National Laboratory of Applied Organic Chemistry[1], College of Chemistry and Chemical Engineering and Department of Biology[2] , Lanzhou University, Lanzhou, China

Cytotoxic triterpenes from *Ligulariopsis shichuana*

WEN-SHU WANG[1], KUN GAO[1], CHUN-MING WANG[2], ZHONG-JIAN JIA[1]

Received July 26, 2002, accepted September 3, 2002

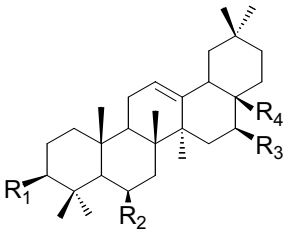
Prof. Zhongjian Jia, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, Gansu 730000, P. R. China
Jiazj@lzu.edu.cn

Pharmazie 58: 148–150 (2003)

Five olean-12-ene triterpenes (**1**–**5**) were isolated from the whole plant of *Ligulariopsis shichuana*. Their structures were elucidated by spectroscopic methods, including IR, EIMS positive HRSIMS, 1DNMR, and 2DNMR. Among them, 16β,28-dihydroxyolean-12-en-3-one (**3**), olean-12-en-3β,6β,16β,28-tetraol (**4**), 6β,16β-dihydroxyolean-12-en-3-one (**5**) are new compounds. In addition, compounds **4** and **5** showed cytotoxic activities on human hepatoma cells (SMMC-7721), human ovarian neoplasm cells (HO-8910) and human hepatocytes cells (LO2).

1. Introduction

Ligulariopsis shichuana is the only species in genus *Ligulariopsis* (Compositae) [1]. Because of its similarity with some *Ligularia* and *Cacalia* species, it has long been incorrectly assigned to the genus *Cacalia* [2]. As part of our research program in investigating plants of Compositae in China [3–5], we collected this special plant in Qinling Mountain and report here the isolation of three new olean-12-ene triterpenes, 16β,28-dihydroxyolean-12-en-3-one (**3**), olean-12-en-3β,6β,16β,28-tetraol (**4**), 6β,16β-dihydroxyolean-12-en-3-one (**5**), as well as two known triterpenes, gummosogenin **(1)** [6], and longispiongenin **(2)** [7, 8]. Furthermore, in order to find new biologically active compounds in Compositae, we chose olean-12-en-3β,6β,16β,28-tetraol **(4)** and 6β,16β-dihydroxyolean-12-en-3-one **(5)** for cytotoxic test.



	R_1	R_2	R_3	R_4
1	OH	H	OH	CHO
2	OH	H	OH	CH_2OH
3	O=	H	OH	CH_2OH
4	OH	OH	OH	CH_2OH
5	O=	OH	OH	CH_3

2. Investigations, results and discussion

Dried and crushed whole plant material was extracted with acetone to give a residue (38 g). After repeated column chromatography with different eluates, new compounds 16β,28-dihydroxyolean-12-en-3-one (**3**), olean-12-en-3β,6β,16β,28-tetraol (**4**), 6β,16β-dihydroxyolean-12-en-3-one (**5**) and two known compounds were indentified.

Compounds **1** and **2** were identified by comparison of their spectroscopic data (EIMS, ^{1}H NMR, ^{13}C NMR) with those of known compounds [6–8].

Compound **3** was obtained as white gum, its molecular formula was deduced as $C_{30}H_{48}O_3$ by the $[M + H]^+$ peak at m/z 457.3724 ($C_{30}H_{49}O_3$, required 457.3676) in positive HRSIMS spectrum. The degree of unsaturation was 7. The IR spectrum contained bands at 3378 cm^{-1} (broad), 1715 cm^{-1} and 1650 cm^{-1} for hydroxy groups, carbonyl group and double bond respectively. In combination with thirty carbon signals in its ^{13}C NMR spectrum (Table 1), and seven methyl singlets in the highfield in the

Table 1: ^{13}C NMR spectra data of compounds 3, 4 and 5

C	**3**	DEPT	**4**	DEPT	**5**	DEPT
1	39.3	CH_2	41.4	CH_2	39.2	CH_2
2	33.4	CH_2	28.2	CH_2	34.4	CH_2
3	216.0	C	79.8	CH	216.4	C
4	47.8	C	41.5	C	46.6	C
5	55.3	CH	56.4	CH	56.6	CH
6	19.6	CH_2	68.6	CH	66.0	CH
7	34.1	CH_2	41.2	CH_2	39.0	CH_2
8	41.0	C	41.1	C	40.7	C
9	47.8	CH	47.9	CH	48.8	CH
10	37.0	C	37.2	C	37.4	C
11	23.6	CH_2	24.2	CH_2	21.4	CH_2
12	122.4	CH	123.7	CH	122.4	CH
13	142.8	C	142.7	C	142.9	C
14	44.8	C	45.5	C	44.5	C
15	36.0	CH_2	36.8	CH_2	36.3	CH_2
16	67.9	CH	69.3	CH	69.2	CH
17	40.9	C	40.0	C	36.3	C
18	44.8	CH	45.5	CH	44.4	CH
19	46.5	CH_2	47.4	CH_2	46.5	CH_2
20	31.8	C	31.6	C	30.6	C
21	32.1	CH_2	34.3	CH_2	30.9	CH_2
22	25.9	CH_2	26.8	CH_2	25.9	CH_2
23	26.7	CH_3	28.7	CH_3	29.7	CH_3
24	15.3	CH_3	17.8	CH_3	16.5	CH_3
25	16.6	CH_3	17.8	CH_3	16.5	CH_3
26	16.5	CH_3	19.0	CH_3	18.7	CH_3
27	26.4	CH_3	27.7	CH_3	27.1	CH_3
28	71.2	CH_2	72.0	CH_2	25.9	CH_3
29	33.1	CH_3	33.9	CH_3	33.2	CH_3
30	23.8	CH_3	24.7	CH_3	23.6	CH_3

^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ, ppm.

^{1}H NMR, δ 0.92 (s, CH_3), δ 1.06 (s, CH_3), δ 1.07 (s, CH_3), δ 1.09 (s, CH_3), δ 1.11 (s, CH_3), δ 1.24 (s, CH_3), δ 1.26 (s, CH_3), this compound should be a pentacyclic triterpene. Furthermore, in the ^{13}C NMR spectrum, the characteristic double bond carbons were δ 122.4 (CH), δ 142.8 (C), indicating this triterpene was an olean-12-ene [9]. Similarities in ^{1}H NMR of compound **3** and **2** showed a hydroxymethyl at C-28, δ 3.21 (d, J = 12.8 Hz, H-28a), δ 4.16 (d, J = 12.8 Hz, H-28b), and a β-hydroxy at C-16, δ 4.32 (dd, J = 10.2, 6.8 Hz, H-16α). In the HMBC spectrum, the carbonyl signal δ 216.0 correlated with two methyls (CH_3-23, CH_3-24) and a CH_2-2 group at δ 2.54 (ddd, J = 12.0, 2.8, 1.2 Hz, H-2α), δ 2.39 (dt, J = 12.0, 4.6 Hz, H-2β), which led to the assignment of C-3 carbonyl. Those characteristic RDA fragments [10] m/z at 232 $[D/E\text{-}H_2O]^+$, 219 $[D/E\text{-}CH_2OH]^+$, 201 $[D/E\text{-}H_2O{-}CH_2OH]^+$ and 206 [A/B ring]$^+$ in the EIMS spectrum supported all the conclusion. Thus this compound was established as 16β,28-dihydroxyolean-12-en-3-one.

Compound **4** was obtained as white powder, $[M + H]^+$ peak at m/z 475.3786 ($C_{30}H_{51}O_4$, requires 475.3782) in positive HRSIMS spectrum and the signals at δ 123.7 (CH) and δ 142.7 (C) in ^{13}C NMR indicated that this compound was another olean-12-ene with the molecular formula $C_{30}H_{50}O_4$. The degree of unsaturation was 6, showing that the four oxygen atoms were belonging to hydroxy groups. Indeed, there was a broad and strong absorption at 3394 cm^{-1} in its IR spectrum. Compared with compound **3**, **1** and **2**, ^{1}H NMR of **4** indicated β-OH at C-3, δ 3.16 (dd, J = 8.7, 7.0 Hz, H-3α), hydroxymethyl at δ 3.21 (d, J = 11.8 Hz, H-28a), δ 4.17 (d, J = 11.8 Hz, H-28b), and β-OH at C-16, δ 4.30 (dd, J = 12.0, 5.0 Hz, H-16α). The fourth hydroxy was assigned on C-6, for the absence of CH_2-6 signal in olean-12-ene, which always appeared at δ 18.0 in ^{13}C NMR spectrum [11]. Actually, there were cross peaks between proton δ 4.60 (m, J = 4.8 Hz, H-6α) and carbons δ 41.1 (C-8), δ 37.2 (C-10) respectively in the HMBC spectrum. Only H-6 in α orientation, where the angles between H-6α and H-5α, H-7α/β were similar and about 40°, leading to δ 4.60 (m, J = 4.8 Hz, H-6α). EIMS of compound **4** also gave a correct series of RDA fragmentation ions at m/z 232 $[D/E\text{-}H_2O]^+$, 219 $[D/E\text{-}CH_2OH]^+$, 201 $[D/E\text{-}H_2O{-}CH_2OH]^+$ and 187 $[A/B\text{-}2H_2O{-}H]^+$. Therefore, compound **4** was deduced as olean-12-en-3β,6β,16β,28- tetraol.

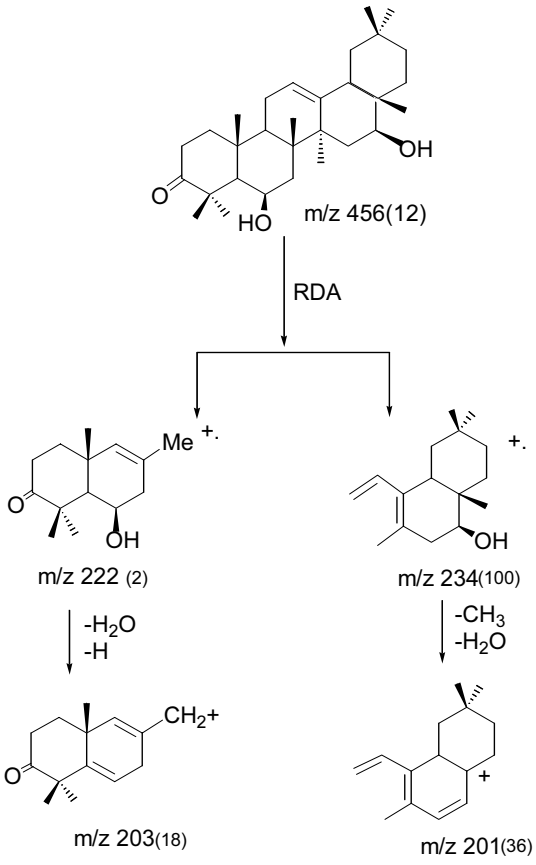
Compound **5** was obtained as a white gum, it had a $[M + H]^+$ peak at m/z 457.3677 ($C_{30}H_{49}O_3$ required 457.3676) in positive HRSIMS, which showed the molecular formula was $C_{30}H_{48}O_3$. There were thirty carbon signals in the ^{13}C NMR spectrum (Table 1), and eight methyl singlets δ 0.82 (s, 3 H), δ 0.90 (s, 3 H), δ 0.92 (s, 3 H), δ 1.18 (s, 3 H), δ 1.19 (s, 3 H), δ 1.37 (s, 3 H), δ 1.43 (s, 3 H) and δ 1.52 (s, 3 H) in the ^{1}H NMR spetrum, both of which indicated that **5** had an olean-12-ene skeleton. Besides the six unsaturated degrees of skeleton, there was one caused by a carbonyl group, which was proved by the absorption of 1710 cm^{-1} in the IR spectrum. Compared with ^{13}C NMR of **3**, δ 216.4 (C) of **5** should be assigned to C-3 carbonyl. Similarities in ^{1}H NMR of compound **5** and **3**, **4** displayed that δ 4.19 (dd, J = 11.8, 4.8 Hz, 1 H) should be H-16α, and δ 4.52 (m, J = 4.5 Hz, 1 H) should be H-6α. Furthermore, the characteristic RDA fragmentation ions m/z at 234 [D/E ring]$^+$, 219 $[D/E\text{-}CH_3]^+$, 201 $[D/E\text{-}CH_3{-}H_2O]^+$, and 222 [A/B ring]$^+$, 203 $[A/B\text{-}H_2O{-}H]^+$ (Scheme) in EIMS verified all the above deduction. This olean-12-ene was then established as 6β,16β-dihydroxyolean-12-en-3-one.

Compounds **4** and **5** showed strong cytotoxity to human hepatoma cells (SMMC-7721), and showed cytotoxity to human ovarian neoplasm (HO-8910) cells and human hepatocytes (LO2), when compared with the reference compound vincristine (Table 2).

Table 2: IC_{50} (μg/ml) of compounds 4 and 5

Compd.	SMMC-7721 cells	HO-8910 cells	LO2 cells
Vincristine	30.35	20.74	17.25
4	31.84	48.50	44.86
5	27.87	54.41	35.39

Scheme



3. Experimental

3.1. Equipment

All optical rotations were measured on Perkin-Elmer model 341 polarimeter. IR spectra were taken on a Nicolet AVATAR 360 FT-IR spectrometer. Positive HRSIMS were tested on Bruker Daltonics APEX II 47e Specifications. ^{13}C NMR (100 MHz, $CDCl_3$) spectra and ^{1}H NMR spectra (400 MHz, $CDCl_3$) were recorded on a Bruker AM 400 FT-NMR spectrometer with TMS as internal standard. EIMS data were recorded on HP-5988 MS spectrometer. Silica gel (200–300 mesh) was used for CC and silica GF_{254} for TLC. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2. Plant material

The whole plant of *L. shichuana* was collected in Qingling Mountain, Shaanxi Province, P. R. China, in September 2000. The plant material was identified by Prof. Yao-Jia Zhang, Department of Biology, Lanzhou University, Lanzhou, P. R. China. The voucher specimen (No. 2000823) was deposited at College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried and powdered whole plants of *L. shichuana* (750 g) were extracted with acetone (3 L) (5 days × 3) at room temperature. The combined extracts were evaporated *in vacuo* to yield 38 g of residue, which was chromatographed over silica gel (500 g). The column was eluted with petroleum ether-acetone (20 : 1, 15 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 1 : 1, V/V) solvent system. The eluted fractions were monitored by TLC, the combination afforded 11 fractions (A-K). Compound **1** (5 mg) was deposited from fraction C (petroleum ether-acetone, 10 : 1, 0.4 g) and recrystalized from MeOH. Fraction C was further separated by column chromatography on silica gel using petroleum ether-ethyl acetate 8 : 1, eight subfractions were yielded and subfractions 4–6 were combined to give **3** (4 mg). Fraction F (petroleum ether-acetone 5 : 1, 0.2 g) was subjected to column chromatography on silica gel eluted with CH_3Cl-acetone 6 : 1 repeatedly to give **5** (3 mg). From fraction G (between petroleum ether-acetone 5 : 1 and petroleum ether-acetone 3 : 1, 0.2 g), **2** (6 mg) was deposited and recrystalized from MeOH. Fraction H (petroleum ether-acetone 3 : 1, 0.3 g) was rechromatographed (petroleum ether-acetone 5 : 1) again to afford crude **4**, which was then purified again using different solvent system (CH_3Cl-acetone 3 : 1) to give **4** (5 mg).

3.4. 16β,28-Dihydroxyolean-12-en-3-one (3)

White gum; Rf 0.72 (petroleum ether-acetone 3 : 1); $[\alpha]_D^{20} + 4$ (acetone, c 0.15); IR (ν_{max}^{KBr}, cm^{-1}): 3378 (broad and strong), 1715, 1650, 1031 cm^{-1}; Positive HRSIMS $[M + H]^+$ 457.3724 (calc. for $C_{30}H_{49}O_3$ 457.3676); EIMS (m/z,%) 438 [M-H_2O] (45), 420 [M-2H_2O] (31), 407 [M-H_2O−CH_2OH] (35), 232 [D/E-H_2O] (7), 219 [D/E-CH_2OH] (9), 201 [D/E-H_2O−CH_2OH] (100), 206 [A/B ring] (2), 205 (5); ^{1}H NMR data (δ, $CDCl_3$, 400 MHz): δ 0.92 (s, CH_3), δ 1.06 (s, CH_3), δ 1.07 (s, CH_3), δ 1.09 (s, CH_3), δ 1.11 (s, CH_3), δ 1.24 (s, CH_3), δ 1.26 (s, CH_3), δ 2.54 (ddd, J = 12.0, 2.8, 1.2 Hz, H-2α), δ 2.39 (dt, J = 12.0, 4.6 Hz, H-2β), δ 3.21 (d, J = 12.8Hz, H-28a), δ 4.16 (d, J = 12.8 Hz, H-28b), δ 4.32 (dd, J = 10.2, 6.8 Hz, H-16α), δ 5.23 (t, J = 4.0 Hz, H-12); ^{13}C NMR data see Table 1.

3.5. Olean-12-en-3β,6β,16β,28-tetraol (4)

White powder; Rf 0.40 (CH_3Cl-acetone 3 : 1); $[\alpha]_D^{20}$: + 15 (acetone, c 0.23); IR (ν_{max}^{KBr} cm^{-1}): 3394 (broad and strong), 1644, 1024 cm^{-1}; Positive HRSIMS $[M + H]^+$ 475.3786 (calc. for $C_{30}H_{51}O_4$ 475.3782); EIMS (m/z,%): 456 [M-H_2O] (30), 438 [M-2H_2O] (1), 407 [M-2H_2O−CH_2OH] (2), 232 [D/E-H_2O] (17), 219 [D/E-CH_2OH] (4), 201 [D/E-H_2O−CH_2OH] (100), 187 [A/B-2H_2O−H] (15); ^{1}H NMR data (δ, $CDCl_3$, 400 MHz): δ 0.92 (s, CH_3), δ 0.92 (s, CH_3), δ 1.10 (s, CH_3), δ 1.19 (s, CH_3), δ 1.20 (s, CH_3), δ 1.31 (s, CH_3), δ 1.33 (s, CH_3), δ 2.38 (brdt, J = 13.2, 3.4 Hz, H-22β), δ 1.99 (d, J = 13.2 Hz, H-22α), δ 3.16 (dd, J = 8.7, 7.0 Hz, H-3α), δ 3.21 (d, J = 11.8 Hz, H-28a), δ 4.17 (d, J = 11.8 Hz, H-28b), δ 4.30 (dd, J = 12.0, 5.0 Hz, H-16α), δ 4.60 (m, J = 4.8 Hz, H-6α), δ 5.26 (t, J = 3.2 Hz, H-12); ^{13}C NMR data see Table 1.

3.6. 6β,16β-Dihydroxyolean-12-en-3-one (5)

White gum; Rf. 0.60 (petroleum ether-ethyl acetate 4 : 1); $[\alpha]_D^{20}$: + 5 (acetone, c 0.10); IR (ν_{max}^{KBr} cm^{-1}): 3392, 1710, 1640, 1033 cm^{-1}; Positive HRSIMS $[M + H]^+$ 457.3677 (calc. for $C_{30}H_{49}O_3$ 457.3676); EIMS (m/z,%): 456 $[M]^+$(12), 438 [M-H_2O] (10), 423 [M-H_2O-CH_3] (4), 420 [M-2H_2O] (1), 234 [D/E ring] (100), 219 [D/E-CH_3] (30), 201 [D/E-CH_3-H_2O] (36), 222 [A/B ring] (2), 204 [A/B-H_2O] (6), 203 (18); ^{1}H NMR data (δ, $CDCl_3$, 400 MHz): δ 0.82 (s, CH_3), δ 0.90 (s, CH_3), δ 0.92 (s, CH_3), δ 1.18 (s, CH_3), δ 1.19 (s, CH_3), δ 1.37 (s, CH_3), δ 1.43 (s, CH_3), δ 1.52 (s, CH_3), δ 1.70 (t, J = 11.8 Hz, H-15β), δ 1.66 (dd, J = 11.8, 4.8 Hz, H-15α), δ 2.78 (ddd, J = 14.0, 4.2, 1.2 Hz, H-2α), δ 2.27 (dt, J = 14.0, 6.4, H-2β), δ 4.19 (dd, J = 11.8, 4.8Hz, H-16α), δ 4.52 (m, J = 4.5 Hz, H-6α), δ 5.34(t, J = 3.6 Hz, H-12); ^{13}C NMR data see Table 1.

3.7. Antitumor assays

Cytotoxic activity assays of compounds **4** and **5** were carried out in the Department of Biology of Lanzhou University, according to the MTT method [12].

Acknowledgements: This work was financed by National Natural Science Foundation of China (No. 29972017 and QT program).

References

1 Chen, Y. L.: Acta Phytotaxonomica Sinica **34**, 631 (1996)
2 Institute of Botanic Boreali-Occidentali, Academica Sinica. Flora Tsinlingensis, Vol. 1, Chapter 5, p. 298, Science Publisher, Beijing 1985
3 Wang, W. S.; Gao, K.; Zhu, Q. X.; Jia, Z. J.: J. Chin. Chem. Soc. **47**, 1291 (2000)
4 Gao, K.; Yang, L.; Jia, Z. J.: J. Chin. Chem. Soc. **46**, 619 (1999)
5 Mao, M. J.; Zhang, Q.; Jia, Z. J.: Planta Med. **68**, 55 (2002)
6 Takizawa T.; Kinoshita K.; Koyama K.; Takahashi K.: J. Nat. Prod. **58**, 1913 (1995)
7 Morales G.; Mclaughlin J. L.: J. Nat. Prod. **52**, 381 (1989)
8 Djerassi Carl; McDonald R. M.; Lemin A. J.: J. Am. Chem. Soc. **76**, 5940 (1953)
9 Tori K.; Seo S.; Shimaoka A.; Tomita Y.: Tetrahedron Lett. **48**, 4227 (1974)
10 Cong, P. Z.: Application of MS in Natural Organic Chemistry, 1st editon, p. 684, Science Publisher, Beijing 1987
11 Shashi B. Mathato; Asish P. Kundu.: Phytochemistry **37**, 1517 (1994)
12 Han R.: Research and Development of Anticancer Drugs and Experimental Techniques, p. 284, Associated Press of Beijing Medical University and Xiehe Medical University of China, Beijing 1997