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RESEARCH ARTICLE

LAN Wenjian, LI Houjin

New bioactive triterpenoid from *Oldenlandia cantonensis* How

Abstract A new pentacyclic triterpenoid, urs-12-en-29α-oic acid-3β-ol (1), was obtained from the ethanol extract of Chinese herb *Oldenlandia cantonensis* How. The structure was elucidated by spectroscopy methods, including nuclear magnetic resonance (NMR) (1D and 2D), infrared spectroscopy (IR), and mass spectrometry (MS). 1 exhibited significant inhibitory activity against the human DNA topoisomerase I (hTopo I), the cancer cell lines BEL-7402 and MCG-803, with the IC_{50} values 12.0, 6.5, and 8.0 μg/mL, respectively. The volatile oil, the fraction of petroleum ether: EtOAc = 20:1 (*V*/*V*) on Si gel chromatography, was also quantitatively analyzed by gas chromatography mass spectrometry (GC-MS). As a result, 60 compounds were identified. Among them, the long chain aliphatics, terpenes and steroids, as the representative structure type, were found with percentages of 36.16%, 6.42% and 9.28%, respectively.

Keywords *Oldenlandia cantonensis* How, triterpenoid, urs-12-en-29α-oic acid-3β-ol, topoisomerase inhibitor, cytotoxicity

1 Introduction

Oldenlandia cantonensis How, a traditional Chinese herb, is widely distributed in Southern China, such as in the provinces of Jiangxi, Fujian, and Guangdong. Its leaves and stems have been used for treatment of hepatitis, turgescence and pain caused by vespid stinging. Its medical uses are recorded in the book "Records on Herbal Medicine from Jiangxi Province". The chemical composition of *Oldenlandia cantonensis* How has not been reported up to now. As a part of our ongoing program on finding biologically active components from Chinese herbs, we carried out a chemical investigation on *Oldenlandia cantonensis* How which was collected from Jiangxi Province. As a result, a new pentacyclic triterpenoid, urs-12-en-29α-oic acid-3β-ol (**1**) (Fig. 1) was obtained from the ethanol extracts, together with the known compound β-sitosterol. 1 exhibited significant bioactivities against the human DNA topoisomerase I (hTopo I), the cancer cell lines BEL-7402 and MCG-803. In addition, 60 compounds were also detected in the volatile oil by gas chromatography mass spectrometry (GC-MS) analysis.

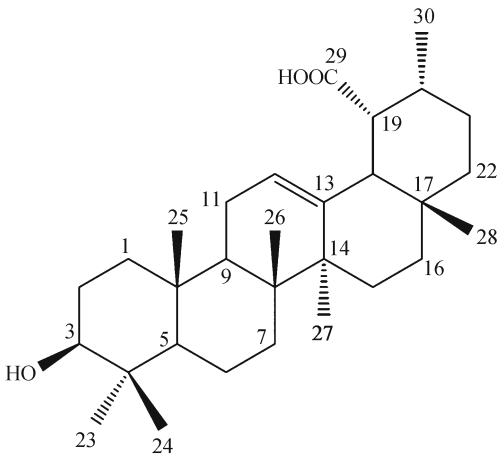

Fig. 1 The structure of **1**

2 Experimental

2.1 Materials and reagents

Oldenlandia cantonensis How was collected from the Gannan area of Jiangxi Province in July 2003. Whole *Oldenlandia cantonensis* How was dried in air. Si gel H, petroleum ether, ethyl acetate, methanol and ethanol were all AR grade.

2.2 Instruments

Melting point was measured on automated melting point apparatus X-6 and is uncorrected. Optical rotation was acquired using a SCHMDT + HAENSCH HNQW5 polarimeter. Elemental analysis was carried out on a Vario EL CHNS-O elemental analysis spectrometer. IR spectra was

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LAN Wenjian
School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510080, China

LI Houjin (✉)
School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China
E-mail: ceslhj@mail.sysu.edu.cn

recorded on a Bruker EQUNOX55-A590/3F Fourier transform infrared spectroscopy. NMR spectra were recorded on a Varian Inova-500 NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, employing TMS as the internal standard. FAB MS was obtained on VG ZAB-HS double focusing mass spectrometry. GC-MS was performed using Agilent HP 6890/5973 gas chromatography mass spectrometry.

2.3 Extraction and isolation

Two kilograms of dried *Oldenlandia cantonensis* How was cut to pieces, then was soaked in 95% ethanol (6 L) for 12 h at room temperature. The extraction procedure was repeated three times. The combined ethanol solution was evaporated under reduced pressure to give 230 g of black extract. The extract was further partitioned with 400 mL EtOAc-H_2O (1:1, *V*/*V*) three times, and the organic layer was concentrated under reduced pressure to afford 160 g EtOAc extract. The EtOAc extract was fractionated on Si gel flash column chromatography using a petroleum ether-EtOAc-methanol solvent system by increasing the polarity. In the first fraction, about 35 g aromatic liquid oil was obtained using 1 000 mL petroleum ether-EtOAc (20:1, *V*/*V*) as eluent. The fraction eluted with petroleum ether-EtOAc (3:1, *V*/*V*) was concentrated and recrystallized to yield sitosterol (3.9 g). The semi-pure fraction concentrated from EtOAc-methanol (10:1, *V*/*V*) solvent was further recrystallized with petroleum ether-EtOAc (1:3, *V*/*V*) solvent to yield 4.8 g of 1.

2.4 Urs-12-en-29α-oic acid-3β-ol (**1**)

White powder solid. Mp > 250°C; $[\alpha]_D^{20} = +40°$ (MeOH, $c = 0.13$); elemental analysis: C 78.83, H 10.90, N 0.000; FAB MS: *m/z* 457 $[M+H]^+$, 439 $[M-H_2O+H]^+$, 411 $[M-COOH]^+$, 393, 355, 341, 327, 289, 279, 248, 203, 189, 133, 119, 69, 55; IR(KBr), ν_{max}: 3 420, 2 970, 2 928, 2 871, 1 693, 1 575, 1 455, 1 407, 1 384, 1 370, 1 313, 1 279, 1 249, 1 142, 1 091, 1 032, 996, 758, 661, 568 cm^{-1}; NMR data were listed in Table 1.

2.5 GC-MS analysis

The volatile oil, the first fraction on flash Si gel chromatography eluted with petroleum ether-EtOAc (20:1, *V*/*V*), was dissolved in chloroform for GC-MS analysis. GC-MS analysis was carried out with an Agilent Technologies 5973 Network Mass Selective Detector coupled with the 6890N network GC System equipped with a fused silica capillary column (HP25MS 30 m × 0.25 mm × 0.25 μm). The oven temperature of the gas chromatograph was maintained at 50°C for 3 min, followed by a gradual increase in temperature at the rate of 5°C /min till a temperature of up to 250°C was attained. The injector and the detector temperatures were 230°C. Helium was used as the carrier gas and was adjusted to a linear flow velocity of 0.5 mL/min, with a split ratio of 20:1. The injection volume was 1 μL. The mass selective detector was operated in the electron ionization (EI) mode, with the ion source temperature being 230°C; the ionization energy, 70 eV; and the mass range, *m/z* 40–500.

Table 1 NMR data of 1 (DMSO-d6, TMS, δ_H at 500 MHz and δ_C at 125 MHz)

Position number	DEPT	δ_C	δ_H
1	CH_2	38.2	1.50 (dt, 13.0, 3.5), 1.91 (dt, 13.0, 4.5)
2	CH_2	23.7	1.54 (m)
3	CH	76.8	3.00(dd, 10.0, 5.5)
4	C	38.3	–
5	CH	54.7	0.66 (dd, 10.0, 1.0)
6	CH_2	17.9	1.46 (m)
7	CH_2	32.6	1.57 (m)
8	C	40.7	–
9	CH	46.9	1.45 (m)
10	C	36.2	–
11	CH_2	22.7	1.86 (m)
12	CH	124.5	5.13 (dd, 3.5, 3.5)
13	C	138.1	–
14	C	41.5	–
15	CH_2	27.5	1.80 (m)
16	CH_2	26.9	1.46 (m), 1.30 (m)
17	C	34.1	–
18	CH	52.3	2.11 (dd, 12.0, 1.0)
19	CH	38.3	1.94 (dd, 12.0, 3.0)
20	CH	38.4	1.85 (m)
21	CH_2	36.2	1.51 (m)
22	CH_2	41.6	1.25 (m), 1.43 (m)
23	CH_3	28.1	0.90 (s)
24	CH_3	15.9	0.68 (s)
25	CH_3	15.1	0.87(s)
26	CH_3	16.9	0.76 (s)
27	CH_3	23.2	1.04 (s)
28	CH_3	20.9	0.92 (s)
29	C	178.1	–
30	CH_3	16.8	0.82 (d, 6.5)
3-O<u>H</u>	–	–	4.15 (brs)
29-COO<u>H</u>	–	–	11.8 (brs)

2.6 Bioassay

The screening of human DNA topoisomerase I inhibitor was performed using the method previously reported by Hsiang et al. [1] and Chowdhury et al. [2]. The cytotoxic activities *in vitro* of 1 were carried out on cancer cell lines BEL-7402 and MCG-803, using the standard modified tertrozalium salt (MTT) method.

3 Results and discussion

3.1 Structure determination of urs-12-en-29α-oic acid-3β-ol (**1**)

The positive result in the Liebermann-Burchard experiment suggested compound 1 was a terpene. FABMS *m/z* 457 $[M+H]^+$ revealed a molecular weight of 456. Elemental analysis indicated it was composed of C, H and O. The ^{13}C

NMR spectrum showed the presence of thirty carbons in the molecule, including seven quaternary carbons, seven methines, nine methylenes and seven methyls. Based on the analysis above, the molecular formula of 1 was established as $C_{30}H_{48}O_3$. Seven degrees of unsaturation were required by the formula. One trisubstituted double bond [δ_C 138.1(C) and δ_C 124.5 (CH)] and one carboxylic resonance at δ_C 178.1, accounted for two degrees of unsaturation, so five rings existed in 1. Six methyl singlets and a methyl doublet (δ_H 0.82, J = 6.5 Hz) were observed in the ^{1}H NMR spectrum. The absorptions at 1 384 and 1 370 cm^{-1} in IR spectrum, suggested that there was a geminal dimethyl group in the molecule. Broad absorption at 3 420 and 1 693 cm^{-1} in IR spectrum, fragment ions *m/z* 411[M−COOH]$^+$ in FAB MS spectrum, as well as δ_C 178.1 and a broad singlet at δ_H 11.8 in NMR spectra indicated the existence of a carboxylic group in the molecule. In addition, a hydroxyl group was connected to methine, which was confirmed by a CH (δ_C76.8, δ_H 3.00, dd, J = 10, 5.5 Hz) and OH resonance at δ_H 4.15. Comprehensive analysis of the 2D NMR data (^{1}H−^{1}H COSY, HMQC and HMBC) (see Fig. 2) allowed us to assemble the structure of 1 to be a new pentacyclic triterpenoid, urs-12-en-29*α*-oic acid-3*β*-ol. **1** has the same skeleton with ursane [3]. The major differences identified were one methyl group connecting to C-19 in ursane, with the carboxylic group connecting to C-19 and the hydroxyl group linking to C-3 in **1**.

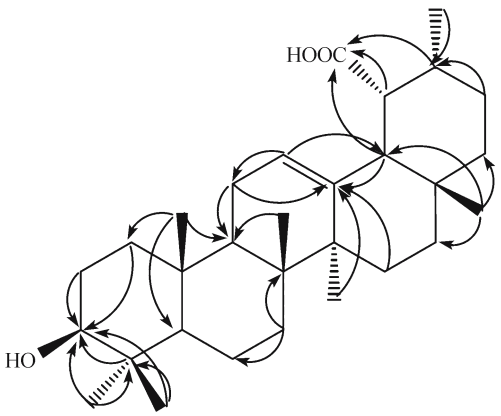

Fig. 2 The key HMBC correlations of **1**

Stereochemistries of A, B and C rings in **1** were established by comparing NMR data with urs-12-en-3-ol. Furthermore, the stereochemistries of H-18, H-19 and H-20 were established by the coupling constants. Based on the coupling constant between H-18 and H-19 (J = 12.0 Hz), we undoubtedly placed them at the axial position. The small coupling constant (3.0 Hz) between H-19 and H-20 indicated that H-20 was equatorial [4,5]. From the analysis above, H-18, H-19 and H-20 were placed at *α*, *β* and *β*, respectively.

Plectranthoic acid (urs-12-en-29*β*-oic acid-3*α* -ol) was isolated by Razdan and his colleagues from *Plectranthus rugosus* in 1982 [6], but its ^{1}HNMR ,^{13}CNMR data and physiological activity had not been reported yet.

Table 2 The analysis of volatile oil by GC-MS

Number	Compound identified	Area/%	Quality
1	3,3,6-trimethyl-2,5-heptanedione	0.10	73
2	6,10,14-trimethyl-2-pentadecanone	1.25	99
3	9-hexadecenoic acid, methyl ester	0.21	99
4	8-(*Z*)-hexadecene	0.75	98
5	Hexadecanoic acid, ethyl ester	1.64	81
6	1,1-dimethoxy-dodecane	0.17	78
7	8,11-octadecadienoic acid, methyl ester	1.43	99
8	9-(*Z*)-octadecenoic acid, methyl ester	2.14	99
9	Octadecanoic acid, methyl ester	0.78	99
10	1-heptadecene	0.13	65
11	9,12-octadecadienoic acid, ethyl ester	0.44	99
12	Ethyl oleate	0.51	99
13	Octadecanoic acid, ethyl ester	0.42	99
14	5-(*Z*)-nonadecene	0.22	97
15	3,7,11,15-tetramethyl-2-hexadecene-1-ol	0.35	75
16	Eicosanoic acid, methyl ester	0.49	99
17	Tricosane	0.77	98
18	2,4-bis(1-methyl-1-phenyl)-ethyl- phenol	0.74	93
19	Docosanoic acid, methyl ester	1.15	99
20	Di-*n*-octyl phthalate	1.04	93
21	2-dodecyl-tetradeca hydro-phenanthrene	0.47	78
22	Tricosanoic acid, methyl ester	1.16	99
23	Pentacosane	1.25	97
24	1-hexacosene	1.32	89
25	Tetracosanoic acid, methyl ester	1.32	96
26	3-formoxy-11-ol-17-one-androstane	0.40	90
27	11,13-dimethyl-12-tetradecen-1-ol, acetic ester	0.84	87
28	Tert-hexadecanethiol	1.13	82
29	2-dodecen-1-succinic anhydride	0.39	86
30	Docosanoic acid, methyl ester	0.71	94
31	9,9'-dimethoxy-2,7-dimethyl-nonan-2-ol	0.73	89
32	Octacosanol	0.92	88
33	2-heptacosanone	0.90	74
34	Hexacosanoic acid, methyl ester	0.80	86
35	Tetradecanoic acid, tetradecyl ester	0.99	79
36	Undecanal dimethyl acetal	0.81	72
37	Hexacosane	0.67	67
38	2-methyl-propanoic acid, docosyl ester	0.90	83
39	28-nor-17α-(*H*)-hopane	0.54	66
40	1-en-3-one-10-dimethoxymethyl-17-acetyloxo-androstane	0.48	69
41	3,5,22-trien-stigmastane	0.57	85
42	2-nonacosanone	1.26	69
43	Heptacosanoic acid, methyl ester	0.72	90
44	Vitamin E	1.08	95
45	Tetradecanoic acid, hexadecyl ester	2.16	99
46	Undecanal dimethyl acetal	0.93	69
47	11-methylnonacosane	0.79	75
48	Eudesmol	0.76	78
49	Butanoic acid, cyclohexyl ester	1.51	77
50	1,1-dimethoxy-9-(*Z*)-octadecene	1.51	81
51	Friedelin	0.66	86
52	3,5-diene-3,17-bis-(trimethylsilanyloxy)-androstane	1.63	67
53	Hexadecyl oxirane	0.89	80
54	7-one-3-acetyloxy-cholestane	1.34	88
55	1-amine-4-methyl-piperazine	1.21	72
56	5,9(11)-dien-3*β*-acetyloxy -cholestane	1.39	79
57	22,23-dihydro-stigmasterol	1.73	93
58	3-one-amyrin	0.95	94
59	3*β*,17*β*-bis- acetyloxy-5-androsten	1.74	93
60	9-hexadecenoic acid, eicosyl ester	2.07	90

3.2 Bioactivity of urs-12-en-29α-oic acid-3β-ol (**1**)

The inhibitory activity of **1** against hTopo I was evaluated using rapid screening. Additionally, the antitumor activities *in vitro* of 1 were tested using the MTT method. 1 exhibited a significant inhibitory activity against hTopo I, the cancer cell lines BEL-7402 and MCG-803, with the IC_{50} values of 12.0, 6.5 and 8.0 μg/mL, respectively. DNA topoisomerase I can induce the DNA break and reunion. DNA breakage *in vitro* is immediate and reversible. Some cytotoxic drugs, such as camptothecin can inhibit the catalytic activity of topoisomerase I, and block the rejoining step of the breakage-reunion reaction. This blockage results in the accumulation of a cleavable complex. HTopo I of eukaryotes has been considered as an effective target in antitumor drugs screening [7,8]. Because our studies suggested that 1 was a cytotoxic compound target on topoisomerase I, further pharmacological and genetic studies are inspired in antitumor drug development.

3.3 GC-MS analysis of volatile oil

The volatile oil was analyzed by GC-MS and 60 compounds were identified (see Table 2). The result showed that long chain aliphatics, including saturated and unsaturated fatty acids and their esters, were dominant with a percentage of 36.16%. Furthermore, a variety of terpenes were detected with a percentage of 6.42% in total. The representative monoterpenes were 3,3,6-trimethyl-2,5-heptanedione and 9,9′-dimethoxy-2,7-dimethyl-nonan-2-ol. The diterpenes found were 3,7,11,15-tetramethyl-2-hexadecene-1-ol, 6,10,14-trimethyl-2-pentadecanone and Vitamin E. The triterpenes included were 28-nor-17α-(H)-hopane, eudesmol, friedelin and 3-one-amyrin. Additionally, another important structure type in the volatile oil was steroid with the percentage of 9.28%. The typical compounds were 3-formoxy-11-ol-17-one-androstane, 1-en-3-one-10-dimethoxymethyl-17-acetyloxo-androstane, 3,5,22-trien-stigmastane, 3,5-diene-3,17-bis-(trimethylsilanyloxy)-androstane, 7-one-3-acetyloxy-cholestane, 5,9(11)-dien-3β-acetyloxy-cholestane, 22,23-dihydro-stigmasterol and 3β,17β-bis-acetyloxy-5-androsten.

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