A NEW TRITERPENE FROM THE LEAVES OF *Craibiodendron yunnanense*

Hui-Ling Ma,[1] Jin-Yi Qin,[1] Lei Wang,[1] Sheng-Xiang Yang,[1,2,3] Ming-Hua Chiu,[2*] Xian-Min Zhang,[2] and Jin-Ming Gao[1,3*]

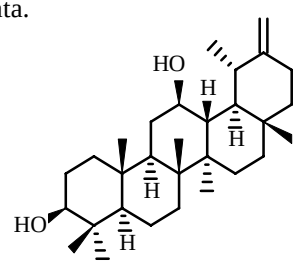
UDC 547.918

*A new triterpene, taraxast-20(30)-en-3*β*,12*β*-diol (**1**), and eight known compounds were isolated from the leaves of* Craibiodendron yunnanense*. Their structures were established on the basis of spectral evidence.*

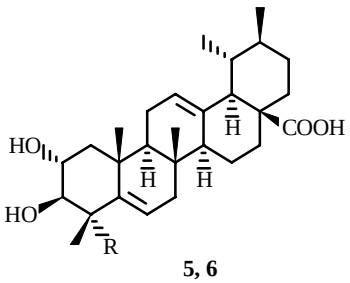
Key words: *Craibiodendron yunnanense*, taraxast-20(30)-en-3*β*,12*β*-diol, triterpenoid, Ericaceae.

Craibiodendron yunnanense W. W. Smith (Ericaceae), a well-known toxic plant, is an evergreen tree or shrub distributed mainly in hilly and valley regions of south, central, and northwest Yunnan Province of China. It has been reported that eating seven pieces of the plant leaves would put people in a coma state for more than one day [1]. The dried plant leaves have been used in Chinese folk medicine for relieving pain of arthritis, stomach algia, and paralysis, and as an insecticide in the southwest region [2]. More recently, ten antifeedant grayanane diterpenoids [2], five flavonoids [3, 4], and two triterpenoids [5] have been isolated from this plant.

In a continuing research of bioactive metabolites from medicinal plants in Yunnan Province of China, an investigation into chemical constituents from the leaves of *Craibiodendron yunnanense* led to isolation of a new urs-type triterpene derivative **1**, together with eight known compounds, namely ursolic acid (**2**), *α*-amyrin (**3**), *β*-amyrin (**4**) [6 – 8], 2*α*,3*β*-dihydroxyurs-5,12-dien-28-oic acid (**5**) [9], 2*α*,3*β*,23-trihydroxyurs-12-en-28-oic acid (**6**) [10], (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(*β*-D-glucopyranosyl)-2-[(*R*)-2′-hydroxydocosanoyl]amino-8-octadecene-1,3,4-triol (**7**) [11], and (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(*β*-D-glucopyranosyl)-2-(palmitoyl) amino-8-octa- decene-1,3,4-triol (**8**) [11], and quercetin-3-*O*-*β*-D-glucopyranoside (**9**) [12]. Their structures were elucidated by spectral data.

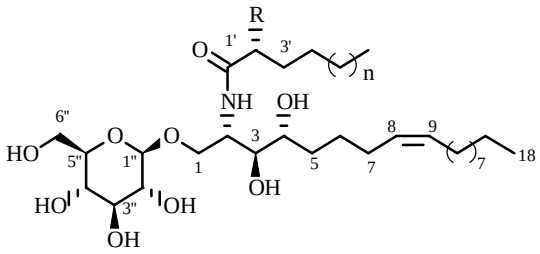


1



5, 6

5: R = CH_3; **6:** R = CH_2OH



7, 8

7: R = OH, n = 17; **8:** R = H, n = 11

Compound **1** was obtained as a colorless powder, $[\alpha]_D$ +6.4° (*c* 0.54, CH_3OH). The IR spectrum indicated absorptions of hydroxyls at 3406 and the olefinic bond at 1639 cm^{-1}. The molecular formula was determined as $C_{30}H_{50}O_2$, with six degrees of unsaturation, on the basis of the mass spectrum and ^{13}C NMR (DEPT) spectral data (Table 1), and further confirmed by positive high-resolution TOF-MS, which exhibited the molecular ion peak at *m/z* 465.3706 ($[M+Na]^+$, calcd. 465.3708). The mass spectra (EI) displayed the occurrence of diagnostically prominent peaks at 205 (30%) and 189 (46%) arising from a retro-Diels-Alder fragmentation, indicating a pentacyclic triterpenoid compound.

1) College of Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China, e-mail: jinminggaocn@yahoo.com.cn, e-mail: mhchiu@mail.kib.ac.cn; 2) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China; 3) National Phytochemistry Engineering Research Center in West China, Yangling, Shaanxi 712100, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 35-37, January-February, 2008. Original article submitted November 23, 2006.

0009-3130/08/4401-0044

TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data for **1** (C_5D_5N, TMS, δ, ppm)

Atom	δ_C (DEPT)	δ_H (Mult. J/Hz)	HMBC
1	38.7 t	1.74 (m), 0.95 (m)	H-2, 3, 25
2	27.0 t	1.62 (m)	H-1
3	75.6 d	3.63 (dd, J = 12.0, 5.0)	H-5, 23, 24
4	39.6 s		H-3, 5, 23, 24
5	53.9 d	0.82 (bd, J = 12.0)	H-23, 24
6	18.5 t	1.89 (m), 1.58 (m)	H-5, 7
7	34.7 t	1.43 (m)	H-5, 26
8	41.9 s		H-26
9	52.2 d	1.77 (m)	H-25, 26
10	42.5 s		H-5, 25
11	24.7 t	3.18 (m), 1.36 (m)	
12	79.6 d	3.83 (m)	H-9, 13, 27
13	39.7 d	1.66 (m)	H-27
14	44.2 s		H-27
15	27.0 t	1.69 (m), 0.99 (m)	H-27
16	39.6 t	1.31 (m), 1.16 (m)	H-15, 28
17	34.7 s		H-19, 21, 22, 28
18	48.8 d	0.97 (dd, J = 10.5, 7.0)	H-19, H-22, H-29
19	39.4 d	2.14 (m)	H-21, H-29, 30
20	155.0 s		H-19, 21, 29
21	26.0 t	2.49 (m), 2.22 (m)	H-19, 22, 30
22	39.3 t	1.42 (m), 1.38 (m)	H-21, 28
23	28.7 q	1.25 (s)	H-3, 5, 24
24	16.6 q	1.12 (s)	H-3, 5, 23
25	15.0 q	1.00 (s)	H-5
26	16.1 q	1.13 (s)	
27	13.2 q	1.27 (s)	
28	19.9 q	0.94 (s)	H-22
29	25.5 q	1.05 (d, J = 6.6)	H-19, 30
30	107.5 t	4.79 (s), 4.72 (s)	H-19, 21

The gross structure of **1** was deduced from detailed analyses of ^{1}H and ^{13}C NMR data aided with 2D NMR experiments. A close inspection of the ^{13}C NMR (Table 1) and DEPT spectra of **1** revealed the presence of 30 signals which were attributed to seven methyls, ten methylenes, and seven methines and six quaternary carbon atoms, including an exocyclic double bond (δ 155.0, 107.5) and two axially oriented carbinolic methine groups (δ 79.6, 75.6). In the ^{1}H NMR spectra (Table 1) of **1**, two protons at δ 3.63 (dd, J = 12.0, 5.0 Hz) and at δ 3.83 (m) were linked to two oxygen carbon atoms C-3 and C-12, respectively. This was confirmed by the HMBC correlations (Table 1) of H-3 with C-1, C-23, and C-24, and of H-12 with C-9, C-14, and C-27. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of taraxasterol [13 – 15], and this suggested that they possess the same skeleton. The distinct difference in ^{13}C NMR between **1** and taraxasterol was that the signal at δ 26.2 (C-12) in taraxasterol was replaced by one at δ 79.6 in compound **1**, namely, the hydroxyl group at C-12 of **1** is absent in taraxasterol.

The relative configuration of the hydroxy group at C-12 position in the molecule was deduced from ROESY experiments. Observed NOE correlations between H-12 and H-9α, H-27 indicated that this hydroxyl was assigned to the β-orientation. In summary, compound **1** was established as taraxast-20(30)-en-3β,12β-diol.

EXPERIMENTAL

General Procedures. Melting points (mp) are determined on an XRC-1 apparatus and uncorrected. Optical rotation was measured with a Horiba model a SEPA-300 polarimeter. IR spectra were obtained with a Nexus 870 FT-IR spectrophotometer with KBr pellets. NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers in C_5D_5N

with TMS as an internal standard, δ in ppm, J in Hz. EI-MS spectra were recorded with a VG Autospec-3000 spectrometer, *m/z* (rel. int.). HR TOF-MS was recorded with an API QSTAR Pulsar 1 spectrometer.

Column chromatography (CC) was carried out on silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). Fractions were monitored by TLC and spots were visualized on precoated silica gel plates by spraying with 10% H_2SO_4 in ethanol followed by heating.

Plant Material and Extraction and Isolation. The fresh leaves of *C. yunnanense* were collected at Kunming in Yunnan province, China, in November, 2005 and identified by Dr. Shui Y. M., Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen was deposited at the herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences. The air-dried and powdered leaves of *C. yunnanense* (1.5 kg) were extracted three times with commercial methanol at reflux. The combined organic layer was concentrated in vacuo to give a deep brown gum (100 g), which was suspended in H_2O and successively extracted with EtOAc and *n*-butanol. The two organic layers were concentrated in vacuo to give EtOAc extract (25 g) and *n*-butanol extract (30 g). EtOAc residue was subjected to repeated silica gel column chromatography and eluted with chloroform/methanol (100:0, 98:2, 95:5, 90:10, 80:20, 50:50, 0:100, v/v). The fraction IV (5 g) eluted with petroleum ether/acetone 10:1 was further purified by repeated CC (silica gel; petroleum ether/acetone 15:1 (v/v), petroleum ether/acetone 10:1 (v/v)) and subjected to Sephadex LH-20 chromatography (methanol) to afford compounds **1** (13 mg) and **2** (15 mg). The fraction III (7 g) eluted with petroleum ether/acetone 15:1 was further purified by repeated silica gel CC (petroleum ether/ ethyl acetate, 15:1 (v/v)) and subjected to Sephadex LH-20 chromatography (methanol) to afford compounds **3** (10 mg) and **4** (14 mg). The *n*-butanol extract was dissolved in MeOH and passed through macroporous absorption resin D101 (MeOH/H_2O, 30:1, 60:1, 90:1, 100:0). Fraction (8 g) eluted with (MeOH/H_2O, 90:1) was submitted to Si gel column chromatography ($CHCl_3$/MeOH, 20:1, 15:1, 10:1, 5:1, 1:1) to form fractions A-E. Fraction A was chromatographed on RP-18 (MeOH/H_2O, 65:35) and Sephadex LH-20 (MeOH) to afford **5** (8 mg) and **6** (6 mg). Fraction B was subjected to RP-18 CC (MeOH/H_2O, 45:55) and further purified by preparative HPLC (MeOH/H_2O, 50:50) to produce **7** (10 mg) and **8** (5 mg). Fraction C was submitted to RP-18 CC (MeOH/H_2O, 40:60) and further purified by Sephadex LH-20 (MeOH) CC to furnish **9** (12 mg).

Taraxast-20(30)-en-3*β*,12*β*-diol (1): Colorless powder. mp 261–262°C, $[\alpha]_D$ +6.4° (*c* 0.54, CH_3OH); IR (KBr, ν, cm^{-1}): 3406, 2939, 1639, 1462, 1382, 1184, 1039, 998, 879, 634; ^1H and ^{13}C NMR: See Table 1; HRTOF-MS: 465.3706 ($[M+Na]^+$, $C_{30}H_{50}O_2$; calcd. 465.3708); MS (EI, 70 eV) *m/z* (%): 442 $[M]^+$ (7), 424 $[M-18]^+$ (6), 229 (18), 219 (14), 207 (4), 206 (12), 205 (30), 203 (29), 189 (46), 175 (39), 161 (25), 147 (34), 135 (43), 121 (76), 109 (100), 95 (84), 81 (57), 69 (40), 55 (39).

Ursolic Acid (2): White powder, EIMS m/z (%): 456 (4), 248 (100), 203 (52), 189 (14), 175 (12), 133 (40).

α-**Amyrin (3):** White powder, EIMS *m/z* (%): 426 (10). All spectroscopic data are consistent with literature.

β-**Amyrin (4):** White powder, EIMSm/z (%): 426 (22).

2*α*,3*β*-Dihydroxyurs-5,12-dien-28-oic acid (5): White powder, FAB-MS *m/z*: 471 $[M+H]^+$ (30), 437 (32), 409 (65), 248 (100), 203 (70), 133 (65). ^1H NMR (C_5D_5N, 400 MHz, δ, J/Hz): 3.61 (1H, s, H-2*β*), 3.39 (1H, d, J = 9.4, H-3*α*), 4.07 (1H, t, J = 9.7, H-5), 5.47 (1H, br s, H-12), 2.63 (1H, d, J = 11.3, H-18), 1.22, 1.21, 1.19, 1.00, 0.98, 0.96 (each 3H, s, 6×CH_3), 1.06 (3H, d, J = 8.4, 29-CH_3); ^{13}C NMR (C_5D_5N, 100 MHz, δ): 48.2 (t, C-1), 68.7 (d, C-2), 83.9 (d, C-3), 39.9 (s, C-4), 139.4 (s, C-5), 125.6 (d, C-6), 33.6 (t, C-7), 39.5 (s, C-8), 48.2 (d, C-9), 39.6 (s, C-10), 25.0 (t, C-11), 122.5 (d, C-12), 144.9 (s, C-13), 44.0 (s, C-14), 28.7 (t, C-15), 26.2 (t, C-16), 48.1 (s, C-17), 56.0 (d, C-18), 40.1 (d, C-19), 39.9 (d, C-20), 31.1 (t, C-21), 37.5 (t, C-22), 29.4 (q, C-23), 17.6 (q, C-24), 17.5 (q, C-25), 18.9 (q, C-26), 23.8 (q, C-27), 180.2 (s, C-28), 17.0 (q, C-29), 21.4 (q, C-30) [9].

2*α*,3*β*,23-Trihydroxyurs-12-en-28-oic acid (6): White powder, FAB-MS *m/z*: 489 $[M+H]^+$ (25), 543 (4), 488 (5), 419 (7), 356 (6), 299 (14), 282 (12), 264 (17), 207 (100), 172 (43), 115 (50). ^1H NMR (C_5D_5N, 400 MHz, δ, J/Hz): 4.24 (1H, m, H-2*β*), 4.28 (1H, m, H-3*α*), 5.45 (1H, s, H-12), 2.60 (1H, d, J = 11.4, H-18), 3.71 (1H, d, J = 10.5, H-23*α*), 4.23 (1H, d, J = 10.5, H-23*β*), 1.05, 1.11, 1.18, 1.12 (each 3H, s, 4×CH_3), 0.89 (3H, d, J = 5.9 Hz, H-29), 0.84 (3H, br.s, H-30); ^{13}C NMR (C_5D_5N, 100 MHz, δ): 48.0 (t, C-1), 68.9 (d, C-2), 78.2 (d, C-3), 43.7 (s, C-4), 48.0 (d, C-5), 18.5 (t, C-6), 33.2 (t, C-7), 40.1 (s, C-8), 47.9 (d, C-9), 38.3 (s, C-10), 23.8(t, C-11), 125.6 (d, C-12), 139.3 (s, C-13), 42.6 (s, C-14), 28.7 (t, C-15), 24.9 (t, C-16), 48.1 (s, C-17), 53.5 (d, C-18), 39.4 (d, C-19), 39.3 (d, C-20), 31.0 (t, C-21), 37.5 (t, C-22), 66.4 (t, C-23), 14.5 (q, C-24), 17.5 (q, C-25), 23.9 (q, C-27), 180.0 (s, C-28), 17.5 (q, C-29), 21.4 (q, C-30) [10].

(2*S*,3*S*,4*R*,8*Z*)-1-*O*-(*β*-D-Glucopyranosyl)-2-[(*R*)-2′-hydroxydocosanoyl]amino-8-octadecene-1,3,4-triol (7): White powder, FAB^- -MS *m/z*: 814 $[M-H]^-$ (100), 652 $[M-H-C_6H_{11}O_5]^-$ (23), 339 (20), 311 (32), 183 (28), 159 (12), 119 (21), 99 (44), 80 (22); ^1H NMR (C_5D_5N, 400 MHz, δ, J/Hz): 8.59 (1H, d, J = 7.8, -NH), 4.72 (1H, dd, J = 10.6, 6.0, H-1*a*), 4.51 (1H,

dd, J = 10.6, 6.0, H-1b), 4.21 (1H, m, H-3), 4.20 (1H, m, H-4), 5.49 (1H, m, H-8), 5.49 (1H, m, H-9), 4.57 (1H, m, H-2″), 4.94 (1H, d, J = 7.6, H-1″), 1.25 (br s, nCH_2), 0.84 (t, J = 5.4, 2×-CH_3); ^{13}C NMR (C_5D_5N, 100 MHz, δ): 70.5 (t, C-1), 51.7 (d, C-2), 75.9 (d, C-3), 72.4 (d, C-4), 130.9 (d, C-8), 130.2 (d, C-9), 175.7 (s, C-1′), 72.5 (d, C-2′), 105.6 (d, C-1″), 75.2 (d, C-2″), 78.5 (d, C-3″), 71.4 (d, C-4″), 78.6 (d, C-5″), 62.6 (t, C-6″), 23.0-32.0 (t, nCH_2), 14.3 (q, C-18 and C-22′) [11].

(2*S*,3*S*,4*R*,8*Z*)-1-*O*-(*β*-D-glucopyranosyl)-2-(palmitoyl)amino-8-octadecene-1,3,4-triol (8): White powder, FAB^--MS *m/z*: 714 $[M-H]^-$ (100), 552 $[M-H-C_6H_{11}O_5]^-$ (16), 473 (6), 312 (9), 176 (5), 159 (12), 119 (27), 97(34); ^{1}H NMR (C_5D_5N, 400 MHz, δ, J/Hz): 8.42 (1H, d, J = 8.7, -NH), 4.72 (dd, J = 5.4, 10.6, H-1*a*), 4.51 (m, H-3), 4.21 (1H, dd, J = 5.4, 10.6, H-1b), 4.20 (1H, m, H-4), 5.46 (1H, d, J = 4.7, H-8), 5.45 (1H, d, J = 4.7, H-9), 4.57 (1H, m, H-2″), 4.95 (d, J = 7.6 Hz, H-1″), 1.24 (br. s, nCH_2), 0.84 (t, J = 5.0, 2×-CH_3); ^{13}C NMR (C_5D_5N, 100 MHz, δ, J/Hz): 70.4 (t, C-1), 54.5 (d, C-2), 71.2 (d, C-3), 72.4 (d, C-4), 130.7 (d, C-8), 130.2 (d, C-9), 175.6 (s, C-1′), 105.7 (d, C-1″), 75.2 (d, C-2″), 78.5 (d, C-3″), 71.6 (d, C-4″), 78.6 (d, C-5″), 62.7 (t, C-6″), 23.0-35.6 (t, nCH_2), 14.4 (q, C-18 and C-16′) [11].

Quercetin-3-*O*-*β*-D-glucopyranoside (9): Yellow powder, FAB^--MS *m/z* 463 $[M-H]^+$ (100), 447 (5), 300 (22), 171 (17), 97 (24) [12].

ACKNOWLEDGMENT

This work was partially supported by the Program for New Century Excellent Talents in University (NCET). The authors are grateful to Mr. Y.-N. He and Ms. H.-L. Liang of Kunming Institute of Botany, Chinese Academy of Sciences, for measuring NMR and MS data, respectively.

REFERENCES

1. J. S. Chen and S. Zheng, *Chinese Poisonous Plants*, Science Press, Beijing, 1987, p. 218.
2. H. P. Zhang, L. Q. Wang, and G. W. Qin, *Bioorg. Med. Chem.*, **13**, 5289 (2005).
3. B. A. Xu, H. Su, and M. Z. Zhang, *Acta Scientiarun Naturalium Universitatis Pekinensis*, **32**, 700 (1996).
4. T. Wang, J. Yang, and H. Li, *Acta Bot. Sin.*, **39**, 82 (1997).
5. R. T. Li, J. Y. Li, J. K. Wang, Z. Y. Zhu, and H. D. Sun, *Acta Botanica Yunnanica*, **27**, 565 (2005).
6. Y. H. Zhang, L. Zhou, R. B. Shi, Y. J. Guo, and Y. Dong, *China J. Chin. Mater. Med.*, **31**, 1247 (2006).
7. Y. H. Gong, *Chemical Shifts of ^{13}C NMR of Natural Organic Products*, Yunnan Sci. Tech. Press, Kunming, 1985, p.130.
8. T. N. Misra, R. S. Singh, T. N. Oiha, and J. Upadhyay, *J. Nat. Prod.*, **44**, 735 (1981).
9. S. B. Mahato and A. P. Kundu, *Phytochemistry*, **34**, 1389 (1987).
10. I. K. Adnyana, A. Tezuka, H. Banskota, Q. Xiong, Q. T. Kim, and S. Kadota, *J. Nat. Prod.*, **63**, 496 (2000).
11. F. Cateni, J. Zilic, G. Falsone, F. Hollan, F. Frausin, and V. Scarcia, *Farmaco*, **58**, 809 (2003).
12. K. R. Markham, B. Ternai, R. Stanley, H. Geiger, and T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
13. S. B. Mahato and A. P. Kundu, *Phytochemistry*, **37**, 1517 (1994).
14. V. Anjaneyulu, K. Ravi, K. H. Prasad, and J. D. Connolly, *Phytochemistry*, **28**, 1471 (1989).
15. W. F. Reynolds, S. Mclean, and J. Poplawski, *Tetrahedron*, **42**, 3419 (1986).