Chemistry of Natural Compounds, Vol. 48, No. 3, July, 2012 [Russian original No. 3, May–June, 2012]

CHEMICAL CONSTITUENTS OF THE ROOTS OF *Inula helenium*

Huo Yan,[1] Shi Haiming,[2] Guo Cheng,[1*] and Li Xiaobo[2]

UDC 547.918

Inula helenium L. (Compositae) is widely distributed in the north of China. Its roots have been used to treat pain of upper body, emesia, and diarrhea, and kill parasite in traditional Chinese medicine [1]. Moreover, in some European pharmacopoeias, the roots are officially listed as a diuretic, diaphoretic, expectorant, and anthelmintic [2]. Some studies on this plant show that it has some bioactivities [3]. Recently, our research showed that the chloroform fraction of the 95% ethanol extract of roots of the plant exhibited significant cytotoxicity activity *in vitro*. Its bioactivities prompted us to continue to investigate its chemical components. In the present work, the chemical investigation of the chloroform extract of the roots led to the isolation of ten compounds **1–10**: 12(13)-en-betulinic acid methyl ester (**1**), alantolactone (**2**), isoalantolactone (**3**), 11αH,13-dihydroisoalantolactone (**4**), macrophyllilactone E (**5**), 5α,6α-epoxyalantolactone (**6**), 3-oxo-4(5),11-eudesmadien-8,12-olide (**7**), 4α-hydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide (**8**), β-sitosterol (**9**), and stigmasterol (**10**).

Compound 1 was isolated as a white amorphous powder with the molecular formula $C_{31}H_{48}O_3$, as determined on the basis of a molecular ion peak at *m/z* 468 $[M]^+$ in EI-MS. Its ^1H and ^{13}C NMR data were almost the same as the NMR data [4] of betulinic acid methyl ester with some minor differences. In comparison with the ^1H and ^{13}C NMR data of betulinic acid methyl ester, the chemical shifts of C-10 and C-14 of compound **1** are 5.7 and 2.5 ppm downfield, respectively. Meanwhile, a signal of the olefinic proton (δ 6.27) can be found in the NMR spectra of compound **1** with classic olefinic carbon signals (C-12, δ 124.4; C-13, 131.4) that are absent in the NMR spectra of the betulinic acid methyl ester. On the basis of the above observations, the structure of compound **1** was elucidated to be 12(13)-en-betulinic acid methyl ester. A search in the SciFinder and Reaxys database shows that there are only three references about compound **1** [5–7]. One of them [5] is about synthesis of compound **1** from methyl acetylbetulinate. Another one [6] is about the bioactivity of compound **1** prepared by the derivatization of betulinic acid. The third one [7] is about the biotransformation of compound **1**, but there was an error in the NMR data stating that the compound is the betulinic acid methyl ester instead of the 12(13)-en-betulinic acid methyl ester. Thus, this is the first time that compound **1** was isolated from the natural source. The ^{13}C NMR data are shown in Table 1, and its ^1H NMR data are as follows: ^1H NMR ($CDCl_3$, δ, ppm): 0.8–0.9 (15H, m, H-23, 24, 25, 26, 27), 4.68 (1H, s, 2H-9a), 4.72 (1H, s, 2H-9b), 4.46 (1H, m, H-12).

Alantolactone (2) [8]. White powder (MeOH), $C_{15}H_{20}O_2$. EI-MS *m/z* 232 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.40–1.82 (6H, m, H-1, 2, 3), 2.43 (1H, m, H-4), 5.13 (1H, d, J = 8, H-6), 3.56 (1H, m, H-7), 4.80 (1H, m, H-8), 2.09 (1H, dd, J = 6, 6, H-9a), 1.53 (1H, m, H-9b), 6.17 (1H, d, J = 4, H-13a), 5.60 (1H, d, J = 4, H-13b), 1.17 (3H, s, 14-CH_3), 1.07 (3H, s, 15-CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 41.7 (C-1), 16.7 (C-2), 32.7 (C-3), 37.5 (C-4), 149.0 (C-5), 118.8 (C-6), 39.4 (C-7), 76.4 (C-8), 42.6 (C-9), 32.6 (C-10), 139.8 (C-11), 170.3 (C-12), 121.5 (C-13), 22.5 (C-14), 28.5 (C-15).

Isoalantolactone (3) [8]. White powder (MeOH), $C_{15}H_{20}O_2$. EI-MS *m/z* 232 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.50–2.20 (6H, m, H-1, 2, 3), 2.34 (1H, m, H-5), 1.71 (1H, m, H-6a), 1.24 (1H, m, H-6b), 2.97 (1H, m, H-7), 4.48 (1H, m, H-8), 1.99 (1H, m, H-9a), 1.39 (1H, m, H-9b), 6.11 (1H, d, J = 2, H-13a), 5.57 (1H, d, J = 2, H-13b), 0.82 (3H, s, CH_3-14), 4.76 (1H, d, J = 3, H-15a), 4.43 (1H, d, J = 3, H-15b). ^{13}C NMR ($CDCl_3$, δ, ppm): 42.5 (C-1), 22.7 (C-2), 36.8 (C-3), 148.9 (C-4), 46.2 (C-5), 27.5 (C-6), 40.5 (C-7), 76.6 (C-8), 41.3 (C-9), 34.3 (C-10), 142.2 (C-11), 170.5 (C-12), 119.9 (C-13), 17.6 (C-14), 106.6 (C-15).

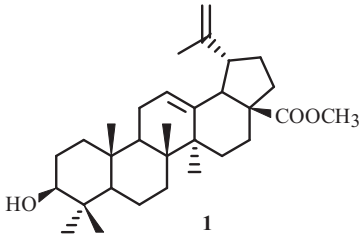
11αH,13-Dihydroisoalantolactone (4) [9]. White powder (MeOH), $C_{15}H_{22}O_2$. EI-MS *m/z* 234 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.40–1.80 (6H, m, H-1, 2, 3), 2.34 (1H, m, H-5), 1.99 (1H, m, H-6a), 1.15 (1H, m, H-6b), 2.39 (1H, m, H-7), 4.46 (1H, m, H-8), 1.47 (1H, dd, J = 9, 9, H-9a), 2.15 (1H, dd, J = 4, 4, H-9b), 2.79 (1H, t, J = 7, H-11), 1.21 (3H, d, J = 15, H-13), 0.80 (3H, s, CH_3-14), 4.77 (1H, d, J = 6, H-15a), 4.47 (1H, d, J = 3, H-15b). ^{13}C NMR ($CDCl_3$, δ, ppm): 41.7 (C-1), 21.3 (C-2), 34.8 (C-3), 149.4 (C-4), 46.5 (C-5), 22.7 (C-6), 42.2 (C-7), 77.8 (C-8), 41.6 (C-9), 36.8 (C-10), 40.3 (C-11), 179.3 (C-12), 9.25 (C-13), 17.8 (C-14), 106.4 (C-15).

1) Department of Pharmacy, 6th Hospital, Shanghai Jiao Tong University, Shanghai, P. R. China, fax: 86 21 24058098, e-mail: gboss@126.com; 2) School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 468–470. Original article submitted March 9, 2011.

 0009-3130/12/4803-0522

TABLE 1. ^{13}C NMR Data of **1** and Betulinic Acid Methyl Ester (**11**) ($CDCl_3$, ppm)

C atom	**1**	**11**	C atom	**1**	**11**
1	35.3	39.3	17	55.9	56.8
2	24.9	28.3	18	40.5	47.6
3	80.9	78.1	19	38.8	49.8
4	37.1	39.5	20	152.7	150.9
5	50.8	55.9	21	28.0	31.0
6	17.7	18.8	22	34.0	37.5
7	29.7	34.8	23	25.7	28.7
8	37.9	41.1	24	16.5	16.4
9	47.8	50.9	25	16.3	16.3
10	31.4	37.1	26	18.2	18.8
11	23.7	21.1	27	15.9	14.9
12	124.4	26	28	171.0	176.5
13	131.4	38.6	29	107.0	110.1
14	45.2	42.7	30	21.4	19.4
15	27.0	30.1	OMe	49.4	51.3
16	28.9	32.4			



1

Macrophyllilactone E (5) [10]. Brown oil (MeOH), $C_{15}H_{20}O_3$. EI-MS *m/z* 248 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.39–1.62 (5H, m, H-1, 2b, 3), 1.82 (1H, m, H-2a), 2.68 (1H, m, H-4), 6.35 (1H, s, H-6), 4.68 (1H, dd, J = 5.8, 5.9, H-8), 2.05 (1H, dd, J = 5.9, 5.8, H-9a), 1.59 (1H, m, H-9b), 9.25 (2H, s, H-13), 1.16 (3H, s, CH_3-14), 1.18 (3H, s, CH_3-15). ^{13}C NMR ($CDCl_3$, δ, ppm): 39.3 (C-1), 17.7 (C-2), 20.3 (C-3), 40.1 (C-4), 159.1 (C-5), 112.7 (C-6), 162.7 (C-7), 76.0 (C-8), 42.9 (C-9), 38.2 (C-10), 118.2 (C-11), 174.5 (C-12), 54.8 (C-13), 29.1 (C-14), 33.7 (C-15).

5α,6α-Epoxyalantolactone (6) [8]. White powder (MeOH), $C_{15}H_{20}O_3$. EI-MS *m/z* 248 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.35–1.83 (7H, m, H-1, 2, 3, 4), 2.91 (1H, d, J = 1, H-6), 3.69 (1H, m, H-7), 4.69 (1H, m, H-8), 1.91 (1H, m, H-9a), 1.60 (1H, m, H-9b), 6.42 (1H, d, J = 1, H-13a), 5.79 (1H, d, J = 1, H-13b), 1.14 (3H, s,CH_3-14), 1.09 (1H, d, J = 5, H-15). ^{13}C NMR ($CDCl_3$, δ, ppm): 39.4 (C-1), 16.3 (C-2), 29.4 (C-3), 37.0 (C-4), 67.4 (C-5), 61.1 (C-6), 37.3 (C-7), 75.0 (C-8), 37.6 (C-9), 32.5 (C-10), 136.6 (C-11), 169.5 (C-12), 123.6 (C-13), 23.8 (C-14), 17.9 (C-15).

3-Oxo-4(5),11-eudesmadien-8,12-olide (7) [11]. White powder (MeOH), $C_{15}H_{18}O_3$. EI-MS *m/z* 246 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 2.08 (1H, m, H-1a), 2.49 (1H, m, H-1b), 2.62 (1H, m, H-2a), 1.80 (1H, m, H-2b), 3.05 (1H, dd, J = 7.3, 7.3, H-6a), 1.95 (1H, dd, J = 4.6, 4.6, H-6b), 3.22 (1H, m, H-7), 4.61 (1H, m, H-8), 1.79 (1H, m, H-9a), 2.23 (1H, m, H-9b), 6.38 (1H, d, J = 3.1, H-13a), 5.72 (1H, d, J = 2.7, H-13b), 1.25 (3H, s,CH_3-14), 1.82 (3H, s, CH_3-15). ^{13}C NMR ($CDCl_3$, δ, ppm): 42.5 (C-1), 34.7 (C-2), 197.8 (C-3), 130.1 (C-4), 158.7 (C-5), 29.7 (C-6), 39.6 (C-7), 74.6 (C-8), 35.23 (C-9), 33.9 (C-10), 138.6 (C-11), 169.8 (C-12), 123.1 (C-13), 11.2 (C-14), 25.0 (C-15).

Compound **7** was isolated from *Inula* genus for the first time.

4α-Hydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide (8) [12]. White powder (MeOH), $C_{15}H_{20}O_3$. EI-MS *m/z* 248 (M^+). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 2.32 (1H, m, H-1), 2.01 (1H, m, H-2a), 1.70 (1H, m, H-2b), 1.81 (1H, m, H-3a), 1.69 (1H, m, H-3b), 1.75 (1H, m, H-5), 2.03 (1H, m, H-6a), 1.72 (1H, m, H-6b), 2.98 (1H, m, H-7), 4.16 (1H, m, H-8), 3.08 (1H, dd, J = 3.5, 3.4, H-9a), 2.38 (1H, m, H-9b), 6.21 (1H, d, J = 4.6, H-13a), 5.52 (1H, d, J = 3.3, H-13b), 5.05 (1H, s, H-14a), 4.95 (1H, s, H-14b), 1.29 (3H, s, H-15). ^{13}C NMR ($CDCl_3$, δ, ppm): 46.4 (C-1), 25.9 (C-2), 41.2 (C-3), 81.1 (C-4), 54.1 (C-5), 26.9 (C-6), 45.2 (C-7), 80.7 (C-8), 45.1 (C-9), 146.6 (C-10), 140.7 (C-11), 169.8 (C-12), 119.6 (C-13), 110.8 (C-14), 23.9 (C-15).

β-Sitosterol (9). mp 135–137°C. EI-MS *m/z:* 396, 329, 301, 273. Its IR, ^1H, and ^{13}C NMR data were identical to those reported in the literature [13].

Stigmasterol (10). mp l68–169°C. Its IR, ^1H, and ^{13}C NMR data were identical to those reported in the literature [13].

Plant Material and Apparatus. The plant material was purchased from the herbal market of Bo Zhou, An Hui Province, People′s Republic of China, in July, 2008. The specimen was identified by Prof. Li Xiao-Bo, School of Pharmacy, Shanghai Jiao Tong University. Voucher specimens have been deposited in the School of Pharmacy, Shanghai Jiao Tong University.

ESI-MS spectra were taken with a Varian Mat-212 mass spectrometer. 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. Chemical shifts (δ) are 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 HGF254 plates (Yantai Zhibu Huangwu Silica Experimental plant).

Extraction and Isolation. Air-dried powdered roots of *I. helenium* (17 kg) were extracted consecutively with 95% EtOH at room temperature thrice. After the removal of EtOH solvent under reduced pressure, the residue was suspended in water and extracted with chloroform. The chloroform extract (159 g) was subjected to column chromatography on silica gel (100–200 mesh) and eluted with gradient mixtures of petroleum ether (60–90°C)–acetone (100:1–1:1, v/v) successively to yield Fractions I–X. Fraction I eluted with petroleum ether–acetone 100:1 was rechromatographed over silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:0.5–100:5, v/v) to yield compound **1** (500 mg). Fraction III eluted with petroleum ether–acetone 100:4 was rechromatographed over silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:0.5–100:5, v/v) to yield three fractions. Fraction III-1 was separated by RP C_{18} column chromatography with gradient mixtures of MeOH–H_2O (20:80–100:0, v/v) to yield alantolactone (**2**) (1000 mg) and isoalantolactone (**3**) (600 mg). Fractions III-2 and III-3 were rechromatographed over silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:1–100:6, v/v) to yield compound **4** (15 mg) and compound **5** (100 mg). Fraction IV was rechromatographed over silver nitrate-silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:4–100:10, v/v) to yield compound **6** (20 mg). Fraction VIII was rechromatographed over silver nitrate–silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:8–100:15, v/v) to yield compound **7** (10 mg). Fraction X was rechromatographed over silver nitrate–silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:15–100:30, v/v) to yield compound **8** (20 mg). Fraction II was rechromatographed over silica gel (200–300 mesh) eluting with gradient mixtures of petroleum ether (60–90°C)–acetone (100:1–100:5, v/v) to yield a mixture of β-sitosterol (**9**) and stigmasterol (**10**) (1:1).

ACKNOWLEDGMENT

The authors thank 6th Hospital, Shanghai Jiao Tong University for providing financial assistance (No. Yuan-1441).

REFERENCES

1. Jiangsu New Medical College, *A Comprehensive Dictionary of Traditional Chinese Materia Medica*, Shanghai, Shanghai Scientific & Technical Press, 1977, p. 80.
2. A. Stojakowska, B. Kedzia, and W. Kisiel, *Fitoterapia*, **76**, 687 (2005).
3. Y. Huo, H. M. Shi, M. Y. Wang, and X. B. Li, *Pharmazie*, **63**, 699 (2008).
4. S. B. Mahato and A. P. Kundu, *Phytochemistry*, **37**, 1517 (1994).
5. J. M. Allison, W. Lawrie, J. McLean, and G. R. Taylor, *J. Chem. Soc*., 3353 (1961).
6. S. Y. Ryu, S. U. Choi, S. H. Lee, C. O. Lee, Z. No, and J. W. Ahn, *Arch. Pharm. Res*., **17**, 375 (1994).
7. J. Zhang, Z. H. Cheng, B. Y. Yu, G. A. Cordell, and S. X. Qiu, *Tetrahedron Lett*., **46**, 2337 (2005).
8. S. G. Klochkov, S. V. Afanas′eva, and A. N. Pushin, *Chem. Nat. Comp*., **42**, 400 (2006).
9. B. Gonzalo, C. Luz, G. Begona, J. R. Pedro, and A. Serrano, *Tetrahedron*, **48**, 5265 (1992).
10. B. Fu, B. N. Su, Y. Takaishi, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov, and O. Ashurmetov, *Phytochemistry*, **58**, 1121 (2001).
11. L. Adewole, Okunade, and D. F. Wiemer, *Phytochemistry*, **24**, 1199 (1985).
12. M. C. Yang, S. U. Choi, W. S. Choi, S. Y. Kim, and K. R. Lee, *J. Nat. Prod*., **71**, 678 (2008).
13. S. L. Feng, L. He, M. Wang, and K. J. Jiao, *China J. Chin. Mater. Med*., **19**, 611 (1994).