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CHEMICAL CONSTITUENTS OF *Carlesia sinensis*

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Carlesia sinensis Dunn from the family Apiaceae comprises about 100 genera (ten endemic) and 614 species (340 endemic) in China, of which *C. sinensis*, a herb of endemic species, belongs to the monospecies genera *Carlesia* with a height of 10 to 30 cm and having usually a digitate-branched long conical stout taproot . This herb, found in Liaoning Province (Zhuanghe City) and Shandong Province (Muping, Weihai, Yantai) of China, is widely grown on dry mountain slopes and rock crevices [1]. In the ethnic applicaton, this herb has been used in the treatment of stomachache. However, its phytochemical studies have never been reported. This systematic study we recently made on the chemical constituents of this herb was targeted to validate the medical use of this herb and explore the chemosystematic relevance of the genus. In this paper, we describe the isolation and structural elucidation of nine compounds from its aerial parts and seven compounds together with a large amount of oil fractions from its root parts, which were all isolated from this plant for the first time.

From the air-dried aerial parts (mainly leaves, 415 g), eight coumarins were isolated together with D-mannitol (**1**) and a waxlike substance (3140 mg), which were identified as umbelliferone (**2**, 47.4 mg) [2, 3], aurapten (**3**, 1283.2 mg) [4], isoarnottinin (**4**, 17.3 mg) [5, 6], isoarnottinin 4′-glucoside (**5**, 809.0 mg) [7], bergapten (**6**, 145.1 mg) [4, 7], xanthotoxin (**7**, 251.5 mg) [8, 9], isopimpinellin (**8**, 646.1 mg) [4], and columbianetin (**9**, 22.4 mg) [10].

From the air-dried root parts (220 g), five coumarins were isolated and identified as umbelliferone (**2**, 1.5 mg) [2, 3], aurapten (**3**, 15.2 mg) [4], bergapten (**6**, 5.0 mg) [4, 7], xanthotoxin (**7**, 2.1 mg) [8], and isopimpinellin (**8**, 3.0 mg) [4], together with a large amount of oil fractions (5.39 g, mainly the linoleic/linolenic ester of glycerol), sugar (14.76 g), and a small amount of β-sitosterol (13.1 g). The structure of these compounds was deduced from spectroscopic experiments and by comparison with literature data.

In Apiaceae, coumarins were often found in large quantities in roots or fruits but less in aerial parts, which seemed contrary to our study. From the chemical aspect, it is thus taxonomically reasonable to categorize this species alone as a monospecies genus. According to the Flora of China, *C. sinensis* is assumed to be the middle-evolved tribe Ammineae. From this species, simple coumarins **2**–**5**, linear furanocoumarins **6**–**8**, and dihydro angular furanocoumarin **9** were isolated, but neither angular furanocoumarin nor pyranocoumarins were found. This pattern seems to be consistent with the middle-evolved level definition. Two uncommon simple coumarins [isoarnottinin (**4**) and isoarnottinin 4′-glucoside (**5**)] were firstly isolated from *Ammi majus* in 1993. This fact to some extent indicates the relation between these two species.

Secondary metabolites are generated during the growth and development of plants along with their adaptation to the outside environment. *C. sinensis* distributed in Liaodong and Shandong Peninsulas of China, where there is enough rainfall, only grows in the gaps between rocks on the topside of stone mountains, where moisture in the soil is rare. Therefore, besides some morphological characters, some chemical characters could also be considered as due to the ecological evolution of this species. To survive in such an environment, *C. sinensis* develops long roots (up to more than 30 cm) to store large concentrations of nutrients such as oil and sugar, but less coumarins, and short aerial parts (less than 30 cm) containing wax to prevent the herb from losing water and large amounts of coumarins to keep insects away from the herb.

Plant Material, Extraction, and Isolation. Fresh samples of *Carlesia sinensis* Dunn were collected in Kun Yu Shan Mount, Shandong Province in China, in August, 2006. The plant material was identified by one of the authors (Prof. Nianhe Wang). A voucher specimen (No. WNH06801) was deposited at the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences, Nanjing Botanical Garden Mem. Sun Yat-sen, Nanjing, P. R. China.

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The air-dried roots and aerial parts of *C. sinensis* were chopped and extracted with EtOAc and then MeOH. Then the extracts were subjected to column chromatography on silica gel with hexane–EtOAc and $CHC1_3$–MeOH gradient repeatedly to afford the compounds.

General Experimental Procedures. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were recorded by a Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard. Column chromatography was performed on silica gel (Merck, 60–120 mesh), and thin-layer chromatography on silica gel G coated TLC plates (Merck).

The physicochemical and spectral data of compounds **4** and **5** are reported below.

Isoarnottinin (4). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.88 (3H, d, J = 1.4, H-5′), 3.29 (3H, s, OMe), 3.64 (2H, br.d, J = 7.3, H-1′), 3.83 (2H, br.s, H-4′), 5.56 (1H, tq, J = 7.3, 1.4, H-2′), 6.24 (1H, d, J = 9.6, H-3), 6.73 (1H, d, J = 8.5, H-6), 7.18 (1H, d, J = 8.5, H-5), 7.60 (1H, d, J = 9.6, H-4). ^{13}C NMR spectrum (δ, ppm): 161.36 (C-2), 112.56 (C-3), 143.95 (C-4), 112.72 (C-4a), 126.50 (C-5), 112.80 (C-6), 157.74 (C-7), 114.46 (C-8), 153.25 (C-8a), 21.56 (C-1′), 123.58 (C-2′), 134.88 (C-3′), 78.09 (C-4′), 14.07 (C-5′), 57.73 (OMe).

Isoarnottinin 4′-Glucoside (5). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.81 (3H, s, H-5′), 2.93–4.08 [sugar moiety, 4.08 (2H, d, J = 7.8, anomeric H)], 3.45 (1H, dd, J = 13.8, 7.3, H-1′), 3.42 (1H, dd, J = 13.8, 7.3, H-1′), 3.85 (1H, br.d, J = 11.8, H-4′), 4.11 (1H, br.d, J = 11.8, H-4′), 5.47 (1H, t, J = 7.3, H-2′), 6.19 (1H, d, J = 9.6, H-3), 6.84 (1H, d, J = 8.5, H-6), 7.38 (1H, d, J = 8.5, H-5), 7.91 (1H, d, J = 9.6, H-4). ^{13}C NMR spectrum (δ, ppm): 160.39 (C-2), 110.89 (C-3), 144.82 (C-4), 111.30 (C-4a), 126.80 (C-5), 112.20 (C-6), 158.71 (C-7), 113.95 (C-8), 152.99 (C-8a), 20.97 (C-1′), 123.95 (C-2′), 132.28 (C-3′), 73.40 (C-4′), 13.92 (C-5′), 101.68 (C-Glc1), 73.26 (C-Glc2), 76.64 (C-Glc3), 69.90 (C-Glc4), 76.67 (C-Glc5), 60.91 (C-Glc6).

REFERENCES

1. *Reipublicae Popularis Sinicae, Flora*, Beijing, Science Press, **55** (2) (1985), p. 151.
2. X. W. Yang, J. Y. Wang, Zh. K. Yan, and D. Y. Liu, *J. Chin. Med. Mater*., **17**, 30 (1994).
3. J. H. Liu, Y. Tan, Y. P. Chen, S. X. Xu, and X. Sh. Yao, *Chin. Trad. Herbal Drugs*, **6**, 288 (1994).
4. L. Yu and D. M. Zhang, *China J. Chin. Mater. Med.*, **31**, 2049 (2006).
5. M. H. A. Elgamal, M. M. Shalabyn, H. Duddeck, and M. Hiegemann, *Phytochemistry*, **34**, 819 (1993).
6. I. Hisashi and I. Tsutomu, *Chem. Pharm. Bull*., **23**, 934 (1975).
7. S. Harkar, T. K. Razdan, and E. S. Waight, *Phytochemistry*, **23**, 419 (1984).
8. F. Toshihiro, F. Kazuhiro, F. Hiroko, O. Hikaru, M. Kunihide, N. Yukitaka, M. Hisashi, K. Mitsuo, and M. Masato, *Chem. Pharm. Bull*., **47**, 96 (1999).
9. Y. Y. Liu, C. Zhang, L. Li, and Y. Q. Xiao, *China J. Chin. Mater. Med.*, **31**, 309 (2006).
10. Sh. Q. Chen, H. Q. Zhang, Ch. Q. Yuan, Y. Q. Deng, and G. Y. Chen, *Acta Pharm. Sin*., **17**, 393 (1982).