Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2274–2277

Novel skeleton terpenes from *Celastrus hypoleucus* with anti-tumor activities

Kui-wu Wang,[a] Jian-shan Mao,[b] Yuan-po Tai[a] and Yuan-jiang Pan[a,*]

[a]*Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China*
[b]*Department of Gastroenterology, The Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310009, PR China*

Received 6 December 2005; revised 23 December 2005; accepted 6 January 2006
Available online 7 February 2006

Abstract—Celahypodiol **1**, an unusual 17-membered carbon diterpenoid with a novel skeleton, and a new triterpenoid 12-oleanene-3β,6α-diol **2**, together with four known compounds furreginol **3**, suigol **4**, 20(30)-lupene-3β, 29-diol **5**, and 20(29)-lupene-1β,3β-diol **6**, were isolated from the stalks of *Celastrus hypoleucus* (Oliv.) Warb. Their structures were established by means of spectroscopic analysis, including 2D NMR. The new compounds exhibited anti-tumor activities against a panel of human tumor cell lines.

Some plants belonging to Celastraceae family have long been used as traditional herb medicine to treat fever, chill, joint pain, edema, rheumatoid arthritis, and bacterial infection in Chinese folk medicine.[1] A lot of sesquiterpenes and triterpenes with anti-tumor and anti-HIV activities have been isolated from this family.[2] Our investigation of bioactive constituents of *Celastrus hypoleucus* (Oliv.) Warb., a perennial plant belonging to the family celastraceae, led to the isolation[3] of a new 17-membered carbon diterpenoid with a novel skeleton: celahypodiol **1**, and a new triterpene: 12-oleanene-3β,6α-diol **2** together with four known compounds furreginol **3**, suigol **4**, 20(30)-lupene-3β, 29-diol **5**, and 20(29)-lupene-1β,3β-diol **6**. The new compounds were tested for in vitro anti-tumor activity against four human-tumor cell lines and showed anti-tumor activities.

Celahypodiol **1** was obtained as a white powder (mp 168–170 °C) and was positive to $FeCl_3$ reagent. The molecular formula was deduced as $C_{17}H_{20}O_3$ by the FT-ICR-MS [*m*/*z*: 273.1485 $[M+H]^+$, calcd for $C_{17}H_{21}O_3^+$, 273.1485]. Its IR spectrum showed characteristic absorption bands for the hydroxyl group, the carbonyl group, phenyl group, and double bonds (3408, 1654, 1582, 1513, 1209, 1078, 892 cm^{-1}). These assignments were confirmed by its ^{13}C and ^1H NMR spectral data (δ_C 196.5 (s) ppm, 151.6 (s), 149.0 (s), 144.2 (s), 125.2 (s), 114.4 (d), 111.1 (d) ppm, 126.6 (s), 125.9 (s) ppm, and δ_H 6.93, 7.44 ppm) (Table 1). Since six out of eight degrees of unsaturation were accounted for, celahypodiol **1** was inferred to contain two more rings.

The ^1H NMR spectrum showed two *para* aromatic proton signals at δ_H 6.93 (1H, s, H-11) and δ 7.44 (1H, s, H-14), three tertiary-linked methyl signals at δ_H 1.07, 1.65, and 1.67 (each 3H, s, Me-17, 15, 16). The ^{13}C NMR spectrum displayed 17 carbon signals, which were assigned by DEPT experiments as three methyls, three methylenes, three methines, and eight quaternary carbon signals.

From the ^1H, ^1H-COSY, and HMQC spectra, the carbon signals at δ_C 33.5 and 29.8 could be assigned to C-1 and C-2, respectively. In the HMBC spectrum of **1** (Table 1), the signals at δ_H 2.11 (H-2β)[4] and 2.26 (H-2α) showed correlation with the signals at δ_C 33.5 (t, C-1), 126.6 (s, C-3), 125.9 (s, C-4), 36.4 (s, C-10), and 19.5 (q, C-16); the signals at δ_H 2.70 (1H, d, $J = 14.0$, H-5) correlated with the signals at δ_C 33.5 (t, C-1), 126.6 (s, C-3), 125.9 (s, C-4), 196.5 (s, C-7), and 21.8 (q, C-17); the signals at δ_H 1.65 (3H, s, Me-15) exhibited cross peaks with both signals at δ_C 126.6 (s, C-3) and 45.7 (d, C-5); the signals at δ_H 1.67 (3H, s, Me-16) showed correlation with δ_C 29.8 (t, C-2) and 125.9 (s,

Keywords: Novel skeleton; Diterpenoid; Triterpenoid; Isolation; Structure identification; Anti-tumor activities.
* Corresponding author. Tel./fax: +86 571 87951264; e-mail: cheyjpan@zju.edu.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.021

Table 1. NMR data of compound **1** (in acetone)[a]

Compound	^1H[b]	^{13}C[c]	^1H, ^1H-COSY	HMBC	NOESY
1α	1.59 m	33.5 (t)[d]	H-2	C-2, 3, 5, 10, 17	H-5, 11
1β	2.28 m				H-11, 17
2α	2.26 m	29.8 (t)	H-1	C-1, 3, 4, 10, 16	
2β	2.11 m				
3		126.6 (s)			
4		125.9 (s)			
5α	2.70 d (14.0)	45.7 (d)	H-6	C-1, 3, 4, 7, 17	H-1α
6α	2.79 d (17.7)	38.7 (t)	H-5	C-4, 5, 7, 10	H-15
6β	2.41 dd (14.0, 17.7)				H-15, 17
7		196.5 (s)			
8		125.2 (s)			
9		149.0 (s)			
10		36.4 (s)			
11	6.93 s	111.1 (d)		C-8, 10, 12, 13	H-1α, 1β, 17
12		144.2 (s)			
13		151.6 (s)			
14	7.44 s	114.4 (d)		C-7, 9, 12, 13	
15	1.65 s	15.4 (q)		C-3, 5	H-6α, 6β
16	1.67 s	19.5 (q)		C-2, 4	
17	1.07 s	21.8 (q)		C-1, 5, 9, 10	H-1β, 6β, 11

[a] TMS was used as internal standard, δ in ppm, J in Hz.
[b] 500 MHz.
[c] 125 MHz.
[d] Multiplicities DEPT experiments in parentheses: s: quaternary; d: CH; t: CH_2, and q: Me C-atoms.

C-4); while the signals at δ_H 1.07 (3H, s, Me-17) showed correlation with δ_C 33.5 (t, C-1), 45.7 (d, C-5), 149.0 (s, C-9), and 36.4 (s, C-10). These indicated the presence of structure unit ring A with a double bond at C-3 and C-4, a methyl group at C-10, and two methyl groups linked to C-3 and C-4, respectively. Ring B was formed by connecting the structural unit –CH_2–CO–C=C– through C-5 and C-10, which was judged from the HMBC correlation from the two proton signals at δ_H 2.41 (1H, dd, J = 14.0, 17.7, H-6β) and 2.79 (1H, d, J = 17.1, H-6α) to the carbon signals at δ_C 125.9 (s, C-4), 45.7 (d, C-5), 196.5 (s, C-7), and 36.4 (s, C-10). HMBC correlations

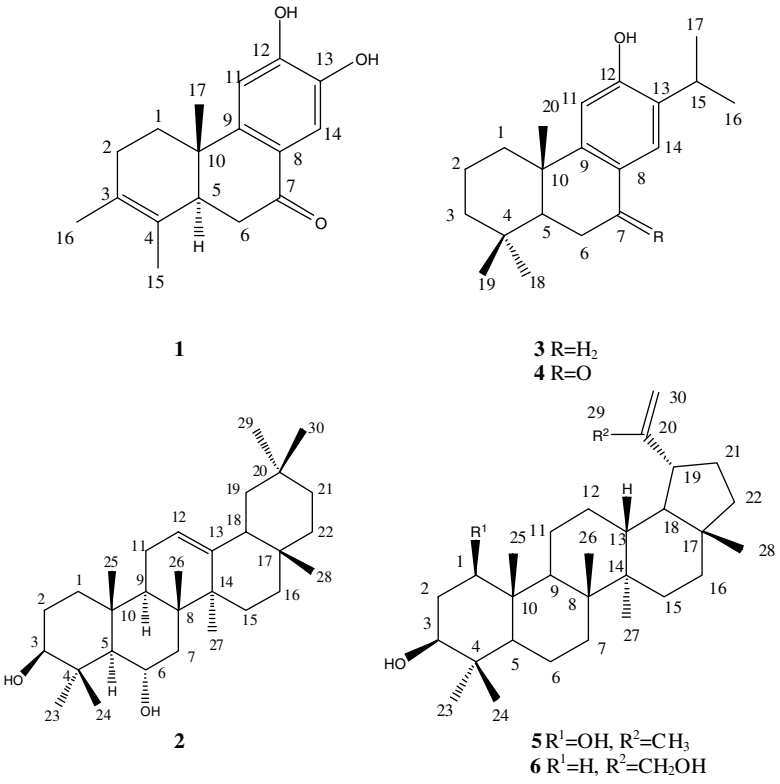

Figure 1. The structure of compounds **1**–**6**.

from the proton signal at δ_H 6.93 (s, H-11) to the carbon signals at δ_C 125.2 (s, C-8), 36.4 (s, C-10), 144.2 (s, C-12), and 151.6 (s, H-13); from the proton signal at δ_H 7.44 (s, H-14) to the carbon signals at δ_C 144.2 (s, C-7), 149.0 (s, C-9), 144.2 (s, C-12), and 151.6 (s, C-13) suggested that ring C was constructed by connecting the structural unit –CH=CH–CH=CH– through C-8 and C-9. The above conclusions were further confirmed by ^{1}H, ^{1}H-COSY spectral data of **1** (Table 1).

Thus, the structure of celahypodiol **1** was elucidated to be 12,13-dihydroxy-3,8,11,13-celastratrtraen-7-one. This evidence indicated that compound **1** was a diterpenoid with a novel skeleton containing a 3,4-dimethyl structural unit but no isopropyl group substituent at C-13, which is different from 19 [4 → 3] *abeo*-*O*-demethyl cytojaponol[5] (*neo*-clerodane skeleton) and (+)-podocarpic acid[6] (podocarpane skeleton).

The relative stereochemistry of **1** was determined through 2D NOESY analysis. The observation of a NOESY correlation from H-1α (δ_H 1.59) to H-5 suggested that H-1α and H-5 are on the same face of the molecule and H-5 is an α-configuration. Similarly, a NOSEY correlation from H-1β (δ_H 2.28) and H-6β (δ_H 2.41) to Me-17 placed Me-17 on the opposite face of the molecule from H-5, suggesting an axial orientation for Me-17 (β-configuration) and hence a *trans* ring junction. So, the relative stereochemistry of **1** is proposed as shown in Figure 1.

Compound **2** was obtained as colorless needle crystals (mp 239–241 °C) and showed positive Liebermann–

Table 3. Cytotoxic activities of compounds **1**, **2** and Mitomycin (IC_{50} values in μg/ml)

	Compound **1**	Compound **2**	Mitomycin
Bcap 37	24.42	14.56	2.33
RKO	27.16	12.20	1.75
SMMC 7721	38.03	22.69	2.44
K562	16.21	11.21	3.35

Table 2. NMR Data of compound **2** (in $CDCl_3$)[a]

Compound	^{1}H[b]	^{13}C[c]	^{1}H, ^{1}H-COSY	HMBC	NOESY
1	1.02 m	38.6 (t)[d]	H-2	C-3, 5, 10, 25	
	1.64 m				
2	0.96 m	27.1 (t)	H-1, 3	C-1, 3	
	1.00 m				
3	3.20 dd (11.2, 4.4)	79.0 (d)	H-2	C-1, 23, 24	H-5
4		39.3 (s)			
5	0.92 m	60.5 (d)	H-6	C-4, 6, 10, 25	H-3, 23
6	4.08 t, d (10.8, 4.0)	69.2 (d)	H-5, 7	C-5, 7, 8, 10	H-24, 25, 26
7	1.57 m	45.2 (t)	H-6	C-5, 6, 8, 9,14, 26	
	1.64 m				
8		42.1 (s)			
9	1.97 m	47.4 (d)		C-10, 11, 12, 13, 25, 26	
10		39.3 (s)			
11	1.85 m	23.9 (t)	H-12	C-9, 10, 12, 13, 25, 26	
12	5.19 t (4.8)	121.9 (d)	H-11	C-9, 11, 14, 18	
13		145.1 (s)			
14		41.5 (s)			
15	1.80	26.4 (t)		C-13, 27	
16	1.66 m	27.0 (t)		C-15, 17, 28	
	2.00 m				
17		32.7 (s)			
18	1.62 m	47.3 (d)		C-12, 13, 19	
19	1.02 m	47.0 (t)		C-13, 17, 18, 20, 21, 30	
20		31.3 (s)			
21	1.11 m	34.9 (t)		C-17, 19, 20, 22	
	1.36 m				
22	1.27 m	37.3 (t)		C-16, 17, 20, 28	
	1.43 m				
23	1.34 s	31.2 (q)		C-3, 4, 5, 24	H-5
24	1.02 s	15.9 (q)		C-3, 4, 5, 23	H-6, 25
25	1.00 s	16.6 (q)		C-1, 5, 9, 10	H-6, 24, 26
26	1.06 s	18.4 (q)		C-7, 8, 9, 14	H-6, 25
27	1.17 s	26.2 (q)		C-8, 13, 14, 15	
28	0.81 s	28.6 (q)		C-16, 17, 18, 22	H-18
29	0.87 s	33.6 (q)		C-19, 20, 21, 30	
30	0.81 s	23.9 (q)		C-19, 20, 21, 29	

[a] TMS was used as internal standard, δ in ppm, J in Hz.
[b] 500 MHz.
[c] 125 MHz
[d] Multiplicities DEPT experiments in parentheses: s: quaternary; d: CH; t: CH_2, and q: Me C-atoms.

Buchard reaction. The molecular formula was assigned as $C_{30}H_{50}O_2$ based on FT-ICR-MS (*m/z*: 441.3725 $[M-H]^-$, calcd for $C_{30}H_{49}O_2^-$, 441.3727) and the NMR data (Table 2). Its IR spectrum indicated the presence of hydroxyl group (3424 cm^{-1}) and olefinic (1637 cm^{-1}) group.

The ^1H NMR spectrum showed eight tertiary methyl signals at δ_H 0.81 (6H, s, Me-28, 30), 0.87 (3H, s, Me-29), 1.00 (3H, s, Me-25), 1.02 (3H, s, Me-24), 1.06 (3H, s, Me-26), 1.17 (3H, s, Me-27), and 1.34 (3H, s, Me-23), two protons geminal to secondary alcoholic group at δ_H 3.20 (1H, dd, J = 11.2, 4.4, H-3) and 4.08 (1H, dd, J = 10.8, 4.0, H-6). The latter observation, combined with the shape and position of the olefinic proton signal: triplet δ_H 5.19 (1H, t, J = 4.8, H-12), correlated to the C-atom at δ_C 121.9 (C-12) and the quaternary olefinic C-atom C-13 appearing at 145.1 suggested that the compound was most probably a diol of olean-12-ene type triterpene.[7] The ^{13}C NMR and DEPT spectra of compound **2** allowed the assignment of 30 carbon signals to eight methyls, nine methylenes, six methines groups, and seven quaternary C-atoms.

The coupling constants of H-3 (δ_H 3.20, 1H, dd, J = 11.2, 4.4) in the ^1H spectrum and the ^1H–^{13}C long range correlation of H-3 with C-1, C-23, and C-24 suggested a β configuration for the hydroxyl group at C-3.[7] Then the only point remaining to be established is the position of the second secondary hydroxyl group. The most significant differences between the ^{13}C NMR data of compound **2** and β-amyrin[7] are the resonances of C-5, C-6, C-7, and C-8 atoms. In the ^1H, ^1H-COSY spectrum, H-6 displaying cross peaks with H-5 and H-7 revealed that the second OH is connecting to C-6. The coupling constants and the up-shift of the proton signal (δ_H 4.08, t, d, J = 10.8, 4.0, H-6) compared with those of the 6β-OH type triterpenoid daturadiol[8] (δ_H 4.54, br, s, H-6) and the 6β-OH type triterpenoid 6α-hydroxy-12-oleanene-3-one[9] (δ_H 3.94, t, d, J = 10.4, 4.5) suggested a 6α-OH configuration. This deduction was confirmed by the HMBC and NOESY spectra. The HMBC spectrum showed the correlations from H-6 (δ_H 4.54) to δ_C 60.5 (d, C-5), 45.2 (t, C-7), 42.1 (s, C-8), and 39.3 (s, C-10), respectively, and the cross-peak of H-6 with Me-24, Me-25, and Me-26 was observed in NOESY spectrum. Further NOESY correlation from H-5 to H-3 and Me-23, and from Me-25 to Me-24 and Me-26, from H-18 to Me-28 determined the relative stereochemistry as shown. Thus, compound **2** was identified as 12-oleanene-3β,6α-diol.

Furthermore, four known compounds, furreginol **3**, suigol **4**, 20(30)-lupene-3β, 29-diol **5**, and 20(29)-lupene-1β,3β-diol **6**, were identified by comparison of their spectroscopic data with those of literature.[10]

The new compounds **1** and **2** were tested for in vitro anti-tumor activity against four human tumor cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method,[11] and they showed moderate anti-tumor activity against human mammary carcinoma (Bcap 37), human colon carcinoma (RKO), human hepatocellular carcinoma (SMMC 7721), and human erythroleukemia (K 562) with the IC_{50} values from 11.21 to 38.03 μg/ml, respectively (Table 3). Under the same test condition, the positive control (Mitomycin) exhibited anti-tumor activity at 1.75–3.35 μg/ml, respectively.

Acknowledgment

This work was supported by NSFC of China (20375036).

References and notes

1. Chen, P. D.; Liang, J. Y. *Strait Pharmaceutical Journal* **1999**, *11*, 3.
2. (a) Wang, H.; Tian, X.; Pan, Y. J. *Bull. Korean Chem. Soc.* **2003**, *24*, 541; (b) Brüning, R.; Wagner, H. *Phytochemistry* **1978**, *17*, 1821; (c) Chen, B.; Duan, H. Q.; Takaishi, Y. *Phytochemistry* **1999**, *51*, 683; (d) Chen, K.; Shi, Q.; Kashiwada, Y. *J. Nat. Prod.* **1992**, *55*, 340.
3. The shade-dried stalks (10 kg) were extracted with methanol, and 514 g of extract was obtained, which was partitioned with petroleum ether, EtOAc, and *n*-BuOH successively. The petroleum ether extract (103 g) was subjected to column chromatography (CC) over silica gel (200–300 mesh, 2 kg) eluting with petroleum ether/EtOAc (10:0-0:10, gradients) to afford 5 fractions. Fraction 1 was separated on silica gel CC (300–400 mesh, 100 g) repeatedly, using *n*-hexane/acetone (10:1) as eluent to yield pure **1** (20.1 mg), **3** (10.2 mg), and **4** (23.3 mg). Fraction 3 was rechromatographed on a silica gel (300–400 mesh, 60 g) column with *n*-hexane/acetone (5:1) to give pure **2** (15.3 mg) and **6** (50.4 mg). Fraction 4 on CC over silica gel (300–400 mesh, 100 g) using petroleum ether/acetone (4:1) afforded compound **5** (10.2 mg).
4. H-1α, 2β, 6β are all axial hydrogen atoms, but H-1β, 2α, 6α are all equatorial hydrogen atoms so the chemical shifts assigned as in Table 1. Ning, Y.C., *Structural Identification of Organic Compounds and Organic Spectroscopy (Chinese edition)*. Science Press, 2000, p 31.
5. Galicia, M. A.; Esquivee, B.; Sanchez, A.; Cardenas, T.; Ramamoorthy, T. P.; Rodriguez-Hahn, L. *Phytochemistry* **1988**, *27*, 217.
6. Fujiwara, Y.; Yamato, T.; Banto, T.; Shishido, K. *Tetrahedron: Asymmetry* **1997**, *8*, 2793.
7. Shashi, B. M.; Asish, P. K. *Phytochemistry* **1994**, *37*, 1517.
8. Kocor, M.; Pyrek, J. S. *J. Org. Chem.* **1973**, *38*, 3685.
9. Wang, K. W.; Sun, H. X.; Wu, B.; Pan, Y. J. *Helv. Chim. Acta* **2005**, *88*, 990.
10. (a) Nakanishi, T.; Hitoshi, M.; Masao, N.; Hideko, H.; Kaisuke, Y. *Phytochemistry* **1983**, *22*, 721; (b) Gao, J.; Han, G. *Phytochemistry* **1997**, *44*, 759; (c) Abdel-Mogib, M. *Phytochemistry* **1999**, *51*, 455; (d) Giuseppe, S.; Maurizio, B.; Benjamín, R.; José, L. M. *Phytochemistry* **1987**, *26*, 3305.
11. Tweentyman, P. R.; Luscombe, M. *Br. J. Cancer* **1987**, *56*, 279.