A New Taraxastane-Type Triterpene from *Vitex trifolia* var. *simplicifolia*

by **Ming-Yu Huang**, **Lin-Jing Zhong**, **Jie-Ming Xie**, **Fei Wang**, and **Yong-Hong Zhang***

School of Pharmacy, Fujian Medical University, Fuzhou 350004, P. R. China
(phone/fax: +86-591-22862016; e-mail: zhangyh@mail.fjmu.edu.cn)

3-Oxotaraxer-14-en-30-al (**1**), a new taraxastane-type triterpene, together with 14 known compounds, taraxerone (**2**), 3-epiursolic acid (**3**), 2α,3β-dihydroxyurs-12-en-28-oic acid (**4**), lupeol (**5**), betulinic acid (**6**), casticin (**7**), artemetin (**8**), luteolin (**9**), 4-hydroxybenzoic acid (**10**), docosanoic acid (**11**), tetracosanoic acid (**12**), cerotic acid (**13**), β-sitosterol (**14**), and β-daucosterol (**15**), was isolated from the leaves and twigs of *Vitex trifolia* var. *simplicifolia* . Compounds **2**–**6** were found for the first time in this plant. Their structures were established by spectroscopic analysis, including 2D-NMR techniques. Cytotoxic activities of compounds **3**, and **5**–**10** were tested on the three cancer cell lines, PANC-1, K562, and BxPC-3. Results revealed that **7** exhibited cytotoxicity against PANC-1, K562, and BxPC-3, with IC_{50} values of 4.67, 0.72, and 4.01 μg/ml, respectively, whereas **8** was inactive against these cancer cell lines. The structure–activity relationship of compound **7** and **8** indicated that the 3′-OH group in polymethoxyflavonoids is essential for antitumor activity.

Introduction. – *Vitex trifolia* L. var. *simplicifolia* CHAM. (Verbenaceae) is distributed in tropical and subtropical areas along the sea in many countries, including southern China, and its fruits are used as a Traditional Chinese Medicine to treat headaches, colds, migraine, and eye pain [1]. *Vitex trifolia* var. *simplicifolia* mainly contains diterpenes, flavonoids, and phenolic compounds [2–5]. The present investigation on this plant led to the isolation of a novel taraxastane-type triterpene, 3-oxotaraxer-14-en-30-al (**1**), as well as of 14 known compounds (*Fig. 1*), taraxerone (**2**) [6], 3-epiursolic acid (**3**) [7], 2α,3β-dihydroxyurs-12-en-28-oic acid (**4**) [8][9], lupeol (**5**) [10], betulinic acid (**6**) [11], casticin (**7**) [12], artemetin (**8**) [12], luteolin (**9**) [13], 4-hydroxybenzoic acid (**10**) [14], docosanoic acid (**11**) [15], tetracosanoic acid (**12**) [16], cerotic acid (**13**) [16], β-sitosterol (**14**), and β-daucosterol (**15**). Compounds **2**–**6** were found for the first time in this plant. This article mainly deals with the isolation and structure elucidation of compound **1**, the cytotoxicities of compounds **3**, and **5**–**10**, as well as with the discussion of the structure–activity relationship of compounds **7**–**9**.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder. It gave rise to a positive *Liebermann–Burchard* coloration test, indicating a triterpenoid structure. Its molecular formula was determined as $C_{30}H_{46}O_2$ from its HR-ESI-MS (m/z 461.3384 ($[M+Na]^+$)), which was confirmed by ^{13}C-NMR and DEPT analysis, corresponding to eight degrees of unsaturation. The IR specturm of **1** exhibited CHO (1710, 2660 cm^{-1}), and C=O (1700 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR data of **1** (*Table 1*) indicated a pentacyclic triterpenoid, assignments being confirmed with the help of 2D-NMR (HMBC, HSQC, and NOESY) experiments (*Fig. 2*).

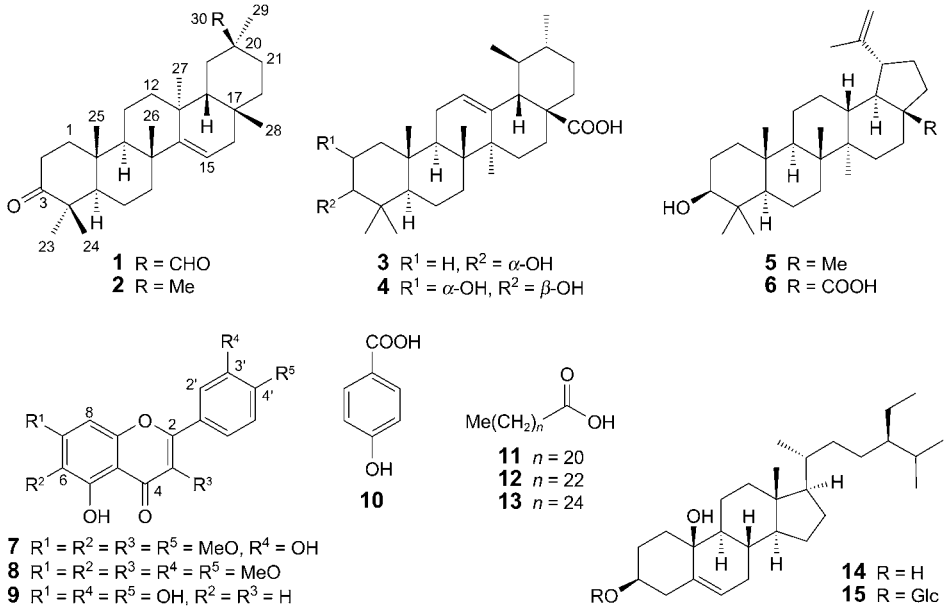

Fig. 1. *Compounds* **1**–**15**, *isolated from* Vitex trifolia *var.* simplicifolia

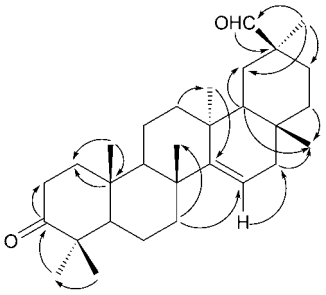

Fig. 2. *Key HMBCs of compound* **1**

The ^{1}H-NMR spectrum exhibited the characteristic signals of triterpenoids in the higher field, *i.e.*, seven *singlet*s for Me groups (δ(H) 0.82, 0.92, 1.04, 1.06, 1.08, 1.10, and 1.16). In addition, a signal of one H-atom on a C=C bond at δ(H) 5.56 (*dd*, $J = 8.0, 2.8$, H–C(15)), together with typical ^{13}C-NMR resonances at δ(C) 157.6 and 117.2, suggested the compound **1** was a taraxer-14-ene derivative [17]. Its ^{1}H-NMR spectrum showed an aldehyde *singlet* (δ(H) 9.50), while its mass spectrum showed a low-intensity M^+ peak at *m*/*z* 438, and a base peak at *m*/*z* 409 ($[M - CHO]^+$). Comparison of the ^{1}H- and ^{13}C-NMR spectra of compound **1** with **2** [6], suggests that compound **1** is different from **2**, with which it shares the same basic skeleton, in the presence of an aldehyde group at C(20). The ^{13}C-NMR spectrum of compound **1** showed a *singlet* at δ 217.6 ppm, assigned to a C(3). A signal at δ 206.9 was attributed to an aldehydic C-atom at C(30). The remaining 28 signals in the ^{13}C-NMR and DEPT spectra indicated the

Table 1. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp.) *Data of Compound* **1**. Recorded in $CDCl_3$; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	1.30–1.32 (*m*), 1.74–1.77 (*m*)	38.3 (*t*)	16	1.58–1.60 (*m*), 1.78–1.84 (*m*)	37.5 (*t*)
2	2.22–2.24 (*m*), 2.27–2.30 (*m*)	34.1 (*t*)	17		38.8 (*s*)
3		217.6 (*s*)	18	0.86–0.89 (*m*)	48.6 (*d*)
4		47.5 (*s*)	19	1.33–1.35 (*m*), 1.96–2.02 (*m*)	40.6 (*t*)
5	1.23–1.25 (*m*)	55.7 (*d*)	20		48.7 (*s*)
6	0.96–0.98 (*m*), 1.48–1.50 (*m*)	19.9 (*t*)	21	1.27–1.29 (*m*), 1.55–1.60 (*m*)	33.0 (*t*)
7	1.19–1.22 (*m*), 1.86–1.88 (*m*)	36.6 (*t*)	22	0.93–0.95 (*m*), 1.26–1.28 (*m*)	35.1 (*t*)
8		37.7 (*s*)	23	1.06 (*s*)	26.0 (*q*)
9	1.43–1.55 (*m*)	48.6 (*d*)	24	1.08 (*s*)	21.3 (*q*)
10		35.7 (*s*)	25	1.04 (*s*)	14.8 (*q*)
11	0.99–1.00 (*m*),1.40–1.42 (*m*)	17.4 (*t*)	26	1.10 (*s*)	29.8 (*q*)
12	1.52–1.54 (*m*)	33.5 (*t*)	27	0.92 (*s*)	25.5 (*q*)
13		37.6 (*s*)	28	0.82 (*s*)	30.1 (*q*)
14		157.6 (*s*)	29	1.16 (*s*)	21.4 (*q*)
15	5.56 (*dd*, *J* = 8.0, 2.8)	117.2 (*d*)	30	9.50 (*s*)	206.9 (*d*)

presence of eight quaternary, five tertiary (CH), ten secondary (CH_2), and eight primary (CH_3) C-atoms. The HMBCs *(Fig. 2)* revealed that compound **1** exhibited three-bond couplings between H–C(2) and C(3), and between H–C(23) and C(3), confirming the structure of ring *A*. The H-atoms of the angular Me(29) group coupled with the aldehydic C-atom, locating CHO at C(20). The linkage of the CHO group to C(20) was evident from the HMBC from the downfield-shifted aldehydic H-atom at signal δ 9.50 to the quaternary C-atom signal at δ 48.7 (C(20)). The mass spectrum exhibited the molecular-ion peak at *m*/*z* 438, with other significant peaks at 409 ($[M - CHO]^+$), 300, and 285, representing a fragment resulting from the cleavage of ring *D*, followed by loss of H, 218, 205, and 204. Based on these findings and comparisons with spectral data in the literature [6], the structure of compound **1** was elucidated as 3-oxotaraxer-14-en-30-al.

Compounds **3** and **5**–**10** were tested for their *in vitro* cytotoxicities against human pancreatic cancer cells PANC-1, human leukemia cells K562, and pancreatic carcinoma cells BxPC-3, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay [18], the results of which are presented in *Table 2*, revealing that compound **7** exhibited significant cytotoxicity against these tumor cells.

In addition, the cytotoxicity of **7** was more prominent than that of **9** against PANC-1, K562, and BxPC-3 cells. Compound **7** is a polymethoxyflavonoid, while compound **9** is a polyhydroxy substituted flavonol. Thus, polymethoxy substituents could enhance the antitumor activity. As shown in *Fig. 1*, compounds **7** and **8** differ only in the substitution at C(3′). In agreement with findings reported by others [4], we found that a free HO–C(3′) group is essential for significant cytotoxicity of polymethoxyflavonoids (see *Table 2*).

This work was sponsored by the *Natural Sciences Foundation of Fujian Province* (No. 2010J01179) and the *Science and Technology Project of Fujian Province* (No. 2012Y0035).

Table 2. *Cytotoxic Activities of Compounds* **3**, *and* **5**–**10**

Compound	IC_{50} [μg/ml]		
	PANC-1	K562	BxPC-3
3	15.05	6.99	14.80
5	24.85	20.85	32.91
6	22.31	19.83	36.09
7	4.67	0.71	4.01
8	> 80.00	61.25	> 80.00
9	11.67	16.65	12.59
10	> 80.00	> 80.00	> 80.00

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 or 200–300 mesh; *Qingdao Marine Chemical Factory*), *Sephadex LH-20* gel (*Amersham Pharmacia Biotech*), and *MCI* gel *CHP-20P* (75–150 μm; *Mitsubishi Chemical Co.*). TLC: silica gel *G* (*Qingdao Marine Chemical Factory*); visualization under UV light and by spraying with 5% H_2SO_4 in EtOH (*v*/*v*), followed by heating. Optical rotations: *JASCO-20* polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max} in nm. IR Spectra: *Nicolet 170SX* FT-IR spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR spectra: *Bruker NMR* spectrometer; at 400 or 600 (^1H), and 100 or 150 MHz (^{13}C), Me_4Si as internal standard; δ in ppm, *J* in Hz. HR-ESI-MS: *Bruker APEX II* mass spectrometer, EI-MS: *HP-5988* mass spectrometer, in *m*/*z*.

Plant Material. The plants of *Vitex trifolia* L.var. *simplicifolia* CHAM. were collected at the beach of Lang-Qi Island in Fuzhou, Fujian Province, P. R. China, in September 2010, and identified by *Y.-H. Z.*, Fujian Medical University. A voucher specimen was deposited with the Laboratory of the Natural Products, Fujian Medical University, P. R. China.

Extraction and Isolation. Dried and powdered leaves and twigs of *V. trifolia* var. *simplicifolia* (8 kg) were extracted three times with MeOH at r.t. After evaporation of the solvent under reduced pressure, the residue was suspended in H_2O and extracted with petroleum ether (PE), AcOEt, and BuOH, successively. The residue of the PE layer (75.5g) was fractionated by CC (silica gel; stepwise gradient PE/AcOEt 1:0–0:1) to yield 15 fractions, *Frs. 1–15*, and compound **15** (40.0 mg) was isolated from *Fr. 11*. *Fr. 6* (11 g) was purified by repeated CC (silica gel) to give **1** (6.0 mg) and **2** (5.0 mg). *Fr. 7* (13.5 g) was subjected to CC (silica gel; PE/AcOEt 1:0–0:1) to furnish **5** (15.0 mg), **11** (10.0 mg), **12** (16.0 mg), and **14** (15.0 mg).

The AcOEt layer (28.8g) was fractionated by CC (silica gel; stepwise gradient of PE/AcOEt (1:0–0:1) to yield 17 fractions, *Frs. 1–17*, and compound **3** (23.0 mg) was isolated from *Fr. 7*. *Fr. 12* (2.8g) was subjected to CC (silica gel; stepwise gradient of PE/AcOEt 1:0–0:1) to afford seven fractions, *Fr. 12.1–12.7*. *Fr. 12.5* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford **8** (8.0 mg). *Fr. 16* (1.7 g) was subjected to CC (silica gel) to give **9** (10.0 mg), *Fr. 13* was subjected to CC (*MCI*; stepwise gradient H_2O/MeOH 1:0–0:1) to yield **7** (130.0 mg). *Frs. 8* and *11* were purified by repeated CC (silica gel and on *Sephadex LH-20*) to give **6** (12.0 mg) and **10** (100.0 mg), resp.

3-Oxotaraxer-14-en-30-al (**1**). White amorphous powder. M.p. 216–218°. $[\alpha]_D^{23} = +26.0$ ($c = 0.1$, $CHCl_3$). IR: 2926, 2825, 2660, 1710, 1700, 1660, 1454, 1363. ^1H- and ^{13}C-NMR: see *Table 1*. EI-MS: 438 (M^+), 409 ($[M - CHO]^+$), 300, 285, 218, 205, 204. HR-ESI-MS: 461.3384 ($[M + Na]^+$, $C_{30}H_{46}NaO_2^+$; calc. 461.3395).

Taraxerone (**2**). White amorphous powder. M.p. 245–248°. IR: 2930, 2839, 1702, 1665, 1454, 1362. ^1H-NMR (600 MHz, $CDCl_3$): 5.56 (*dd*, *J* = 8.0, 2.8, H–C(15)); 1.14 (*s*, Me(26)); 1.11 (*s*, Me(25)); 1.08 (*s*, Me(24)); 1.07 (*s*, Me(23)); 0.96 (*s*, Me(29)); 0.91 (*s*, Me(30)), 0.83 (*s*, Me(28)). EI-MS: 424 (M^+), 409 ($[M - Me]^+$), 300, 285, 218, 205,121.

3-Epiursolic Acid (**3**). White amorphous powder. M.p. 273–275°. IR: 3392, 2926, 1693, 1457, 1369, 1018. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.87, 0.89, 0.91, 0.98, 1.04, 1.28, 1.31 (7*s*, 7 Me); 3.00 (*t*, J = 8.0, H–C(3)); 5.13 (*d*, J = 4.0, H–C(12)). ESI-MS: 456 (M^+).

2α,3β-Dihydroxyurs-12-en-28-oic Acid (**4**). White amorphous powder. M.p. 245–248°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.76, 0.80, 0.82, 0.84, 0.92, 0.98, 1.10 (7*s*, 7 Me). ESI-MS: 471 ($[M-H]^-$).

Lupeol (**5**). Colorless amorphous powder. M.p. 205–206°. ^{1}H-NMR (400 MHz, CD_3OD): 0.76, 0.83, 0.86, 0.94, 0.98, 1.26, 1.70 (7*s*, 7 Me); 4.62 (*s*, H_a–C(29)); 4.75 (*s*, H_b–C(29)); 3.21 (*dd*, J = 8.0, 4.0, H–C(3)); 2.20 (*s*, H–C(19)). EI-MS: 426 (M^+).

Betulinic Acid (**6**). Colorless amorphous powder. M.p. 228–230°. IR: 3441, 2942, 2871, 1693, 1692, 1452. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.82, 0.85, 0.94, 0.96, 1.09, 1.63 (6*s*, 6 Me); 4.67 (*s*, H_a–C(29)); 4.55 (*s*, H_b–C(29)). ESI-MS: 455 ($[M-H]^-$).

Casticin (**7**).Yellow crystals. M.p. 188–190°. IR: 3463, 3444, 2931, 2849, 1655, 1676, 1587, 1430, 1282, 1210. UV (MeOH): 351, 258. ^{1}H-NMR (400 MHz, (D_6)DMSO): 3.72, 3.79, 3.86, 3.91 (4*s*, 4 MeO); 6.87 (*d*, J = 2.0, H–C(8)); 7.12 (*d*, J = 9.2, H–C(5′)); 7.58 (*d*, J = 2.0, H–C(2′)); 7.59 (*s*, H–C(6′)). ESI-MS: 374 (M^+).

Artemetin (**8**). Yellow crystals. M.p. 161–162°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 3.73–3.92 (*s*, 5 MeO); 6.93 (*s*, H–C(8)); 7.16 (*d*, J = 8.8, H–C(5′)); 7.65 (*d*, J = 2.0, H–C(2′)); 7.72 (*dd*, J = 8.8, 2.0, H–C(6′)). ESI-MS: 388 (M^+).

Luteolin (**9**). Yellow needles. M.p. >300°. IR (KBr): 3392, 1665, 1611, 1507, 1445, 1340, 1305, 1262, 1161, 804. UV (MeOH): 353, 263. ^{1}H-NMR (400 MHz, (D_6)DMSO): 12.79 (*s*, HO–C(5)); 7.42 (*dd*, J = 8.0, 2.0, H–C(6′)); 7.39 (*d* , J = 2.0, H–C(2′)); 6.89 (*d*, J = 8.0, H–C(5′)); 6.67 (*s*, H–C(3)); 6.44 (*d*, J = 2.0, H–C(8)); 6.18 (*d*, J = 2.0, H–C(6)). ESI-MS: 286 (M^+).

4-Hydroxybenzoic Acid (**10**). White needles. M.p. 188–191°. IR: 3388, 1676, 1510, 1422, 1320, 1168. ^{1}H-NMR (400 MHz, CD_3OD): 7.89 (*d*, J = 8.8, H–C(2,6)); 6.82 (*d*, J = 8.4, H–C(3,5)). EI-MS: 138 (M^+), 121 ($[M-OH]^+$), 93 ($[M-COOH]^+$).

Docosanoic Acid (**11**). White amorphous powder. M.p. 78°. EI-MS: 340 (M^+), 312, 284, 213, 199, 185, 171, 157, 143.

Tetracosanoic Acid (**12**). White amorphous powder. M.p. 82°. EI-MS: 367 ($[M-H]^-$), 353 ($[M-COOH]^+$). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*t*, J = 5.7, Me(24)); 1.25 (*m*, H–C(4–23)); 1.63 (*m*, CH_2(3)); 2.34 (*d*, J = 5.9, CH_2(2)).

Cerotic Acid (**13**). White amorphous powder. M.p. 84°. EI-MS: 396 (M^+), 382, 368, 354, 340, 289, 275, 253, 173, 159.

REFERENCES

[1] Institute of Materia Medica, Chinese Academy of Medical Sciences, 'Chinese Materia Medica', People's Medical Publishing House, Beijing, 1984, Vol. 3, p. 679.
[2] M. Ono, M. Yamamoto, T. Yanaka, Y. Ito, T. Nohara, *Chem. Pharm. Bull.* **2001**, *49*, 82.
[3] M. Ono, T. Yanaka, M. Yamamoto, Y. Ito, T. Nohara, *J. Nat. Prod*. **2002**, *65*, 537.
[4] W. G. Ko, T. H. Kang, S. J. Lee, N. Y. Kim, Y. C. Kim, D. H. Sohn, B. H. Lee, *Food Chem Toxicol*. **2000**, *38*, 861.
[5] T. Yoshioka, T. Inokuchi, S. Inokuchi, Y. Kimura, *Naturforsch., C* **2004**, *59*, 509.
[6] N. B. Sakurai, Y. S. Yaguchi, T. K. Inoue, *Phytochemistry* **1987**, *26*, 217.
[7] F. B. Luciane, T. G. Felipe, B. F. Sidnei, S. M. Fábio, L. M. Davyson, *Quim. Nova* **2003**, *26*, 922.
[8] Z. Z. Liang, R. Aquino, V. D. Feo, F. D. Simone, C. Pizza, *Planta Med*. **1990**, *56*, 330.
[9] R. Aquino, F. D. Simone, F. F. Vincieri, C. Pizza, E. Gacs-Baitz, *J. Nat. Prod.* **1990**, *53*, 559.
[10] W. F. Reynolds, S. McLean, J. Poplawski, R. G. Enriquez, L. I. Escobar, I. Leon, *Tetrahedron* **1986**, *42*, 3419.
[11] I. Akira, I. Hideji, *Phytochemistry* **1988**, *27*, 2813.
[12] Azizuddin, M. Talat, I. C. Muhammad, *Turk. J. Chem*. **2010**, 119.
[13] Y. H. Kuo, S. M. Lee, J. S. Lai, *J. Chin. Chem. Soc.* **2000**, *47*, 241.
[14] T. S. Wu, Y. L. Tsai, P. L. Wu, F. W. Lin, J. K. Lin, *J. Nat. Prod.* **2000**, *63*, 692.

[15] A. M. Ivan, A. Hans, *Phytochemistry* **1985**, *24*, 1817.
[16] W. W. Fu, D. Q. Dou, S. Noriko, T. Tadahiro, Y. H. Pei, Y. J. Chen, *J. Nat. Med*. **2006**, *60*, 68.
[17] I. J. Momo, T. H. Dufat, J. Wandji, S. Michel, D. D. Chiozem, *Nat. Prod. Res.* **2013**, *27*, 137.
[18] M. J. Tang, D. D. Shen, Y. C. Hu, S. Gao, S. S. Yu, *J. Nat. Prod*. **2004**, *67*, 1969.

Received November 19, 2012