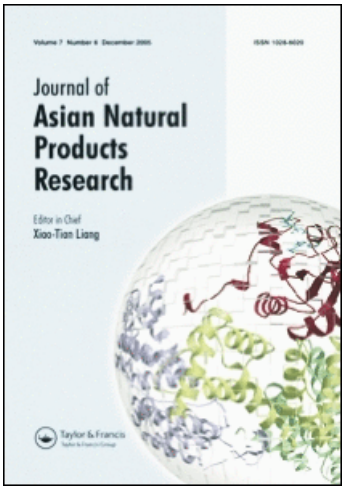

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Triterpenoids from the roots of *Pterospermum heterophyllum* Hance

Shuai Li[a]; Yan Shi[a]; Xiao-Ya Shang[b]; Bao-Song Cui[a]; Yi Yuan[a]; Xiao-Guang Chen[a]; Yong-Chun Yang[a]; Jian-Gong Shi[a]
[a] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China [b] Beijing Key Laboratory of Bioactive Substances and Functional Foods, College of Arts and Science of Beijing Union University, Beijing, China

To cite this Article Li, Shuai , Shi, Yan , Shang, Xiao-Ya , Cui, Bao-Song , Yuan, Yi , Chen, Xiao-Guang , Yang, Yong-Chun and Shi, Jian-Gong(2009) 'Triterpenoids from the roots of *Pterospermum heterophyllum* Hance', Journal of Asian Natural Products Research, 11: 7, 652 — 657
To link to this Article: DOI: 10.1080/10286020902964248
URL: http://dx.doi.org/10.1080/10286020902964248

Journal of Asian Natural Products Research
Vol. 11, No. 7, July 2009, 652–657

Triterpenoids from the roots of *Pterospermum heterophyllum* Hance

Shuai Li[a]*, Yan Shi[a], Xiao-Ya Shang[b], Bao-Song Cui[a], Yi Yuan[a], Xiao-Guang Chen[a], Yong-Chun Yang[a] and Jian-Gong Shi[a]

[a]*Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China;* [b]*Beijing Key Laboratory of Bioactive Substances and Functional Foods, College of Arts and Science of Beijing Union University, Beijing 100083, China*

(*Received 18 February 2009; final version received 13 April 2009*)

Two new triterpenoids taraxer-14-ene-1α,3β-diol (**1**) and 3β-hydroxytaraxer-14-ene-1-one (**2**), together with the known triterpenes taraxerol (**3**), betulin (**4**), betulinic acid (**5**), sumaresinolic acid (**6**), and 5-hydroxy-2-methoxy-1,4-naphthoquinone (**7**), 5,7-dihydroxy-6,8-dimethylchromone (**8**), α-monpalmitin (**9**), palmitic acid (**10**), 6β-hydroxystigmast-4-en-3-one (**11**), β-sitostero1 (**12**), have been isolated from the petroleum ether fraction of the ethanolic extract of *Pterospermum heterophyllum*. Their structures were established by spectroscopic methods including IR, MS, 1D, and 2D NMR experiments. Compounds **1**–**8** were evaluated against several human cancer cell lines. Compound **1** showed *in vitro* selective cytotoxicity against human lung cancer cell lines (A549) with an IC_{50} value of 1.22 μM. Compound **7** showed significant cytotoxicity against the A549, HCT-8, Bel7402, BGC-823, and A2780 cancer cell lines with IC_{50} values of 0.21, 0.55, 0.40, 0.59, and 0.34 μM, respectively. However, the other compounds were inactive ($IC_{50} > 10$ μM).

Keywords: *Pterospermum heterophyllum*; Sterculiaceae; triterpenoid; naphthoquinone; cytotoxic activity

1. Introduction

Pterospermum heterophyllum Hance (Sterculiaceae) is a medium to large tree, and the roots of the plant are used as 'Ban-Feng-He' (Chinese name) in Chinese folk herb for the treatment of rheumatoid arthritis [1]. As a part of our program to assess the chemical and biological diversities of several cultivated traditional Chinese medicines, in our preliminary assay, the crude extract of the roots of *P. heterophyllum* exhibited cytotoxic activity against several human cancer cell lines. During the investigation of its bioactive constituents, two new triterpenoids taraxer-14-ene-1α,3β-diol (**1**) and 3β-hydroxytaraxer-14-ene-1-one (**2**), together with the known triterpenoids taraxerol (**3**), betulin (**4**), betulinic acid (**5**), sumaresinolic acid (**6**), 5-hydroxy-2-methoxy-1,4-naphthoquinone (**7**), 5,7-dihydroxy-6,8-dimethylchromone (**8**), α-monpalmitin (**9**), palmitic acid (**10**), stigmast-4-en-6β-ol-3-one (**11**), β-sitostero1 (**12**), have been isolated from the petroleum ether fraction of the ethanolic extract of *P. heterophyllum*. In this paper, we deal with the isolation and structural elucidation of **1** and **2**, as well as the cytotoxicities of **1**–**8** against several human cancer cell lines.

*Corresponding author. Email: lishuai@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902964248
http://www.informaworld.com

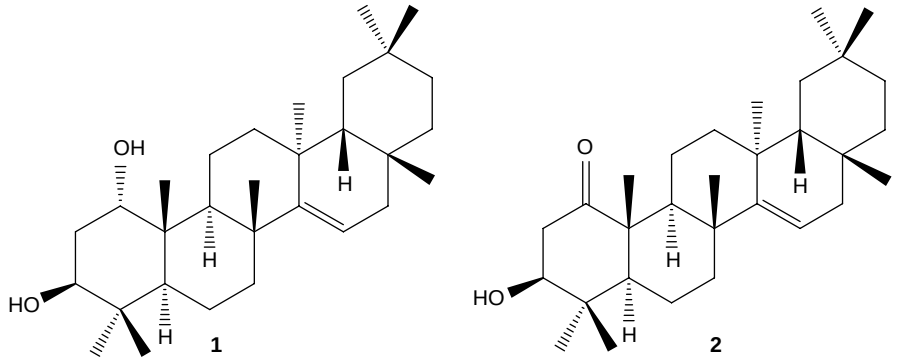

Figure 1. Structures of compounds **1** and **2**.

2. Results and discussion

The EtOH extract of the roots of *P. heterophyllum* was partitioned between water and petroleum ether, EtOAc. The petroleum ether phase was concentrated under vacuum and then subjected to repeated column chromatography over silica gel and Sephadex LH-20 to yield **1** and **2** (Figure 1).

Compound **1** was obtained as a colorless powder from $CHCl_3$–MeOH with m.p. 213–214°C and $[\alpha]_D^{20}$ 49.1 ($c = 0.05$, $CHCl_3$). The IR spectrum showed absorption bands for hydroxyl (3384 cm^{-1}) and double bonds (1461 cm^{-1}). The EI-MS spectrum of **1** gave a molecular ion peak at *m/z* 442 and the HR-EI-MS at *m/z* 442.3813 $[M]^+$ indicated the molecular formula of **1** as $C_{30}H_{50}O_2$. The ^{1}H NMR spectrum of **1** showed eight methyl singlets at δ 0.82 (3H, s, H-24), 0.82 (3H, s, H-28), 0.91 (3H, s, H-30), 0.92 (3H, s, H-27), 0.95 (3H, s, H-25), 0.95 (3H, s, H-29), 1.02 (3H, s, H-23), and 1.11 (3H, s, H-26), an olefinic proton at δ 5.54 (1H, dd, $J = 8.1$, 3.3 Hz, H-15) and a pair of oxygenated methines at δ 3.66 (1H, brd, $J = 2.4$ Hz, H-1) and 3.69 (1H, dd, $J = 12.0$, 4.8 Hz, H-3). The configuration of the hydroxyl group at C-1 was deduced to be in the α-position by a small $^{1,2}J_{H,H}$ coupling constant (2.4 Hz) of the equatorial proton at C-1, and the configuration of the hydroxyl group at C-3 was deduced to be in the β-position by a large $^{2,3}J_{H,H}$ coupling constant (12.0 Hz) of the axial proton at C-3 [2]. Several multiplets with complex coupling patterns attributed to methylenes and methines between δ 1.22 and 2.03 were also shown in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR and DEPT spectra of **1** displayed 30 carbon signals including eight methyls, nine methylenes, six methines (one sp^2 hybrid, two oxygenated carbons), and seven quaternary carbons (one olefinic carbon; Table 1). These spectroscopic data in combination with six degrees of unsaturation required by the molecular formula suggested that **1** was a pentacyclic triterpene containing one double bond and two hydroxyl groups. The olefinic signals at δ 157.9 and 117.0 for C-14 and C-15 in the ^{13}C NMR spectrum indicated the presence of a $\Delta^{14,15}$-taraxeren skeleton in compound **1** [3]. The EI-MS spectrum of **1** showed fragment ion peaks at *m/z* 318, 300, 285, 204, and 189, and confirmed that **1** was a $\Delta^{14,15}$-taraxeren derivative [4].

In order to unambiguously establish the structure, a careful comparison of the NMR spectral data of **1** with those of the known taraxeren [5] and 2D NMR experiments of **1** was carried out. The proton and protonated carbon signals in the NMR spectra of **1** were assigned unequivocally by the HMQC experiment (Table 1). In the HMBC spectrum of **1**, long-range correlations from H-15 to C-16; H-1 to C-3; H-23 to C-3, C-4, C-5,

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2**.

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1	3.66 (1H, brd, $J = 2.4$ Hz)	72.1		212.1
2	1.76 (1H, dt, $J = 13.8$, 4.8 Hz), 2.01 (1H, dt, $J = 13.8$, 12.0, 2.4 Hz)	34.1	3.07 (1H, dd, $J = 12.0$,12.0 Hz), 2.36 (1H, dd, $J = 4.8$, 12.0 Hz)	44.3
3	3.69 (1H, dd, $J = 12.0$, 4.8 Hz)	73.5	3.47 (1H, dd, $J = 4.8$, 12.0 Hz)	79.2
4		38.8		39.4
5	1.24 (1H, m)	47.7	0.96 (1H, m)	54.5
6	1.63 (1H, m), 1.48 (1H, m)	18.5	1.63 (1H, m), 1.48 (1H, m)	18.5
7	1.36 (1H, m), 2.02 (1H, m)	40.7	1.31 (1H, m), 2.00 (1H, m)	40.2
8		38.9		38.7
9	2.02 (1H, m)	40.7	2.02 (1H, m)	41.8
10		41.6		54.1
11	1.68 (1H, m), 1.47 (1H, m)	16.9	1.68 (1H, m), 1.47 (1H, m)	18.2
12	1.63 (1H, m), 1.34 (1H, m)	33.6	1.65 (1H, m), 1.39 (1H, m)	33.9
13		37.5		37.7
14		157.9		157.6
15	5.54 (1H, dd, $J = 8.1$, 3.3 Hz)	117.0	5.51 (1H, dd, $J = 7.8$, 2.8 Hz)	116.9
16	1.91 (1H, dd, $J = 14.4$, 2.4 Hz), 1.62 (1H, m)	37.7	1.92 (1H, brd, $J = 11.6$ Hz), 1.66 (1H, m)	37.7
17		35.8		35.7
18	0.97 (1H, m)	48.7	0.99 (1H, m)	48.9
19	1.32 (1H, m), 0.98 (1H, m)	36.7	1.33 (1H, m), 1.00 (1H, m)	36.6
20		28.8		28.8
21	1.26 (1H, m), 1.34 (1H, m)	33.1	1.26 (1H, m), 1.31 (1H, m)	33.1
22	1.38 (1H, m), 1.02 (1H, m)	35.1	1.39 (1H, m), 1.06 (1H, m)	35.1
23	1.02 (3H, s)	27.9	1.04 (3H, s)	28.4
24	0.82 (3H, s)	15.2	1.04 (3H, s)	15.9
25	0.95 (3H, s)	16.4	1.33 (3H, s)	15.2
26	1.11 (3H, s)	25.9	1.12 (3H, s)	25.8
27	0.92 (3H, s)	21.3	0.99 (3H, s)	21.6
28	0.82 (3H, s)	29.8	0.82 (3H, s)	30.0
29	0.95 (3H, s)	33.3	0.96 (3H, s)	33.4
30	0.91 (3H, s)	29.9	0.91 (3H, s)	30.0

^{1}H NMR spectral data of **1** and **2** were measured at 600 and 500 MHz in $CDCl_3$. ^{13}C NMR spectral data were measured at 150 MHz in $CDCl_3$. The assignments were based on the DEPT, HMQC, and HMBC experiments.

and C-24; H-24 to C-3, C-4, C-5, and C-23; H-25 to C-1, C-5, C-9, and C-10; H-26 to C-14, C-7, C-8, and C-9; H-27 to C-12, C-13, C-14, and C-18; H-28 to C-16, C-17, C-18, and C-22; H-29 to C-19, C-20, C-21, and C-30 (indicated by arrows in Figure 2), together with the chemical shift values of these carbons (Table 1), suggested that the structure of **1** was taraxer-14-ene-1α,3β-diol.

Compound **2** was obtained as a colorless powder ($CHCl_3$–MeOH); m.p. 209–210°C; $[\alpha]_D^{20}$ 55.8 ($c = 0.06$, $CHCl_3$), and showed IR absorption bands for hydroxyl (3454 cm^{-1}), carbonyl (1704 cm^{-1}), and double bond (1539 and 1464 cm^{-1}) functional groups. The EI-MS spectrum of **2** gave a molecular ion peak at *m/z* 440 $[M]^+$, and the HR-EI-MS of **2** at *m/z* 440.3622 indicated a molecular formula of $C_{30}H_{48}O_2$. The ^{1}H NMR spectrum of **2** showed eight methyl singlets at δ 1.04 (3H, s, H-23), 1.04 (3H, s, H-24), 1.33 (3H, s, H-25), 1.12 (3H, s, H-26), 0.99 (3H, s, H-27), 0.82 (3H, s, H-28), 0.96 (3H, s, H-29), and 0.91 (3H, s, H-30), an olefinic proton at δ 5.51 (1H, dd, $J = 7.8$, 2.8 Hz, H-15) and an oxygenated methine at δ 3.47

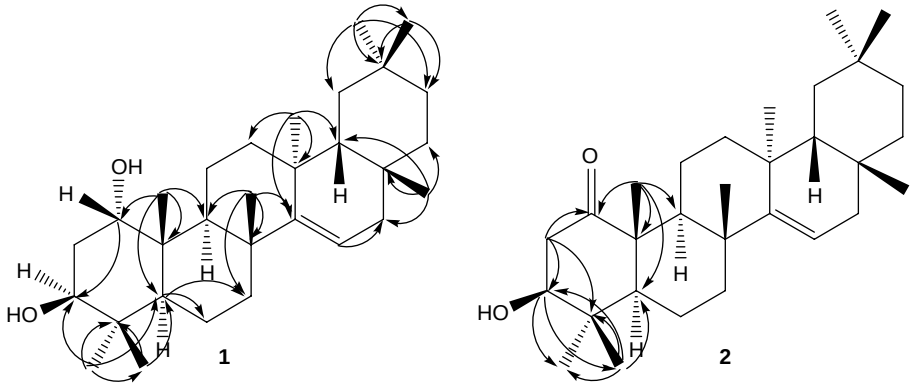

Figure 2. Main HMBC correlations of compounds **1** and **2**.

(1H, dd, $J = 12.0$, 4.8 Hz, H-3). The configuration of the hydroxyl group at C-3 was deduced to be in the β-position by a large $^{2,3}J_{H,H}$ coupling constant (12.0 Hz) of the axial proton at C-3. The ^{13}C NMR and DEPT spectra of **2** displayed 30 carbon signals including eight methyls, nine methylenes, five methines (one sp^2 hybrid, one oxygenated carbon), and eight quaternary carbons (one carbonyl carbon, one olefinic carbon; Table 1). These spectroscopic data in combination with seven degrees of unsaturation required by the molecular formula suggested that **2** was a pentacyclic triterpene containing one carbonyl, one double bond, and one hydroxyl group. The olefinic signals at δ 157.6 and 116.9 for C-14 and C-15 in the ^{13}C NMR spectrum and the EI-MS spectrum of **2** with fragment ion peaks at *m/z* 316, 298, 283, 204, and 189 indicated that **2** was also a $\Delta^{14,15}$-taraxeren derivative. A careful comparison of the NMR spectral data of **2** with those of **1** showed that the structure of **2** was rather similar to that of **1** except for the difference in A ring, namely, the hydroxyl group in the C-1 position of **1** was substituted by the carbonyl group in **2**. This was confirmed by the carbon chemical shift at δ_C 212.1 (C-1) and HMBC correlations from H-2 to C-1, C-3, and C-4; H-3 to C-23 and C-24; H-23 to C-3, C-4, C-5, and C-24; H-24 to C-3, C-4, C-5, and C-23; H-25 to C-1, C-5, C-9, and C-10 (indicated by arrows in Figure 2). Therefore, the structure of **2** was determined as 3β-hydroxytaraxer-14-ene-1-one.

Compounds **1**–**8** were evaluated against several human cancer cell lines including human lung adenocarcinoma (A549), human colon cancer (HCT-8), human hepatoma (Bel7402), human stomach cancer (BGC-823), and human ovarian cancer (A2780) cell lines. Compound **1** showed *in vitro* selective cytotoxicity against the A549 cell lines with an IC_{50} value of 1.22 μM. Compound **7** showed significant cytotoxicity against the A549, HCT-8, Bel7402, BGC-823, and A2780 cell lines with IC_{50} values of 0.21, 0.55, 0.40, 0.59, and 0.34 μM, in which the result of cytotoxicity against the A549 cell lines was similar to the reported data in the literature [6]. However, the other compounds were inactive ($IC_{50} > 10$ μM). The screening results of compounds **1** and **2** against the A549 cell lines indicated that the hydroxyl in the C-1 position may be the active group responsible for the cytotoxicity.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micro-melting-point apparatus and are uncorrected. Optical rotations were

measured with a Perkin-Elmer Model 343 spectropolarimeter. UV spectra were taken on a Thermo Spectronic spectrophotometer, Vision32 software V1.25. IR spectra were recorded as microscope transmission on a Nicolet 5700 FT-IR spectrophotometer. 1D and 2D NMR spectra were obtained at 600 MHz for ^{1}H and 150 MHz for ^{13}C, respectively, on an Inova SYS 600 MHz spectrometer in $CDCl_3$ with solvent peaks as references. EI-MS and HR-EI-MS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (200–300 mesh; Branch of Qingdao Haiyang Chemical Plant, Qingdao, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). TLC was carried out with glass precoated silica gel GF_{254} plates (Branch of Qingdao Haiyang Chemical Plant). Spots were visualized by spraying with 7% H_2SO_4 in 95% EtOH, followed by heating.

3.2 Plant material

The roots of *P. heterophyllum* were collected at Dayao Mountain, Guangxi Province, China, in August 2002, which was identified by Mr Guang-Ri Long (Guangxi Forest Administration, Guangxi 545005, China). A voucher specimen (No. YG2002-120) has been deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, China.

3.3 Extraction and isolation

The air-dried and powdered roots of *P. heterophyllum* (8 kg) were refluxed with 95% EtOH thrice (each 2 h). After the solvent was removed under reduced pressure at $<50°C$, a dark brown residue (510 g) was obtained. The residue was suspended in water and then partitioned with petroleum ether and EtOAc. The petroleum ether phase was concentrated to give a residue (49 g), which was separated by column chromatography over silica gel eluting with a gradient of increasing acetone (0–100%) in petroleum ether (60–90°C) followed by elution with MeOH to give 12 fractions (I–XII) on the basis of TLC analyses. Fraction III (3.8 g) was chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1) to give **3** (103 mg) and **12** (113 mg). Fraction IV (2.9 g) was purified by column chromatography over silica gel eluting with petroleum ether–ethyl acetate (10:1) to give three subfractions D1–D3; D2 was purified by preparation chromatography over silica gel with $CHCl_3$–MeOH (100:4) to yield **4** (9 mg), with petroleum ether–acetone (10:2) to yield **10** (13 mg); D3 was chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1) to give **5** (39 mg) and **7** (7 mg). Fraction V (1.2 g) was subjected to column chromatography over silica gel eluting with petroleum ether–ethyl acetate (1:1) to give **2** (9 mg), **8** (5 mg), and **11** (7 mg). Fraction VI (1.3 g) was purified by column chromatography over silica gel eluting with petroleum ether–acetone (10:1) to give three subfractions F1–F3; F2 was chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1) to give **9** (10 mg); F3 was chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1) to give **6** (16 mg). Fraction VII (0.9 g) was chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1) to give three subfractions, and the second subfraction was purified by column chromatography over silica gel eluting with petroleum ether–acetone (5:1) to yield **1** (12.5 mg).

3.3.1 *Taraxer-14-ene-1α,3β-diol* (**1**)

Taraxer-14-ene-1α,3β-diol **(1)** was obtained as a colorless powder

from $CHCl_3$–MeOH, 12.5 mg; m.p. 213–214°C; $[\alpha]_D^{20}$ 49.1 ($c = 0.05$, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 242.0 (0.88) nm; IR (KBr) ν_{max}: 3384, 2955, 2925, 2869, 1461, 1377, 1037, 1000 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data, see Table 1; EI-MS *m/z* (%): 442 (18) $[M]^+$, 424 $[M-H_2O]^+$ (11), 409 (16), 391 (18), 318 (23), 300 (16), 285 (20), 267 (12), 218 (12), 204 (100), 189 (21), 185 (11), 161 (12), 147 (17), 133 (24), 121 (26), 109 (27), 107 (28), 95 (32), 81 (26), 69 (38), 55 (37); HR-EI-MS *m/z* 442.3813 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 442.3811).

3.3.2 3β-Hydroxytaraxer-14-ene-1-one (**2**)

3β-Hydroxytaraxer-14-ene-1-one (**2**) was obtained as a colorless powder from $CHCl_3$–MeOH, 9.0 mg; m.p. 183–184°C; $[\alpha]_D^{20}$ 55.8 ($c = 0.06$, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204.0 (0.61) nm; IR (KBr) ν_{max} 3454, 2923, 1741, 1704, 1539, 1464, 1379, 1234, 1022, 930, 794, 586 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectral data, see Table 1; EI-MS *m/z* (%) 440 (43) $[M]^+$, 422 (84) $[M-H_2O]^+$, 407 (38), 316 (34), 298 (61), 283 (56), 270 (12), 255 (16), 229 (13), 218 (38), 204 (36), 189 (29), 175 (17), 161 (21), 149 (49), 137 (42), 119 (38), 109 (43), 105 (48), 95 (55), 81 (51), 69 (77), 64 (96), 55 (100); HR-EI-MS *m/z* 440.3622 $[M]^+$ (calcd for $C_{30}H_{48}O_2$, 440.3654).

3.4 Bioassays

For cells and culture condition and cell proliferation assay, see previous report [7].

Acknowledgements

This research was supported by the Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education. We thank Ms Wu Yan for the NMR and Mr Zeper Abliz for the EI-MS and HR-EI-MS measurements.

References

[1] Jiangsu New Medical College, *Dictionary of Chinese Materia Medica* (Shanghai Science and Technology Publishing House, Shanghai, 1998), p. 785.
[2] I. Merfort, J. Buddrus, M.A.M. Nawwar, and J. Lambert, *Phytochemistry* **31**, 4031 (1992).
[3] B. Wiedemanna, H. Lerchea, H. Lottera, A. Neszmelyib, H. Wagnera, and A.A. Muller, *Phytochemistry* **52**, 333 (1999).
[4] H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.* **85**, 3688 (1963).
[5] B.B. Robert, E.J. Neil, N.S. William, and C.S. Chad, *Magn. Reson. Chem.* **36**, 539 (1998).
[6] S.H. Kim, K.S. Lee, J.K. Son, G.H. Je, J.S. Lee, C.H. Lee, and C.J. Cheong, *J. Nat. Prod.* **61**, 643 (1998).
[7] Z. Xue, S. Li, S.J. Wang, Y.H. Wang, Y.C. Yang, J.G. Shi, and L. He, *J. Nat. Prod.* **69**, 907 (2006).