4.79 (2H, t, J = 6, 6, H-6), 6.55 (2H, s, 9,10-OCH_2O-), 7.153 (1H, s, H-4), 7.355 (1H, s, H-1), 7.95 (1H, d, J = 9, H-11), 8.05 (1H, d, J = 9, H-12), 9.925 (1H, s, H-8).

^{13}C NMR (DMSO-d_6): 18.03 (13-CH_3), 55.80 (2-OCH_3), 56.15 (3-OCH_3), 104.55 (9,10-OCH_2O-), 114.57 (C-1), 146.91 (C-10), 119.10 (C-11), 119.32 (C-12), 131.70 (C-12-a), 132.35 (C-13), 135.54 (C-14), 147.18 (C-2), 150.65 (C-3), 120.05 (C-14-a), 111.01 (C-4), 130.41 (C-4-a), 26.72 (C-5), 55.65 (C-6), 142.92 (C-8), 110.81 (C-8-a), 144.60 (C-9)

7-Hydroxyl-4′-methoxyisoflavone (Formononetin) (6) [6]: white needles (MeOH), mp 245–246°C, $C_{16}H_{12}O_4$. ESI-MS (*m/z*): 269.14(M+1), 253.15, 237.12, 226.13, 213.13, 197.13, 181.11, 170.13, 118.09.

^{1}H NMR (DMSO-d_6, J/Hz): 8.33 (1H, s, H-2), 7.97 (1H, d, J = 8.7, H-5), 6.94 (1H, dd, J = 2.1, 8.7, H-6), 6.86 (1H, d, J = 2.1, H-8), 7.51 (2H, d, J = 8.7, H-2, H-6′), 6.99 (2H, d, J = 9.0, H-3′, H-5′), 10.81 (OH), 3.77 (OCH_3).

^{13}C NMR (DMSO-d_6, J/Hz): 153.09(C-2), 124.24 (C-3), 174.61(C-4), 127.34(C-5), 115.19 (C-6), 162.61 (C-7), 102.13 (C-8), 157.45(C-9), 116.59(C-10), 123.14(C-1′), 130.08 (C-2′), 113.59 (C-3′), 158.95 (C-4′), 113.59 (C-5′), 130.08(C-6′), 55.1(OCH_3).

1) Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai 200433, China, Tel:021-25070394, e-mail: lpqin@smmu.edu.cn; 2) Department of Pharmacy, Sixth People's Hospital, Shanghai Jiao Tong University, Shanghai 200233, China; 3) 215 Clinical Department, Dalian Sanatorium of PLA, Dalian 116041, China. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 177-178, March-April, 2007. Original article submitted February 8, 2006.

 0009-3130/07/4302-0214

α-D-Glucopyranosido-β-D-fructofuranoside (Sucrose) (7) [7]: colorless quadrate crystal. $C_{12}H_{22}O_{11}$. ESI-MS (*m/z*): 341.19(M-1), 179.14, 113.08, 89.05.

^{1}H NMR (DMSO-d_6): 5.33(1H, d, 1-H), 3.48 (1H, m, 2-H), 3.68(1H, m, 3-H), 3.39 (1H, d, 4-H), 3.80 (1H, dd, 5-H), 3.74 (1H, m, 6-H), 3.60 (1H, m, 1′-H), 4.14 (1H, m, 3′-H), 3.97 (1H, t, 4′-H), 3.78 (1H, m, 5′-H), 3.75 (1H, m, 6′-H).

^{13}C NMR (DMSO-d_6): 91.68 (C-1), 71.58 (C-2), 72.86(C-3), 69.89(C-4), 72.73(C-5), 60.53 (C-6), 62.05 (C-1′), 103.99 (C-2′), 77.16 (C-3′), 74.35 (C-4′), 82.49 (C-5′), 62.11 (C-6′).

β-Sitosterol (2) [8]: white needles(MeOH), mp 135–137°C. EI-MS *m/z* 396, 329, 301, 273. Positive reaction with Libermann-Buchard reagent. Its IR, ^{1}H, and ^{13}C NMR data were identical to those reported in the literature.

Stigmasterol (3) [8]: white needles (MeOH), mp l68–l69°C. Positive reaction with Libermann-Buchard reagent. Its IR, ^{1}H, and ^{13}C NMR data were identical to those reported in the literature.

Compounds **1**, **3** – **5** and **7** were isolated from *Caragana* Fabr. for the first time.

Plant Material and Apparatus. The plant material was collected from the middle of the Inner-Mongolia Autonomous Region, People's Republic of China, in July, 2004. The specimen was identified by Prof. Zheng Han-Chen, School of Pharmacy, Second Military Medical University of China. Voucher specimens have been deposited in School of Pharmacy, Second Military Medical University of China.

UV spectra were measured on a Shimadzu UV-2501 spectrometer. ESI-MS spectra were taken with a Varian Mat-212 mass spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AM-500 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography was carried out on silica gel (200–400 mesh, Qingdao Ocean Chemical Factory). Reversed phase flash chromatography was done on RP-18 silica gel (25–40 μm, Merck Co.). Thin-layer chromatography was performed on HGF_{254} plates (Yantai Zhibu Huangwu Silica Experimental Plant).

Extraction and Isolation. The plant material of *Caragana microphylla* Lam. (25 kg) was ground and exhaustively extracted with 75% EtOH. The solvent was evaporated in vacuo, and the extract was dissolved in water and partitioned with petroleum, $CHCl_3$, EtOAc, and *n*-BuOH in turn. The $CHCl_3$ fraction was chromatographed over silica gel with $CHCl_3$–MeOH (1:0–0:1) as eluent to give fractions I-X. Fraction I (3 g) was submitted to repeated column chromatography over silica gel with petroleum–EtOAc (50:1-10:1) to afford 3-hydroxy-21-oxo-olean-12-en-28-oic acid (20 mg) (Machaeric acid, **1**). Fraction II (6 g) was submitted to repeated column chromatography over silica gel with $CHCl_3$/MeOH (100:1–10:1) to afford β-sitosterol (400 mg) (**2**). Fraction III (4 g) was submitted to repeated column chromatography over silica gel with $CHCl_3$–MeOH (100:1–10:1) to afford stigmasterol (200 mg) (**3**). Fraction IV (10 g) was submitted to repeated column chromatography over silica gel with $CHCl_3$/MeOH (100:1–1:5). Then the FIV-5 (0.5g) and FIV-6 (1.5g) were subjected to flash column chromatography using Sephadex LH-20 with MeOH–$CHCl_3$ (1:1) to yield 7-hydroxyl-4′-methoxyflavone (Pratol, 20 mg) (**4**) and dehydrothalictrifoline (**5**) (200 mg). Fraction V (2 g) was submitted to repeated column chromatography over silica gel with $CHCl_3$–MeOH (30:1–1:1) to afford 7-hydroxyl-4′-methoxyisoflavone (20 mg) (formononetin, **6**) (20 mg). Fraction X (3 g) was submitted to repeated column chromatography over silica gel with EtOAc–MeOH (30:1–1:1) to afford α-D-glucopyranosido-β-D-fructofuranoside (sucrose, **7**) (400 mg).

REFERENCES

1. *Jiangsu New Medical College, The Dictionary of Traditional Medicine*, Shanghai: Shanghai Scientific&Technical Press, 1985, 392.
2. H. X. Liu, W. H. Lin, and J. S. Yang, *Chin. Pharm. J*., **39**, 327 (2004).
3. C. Djerassi, L. H. Liu, E. Farkas et al., *J. Am. Chem. Soc*., 77, 1200 (1955).
4. Gaydou Emile M. and Bianchini Jean Pierre, *Bull. Soc. Chim. Fr.*, (1-2, Pt. 2): 43 (1978).
5. Kinuko Iwasa, Yumi Nishiyama, Momoyo Ichimaru et al., *Eur. J. Med. Chem.*, **34**, 1077 (1999).
6. J. J. Xu, L. Q. Hai, and X. Q. Wang, WCJ·PS, **20**, 214 (2005).
7. H. M. Liu, Y. Ke, and Z. Z. Liu, *Chin. J. Magn. Reson.*, **20**, 357 (2003).
8. S. L. Feng, L. He, M. Wang et al., *China J. Chin. Mater. Med.*, **19**, 611 (1994).