Chemical Constituents from *Craibiodendron yunnanense*

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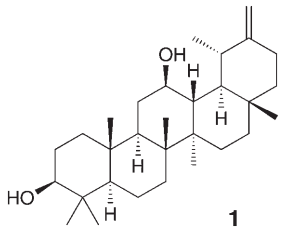
A new triterpene, (3β,12β)-taraxast-20(30)-ene-3,12-diol (=(3β,12β,18α,19α)-urs-20(30)-ene-3,12-diol; **1**), together with the known compounds ursolic acid, α-amyrin, β-amyrin, (2α,3β)-2,3-dihydroxyursa-5,12-dien-28-oic acid, (2α,3β)-2,3,23-trihydroxyurs-12-en-28-oic acid, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-{[(2*R*)-2-hydroxydocosanoyl]amino}octadec-8-ene-1,3,4-triol, and (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-[(palmitoyl)amino]octadec-8-ene-1,3,4-triol, and quercetin 3-(β-D-glucopyranoside) were isolated from the leaves of *Craibiodendron yunnanense*. Their structures were established on the basis of spectral evidence. The last four compounds were identified for the first time in this plant.

Introduction. *Craibiodendron yunnanense* W. W. SMITH (family 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 as a Chinese folk medicine for relieving arthritis pain, stomach algia, and paralysis, and as an insecticide in the southwest region. More recently, ten antifeedant grayanane diterpenoids [2], five flavonoids [3][4], and two triterpenoids [5] have been isolated from this plant. Within the context of our search for bioactive metabolites from medicinal plants in Yunnan Province of China, further investigations of the chemical constituents from the leaves of *C. yunnanense* led to the isolation and identification of the new ursane-type triterpene derivative **1** which is described in this paper.



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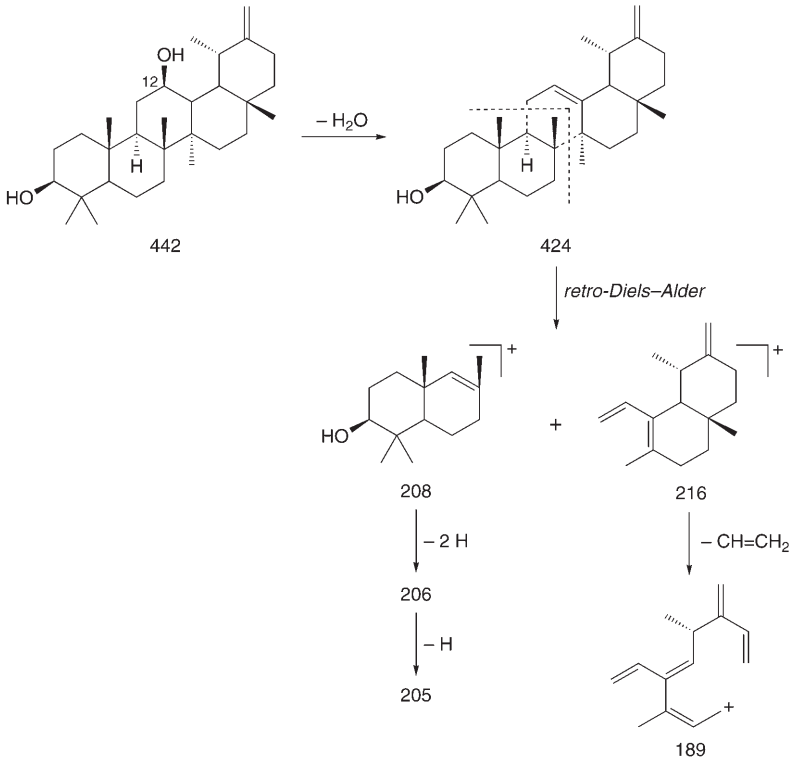
Results and Discussion. – The air-dried leaves of *C. yunnanense* were extracted with MeOH to afford a crude extract. After evaporation, the crude extract was suspended in

H_2O and successively extracted with AcOEt and BuOH. The AcOEt and BuOH extracts were subjected to repeated column chromatography (silica gel and *Sephadex LH-20*) to furnish **1** besides eight known compounds.

Compound **1** was obtained as an optically active colorless powder. Its molecular formula was determined as $C_{30}H_{50}O_2$, with 6 degrees of unsaturation, on the basis of ^{13}C-NMR (DEPT) data and the pseudomolecular ion $[M+Na]^+$ at *m/z* 465.3706 in HR-TOF-MS (positive mode). The IR spectrum indicated absorptions of OH groups at 3406 and an olefinic bond at 1639 cm^{-1}. The EI-MS revealed diagnostic peaks at 216, 208, 207, 206, 205, and 189 arising from a *retro-Diels–Alder* fragmentation of $[M-H_2O]^+$ (*Scheme*), indicating a pentacyclic triterpenoid compound. The structure of **1** as (3*β*,12*β*)-taraxast-20(30)-ene-3,12-diol was deduced from detailed analyses of ^{1}H- and ^{13}C-NMR data (*Table*) supported by 2D NMR experiments (HMBC, ROESY) and comparison with those of taraxasterol (=(3*β*,18*α*,19*α*)-urs-20(30)-en-3-ol) [6–8].

Scheme. *Observed Key EI-MS Fragments* (values in *m/z*) *of Compound* **1**



A close inspection of the ^{13}C-NMR and DEPT spectra of **1** revealed the presence of 30 signals which were attributed to seven Me, ten CH_2, and seven CH groups and to six quaternary C-atoms, including an exocyclic C=C bond (*δ* 155.0 and 107.5) and two axially oriented OH-substituted CH groups (*δ* 79.6 and 75.6). In the ^{1}H-NMR spectra of **1**, two protons at *δ* 3.63 (*dd*, *J* = 12.0, 5.0 Hz) and at *δ* 3.83, 3.85 (*m*) were linked to two O-substituted C-atoms, C(3) and C(12), respectively. This was confirmed by the

Table. *^{1}H-NMR* (500 MHz) *and ^{13}C-NMR* (125 MHz, DEPT) *Data of* **1** in *(D_5)Pyridine.* δ in ppm, J in Hz.

	δ(C)	δ(H)	(C → H)-HMBC
CH_2(1)	38.7 (*t*)	1.73–1.75 (*m*), 0.94–0.96 (*m*)	CH_2(2), H–C(3), Me(25)
CH_2(2)	27.0 (*t*)	1.60–1.63 (*m*)	CH_2(1)
H–C(3)	75.6 (*d*)	3.63 (*dd*, *J* = 12.0, 5.0)	H–C(5), Me(23), Me(24)
C(4)	39.6 (*s*)		H–C(3), H–C(5), Me(23), Me(24)
H–C(5)	53.9 (*d*)	0.82 (br. *d*, *J* = 12.0)	Me(23), Me(24)
CH_2(6)	18.5 (*t*)	1.88–1.91 (*m*), 1.57–1.60 (*m*)	H–C(5), CH_2(7)
CH_2(7)	34.7 (*t*)	1.35–1.50 (*m*)	H–C(5), Me(26)
C(8)	41.9 (*s*)		Me(26)
H–C(9)	52.2 (*d*)	1.74–1.80 (*m*)	H–C(12), Me(25), Me(26)
C(10)	42.5 (*s*)		H–C(5), Me(25)
CH_2(11)	24.7 (*t*)	3.16–3.20 (*m*), 1.30–1.40 (*m*)	
H–C(12)	79.6 (*d*)	3.80–3.85 (*m*)	H–C(9), H–C(13), Me(27)
H–C(13)	39.7 (*d*)	1.65–1.67 (*m*)	Me(27)
C(14)	44.2 (*s*)		H–C(12), Me(27)
CH_2(15)	27.0 (*t*)	1.68–1.71 (*m*), 0.97–1.00 (*m*)	Me(27)
CH_2(16)	39.6 (*t*)	1.29–1.33 (*m*), 1.14–1.18 (*m*)	CH_2(15), Me(28)
C(17)	34.7 (*s*)		H–C(19), CH_2(21), CH_2(22), Me(28)
H–C(18)	48.8 (*d*)	0.97 (*dd*, *J* = 10.5, 7.0)	H–C(19), CH_2(22), Me(29)
H–C(19)	39.4 (*d*)	2.12–2.16 (*m*)	CH_2(21), Me(29), CH_2(30)
C(20)	155.0 (*s*)		H–C(19), CH_2(21), Me(29)
CH_2(21)	26.0 (*t*)	2.45–2.53 (*m*), 2.17–2.27 (*m*)	H–C(19), CH_2(22), CH_2(30)
CH_2(22)	39.3 (*t*)	1.40–1.44 (*m*), 1.36–1.40 (*m*)	CH_2(21), Me(28)
Me(23)	28.7 (*q*)	1.25 (*s*)	H–C(3), H–C(5), Me(24)
Me(24)	16.6 (*q*)	1.12 (*s*)	H–C(3), H–C(5), Me(23)
Me(25)	15.0 (*q*)	1.00 (*s*)	H–C(5)
Me(26)	16.1 (*q*)	1.13 (*s*)	
Me(27)	13.2 (*q*)	1.27 (*s*)	H–C(12)
Me(28)	19.9 (*q*)	0.94 (*s*)	CH_2(22)
Me(29)	25.5 (*q*)	1.05 (*d*, *J* = 6.6)	H–C(19), CH_2(30)
CH_2(30)	107.5 (*t*)	4.79 (*s*), 4.72 (*s*)	H–C(19), CH_2(21)

HMBC correlations (*Table*) of H–C(3) with C(1), C(23), and C(24), and of H–C(12) with C(9), C(14), and C(27). The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of taraxasterol [6–8], suggesting 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)) of taraxasterol was replaced by one at δ 79.6 in the case of **1**, the OH–C(12) of **1** being absent in taraxasterol. The relative configuration of OH–C(12) was deduced from ROESY experiments. The observed NOEs H–C(12)/H–C(9), and Me(27) indicated that this OH group assumes the β-orientation.

Together with **1**, eight known compounds, *i.e.*, the five triterpenes ursolic acid, α-amyrin, and β-amyrin [9–11], (2α,3β)-2,3-dihydroxyursa-5,12-dien-28-oic acid [12] and (2α,3β)-2,3,23-trihydroxyurs-12-en-28-oic acid [13], the two cerebrosides (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-{[(2*R*)-2-hydroxydocosanoyl]amino}octadec-8-ene-1,3,4-triol and (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-[(palmitoyl)amino]octadec-8-ene-1,3,4-triol [14], and the flavonoid quercetin 3-(β-D-glucopyranoside) [15], were also isolated from *C. yunnanense*. Their structures were elucidated by spectral data and

comparison with literature values. The (2α,3β)-2,3,23-trihydroxyurs-12-en-28-oic acid, the two cerebrosides, and the flavonoid were isolated for the first time from this plant.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, *Qingdao Marine Chemical Ltd.*, Qingdao, P.R. China) and *Sephadex LH-20* (*Amersham Biosciences*, Uppsala, Sweden). TLC: precoated silica gel plates; visualization by spraying with 10% H_2SO_4 in EtOH followed by heating. M.p.: *XRC-1* apparatus; uncorrected. Optical rotation: *Horiba SEPA-300* polarimeter. IR Spectra: *Nexus 870-FT-IR* spectrophotometer; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AV-400* and *DRX-500* spectrometers; in C_5D_5N with $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. EI-MS: *VG Autospec-3000* spectrometer; in *m*/*z* (rel. %). HR-TOF-MS: *API-QSTAR-Pulsar-1* spectrometer.

Plant Material. The fresh leaves of *C. yunnanense* were collected at Kunming in Yunnan Province, China, in November 2005 and identified by Dr. *Y. M. Shui*, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimen was deposited at the herbarium of the Kunming Institute of Botany.

Extraction and Isolation. The air-dried and powdered leaves of *C. yunnanense* (1.5 kg) were extracted three times with commercial MeOH under reflux. The combined org. layer was concentrated and the deep brown gum (100 g) suspended in H_2O and successively extracted with AcOEt and BuOH. Concentration of the org. layers gave an AcOEt extract (25 g) and a BuOH extract (30 g). The AcOEt extract was subjected to repeated CC (silica gel, $CHCl_3$/MeOH 100:0, 98:2, 95:5, 90:10, 80:20, 50:50, and 0:100). *Fraction IV* (5 g; eluted with petroleum ether/acetone 10:1, was further purified by repeated CC (silica gel, petroleum ether/acetone 15:1, petroleum ether/acetone 10:1); then *Sephadex LH-20*, MeOH): **1** (13 mg) and ursolic acid (15 mg). *Fr. III* (7 g; eluted with petroleum ether/acetone 15:1) was further purified by repeated CC (silica gel, petroleum ether/AcOEt, 15:1; then *Sephadex LH-20*, MeOH): α-amyrin (10 mg) and β-amyrin (14 mg). The BuOH extract was dissolved in MeOH and subjected to CC (macroporous absorption resin *D101*, MeOH/H_2O 30:1, 60:1, 90:1, and 100:0). The fraction (8 g) eluted with MeOH/H_2O 90:1) was submitted CC (silica gel, ($CHCl_3$/MeOH 20:1, 15:1, 10:1, 5:1, and 1:1): *Fractions A–E*. *Fr. A* was subjected to CC (*RP-18*, MeOH/H_2O 65:35, then *Sephadex LH-20*; MeOH): (2α,3β)-2,3-dihydroxyursa-5,12-dien-28-oic acid (8 mg) and (2α,3β)-2,3,34-trihydroxyurs-12-en-28-oic acid (6 mg). *Fr. B* was subjected to CC (*RP-18*, MeOH/H_2O 45:55) and further purified by prep. HPLC (MeOH/H_2O 50:50): (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-{[(2*R*)-2-hydroxydocosanoyl]amino}octadec-8-ene-1,3,4-triol (10 mg) and (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2-[(palmitoyl)amino]octadec-8-ene-1,3,4-triol (5 mg). *Fr. C* was submitted to CC (*RP-18*, MeOH/H_2O 40:60; then *Sephadex LH-20*, MeOH): quercetin 3-(β-D-glucopyranoside) (12 mg).

(3β,12β)-Taraxast-20(30)-ene-3,12-diol (*= (3β,12β,18α,19α)-Urs-20(30)-ene-3,12-diol;* **1**): Colorless powder. M.p. 261–262°. $[\alpha]_D + 6.4$ ($c = 0.54$, MeOH). IR (KBr): 3406 (OH), 2939, 1639 (C=C), 1462, 1382, 1184, 1039, 998, 879, 634. ^1H- and ^{13}C-NMR: *Table*. EI-MS (70 eV): 442 (7, M^+), 424 (6, $[M-18]^+$), 229 (18), 219 (14), 216 (5.1), 208 (1), 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). HR-TOF-MS: 465.3706 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3709).

REFERENCES

[1] J. S. Chen, S. Zheng, 'Chinese Poisonous Plants', Science Press, Beijing, 1987, p. 218.
[2] H. P. Zhang, L. Q. Wang, G. W. Qin, *Bioorg. Med. Chem.* **2005**, *13*, 5289.
[3] B. A. Xu, H. Su, M. Z. Zhang, *Acta Sci. Nat. Univ. Pekin.* **1996**, *32*, 700.

[4] T. Wang, J. Yang, H. Li, *Acta Bot. Sin*. **1997**, *39*, 82.
[5] R. T. Li, J. Y. Li, J. K. Wang, Z. Y. Zhu, H. D. Sun, *Acta Bot. Yunn.* **2005**, *27*, 565.
[6] S. B. Mahato, A. P. Kundu, *Phytochemistry* **1994**, *37*, 1517.
[7] V. Anjaneyulu, K. Ravi, K. H. Prasad, J. D. Connolly, *Phytochemistry* **1989**, *28*, 1471.
[8] W. F. Reynolds, S. McLean, J. Poplawski, *Tetrahedron* **1986**, *42*, 3419.
[9] Y. H. Zhang, L. Zhou, R. B. Shi, Y. J. Guo, Y. Dong, *China J. Chin. Mater. Med*. **2006**, *31*, 1247.
[10] Y. H. Gong, 'Chemical Shifts of ^{13}C-NMR of Natural Organic Product', Yunnan Science Technology Press, Kunming, 1985, p. 130.
[11] T. N. Misra, R. S. Singh, T. N. Oiha, J. Upadhyay, *J. Nat. Prod.* **1981**, *44*, 735.
[12] S. B. Mahato, A. P. Kundu, *Phytochemistry* **1987**, *34*, 1389.
[13] I. K. Adnyana, A. Tezuka, H. Banskota, Q. Xiong, Q. T. Kim, S. Kadota, *J. Nat. Prod.* **2000**, *63*, 496.
[14] F. Cateni, J. Zilic, G. Falsone, F. Hollan, F. Frausin, V. Scarcia, *Farmaco* **2003**, *58*, 809.
[15] K. R. Markham, B. Ternai, R. Stanley, H. Geiger, T. J. Mabry, *Tetrahedron* **1978**, *34*, 1389.

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