

Full Paper

Two New Triterpenoids from *Photinia serrulata*

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Received: 24 November 2007; in revised form: 11 December 2007 / Accepted: 11 December 2007 / Published: 20 December 2007

Abstract: Two new triterpenoids, $2\alpha,3\beta,11\alpha,13\beta$ -tetrahydroxy-12-ketooleanan-28-oic acid (**1**) and 3β -hydroxy-12-keto-9(11)-ursen-28,13 β -olide (**2**) were isolated from the leaves of *Photinia serrulata*. Their structures were identified by spectral methods. Compounds **1** and **2** were assessed for cytotoxic activity against three human tumor cell lines (A-549, HCT-8, and BEL-7402), and they showed no cytotoxic effects at concentrations up to 5 μ g/mL.

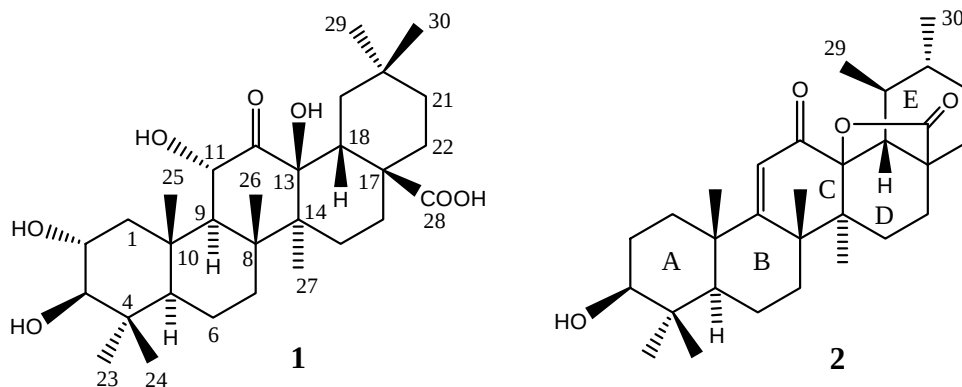
Keywords: *Photinia serrulata*, Rosaceae, triterpenoids, cytotoxic activity

Introduction

Photinia serrulata Lindl. belongs to the Rosaceae family and is found throughout the East and South of Asia [1]. Its tender leaves are used as edible vegetables in the south of China, and the matured leaves, known in China as “Shi-Nan”, are used for the treatment of nephropathy, rheumatism, and spermatorrhea [2]. Despite the wide use of the leaves of this plant in Traditional Chinese Medicine, reports on the chemical constituents of this plant are scarce. During our investigation, two new

compounds including one oleanane- and one ursane-type triterpenoid **1** and **2** (Figure 1) were isolated, and their structures were characterized by means of spectroscopic methods. The cytotoxic activity of compounds **1** and **2** against three human tumor cell lines (A-549, HCT-8, and BEL-7402) were also assessed. In this paper, we report the isolation and structure determination of these new isolates.

Figure 1. Structures of compounds **1** and **2**.



Results and Discussion

Compound **1** was obtained as a white powder. The HRESIMS at m/z 519.3320 $[M-H]^-$ (calcd. for $C_{30}H_{47}O_7$, 519.3321) gave the molecular formula $C_{30}H_{48}O_7$. The IR absorptions at 3430, 1777, and 1725 cm^{-1} indicated the presence of hydroxyl, ketone and carbonyl groups. The ^{13}C -NMR (Table 1) and DEPT spectra of **1** displayed thirty signals, which included seven methyls, eight methylenes, six methines (three oxygenated) and nine quaternary signals, including a ketone and a carbonyl. These data, together with analysis of the corresponding ^1H - ^1H COSY and HMBC spectra suggested that **1** possesses an oleanane-type skeleton [3]. Comparison of the NMR data of **1** with those of maslinic acid and 3β -acetoxy- $11\alpha,13\beta$ -dihydroxyolean-12-one [4,5] disclosed that the two olefinic carbons in maslinic acid were replaced by an oxygenated quaternary carbon at δ 90.0 and a ketone group at δ 200.2, besides, in **1** an oxygen-bearing methine at δ 56.1 was observed, instead of a methylene found in maslinic acid. The ^1H - ^1H COSY coupling of H-9 (δ 2.16) with H-11 (δ 4.31), together with HMBC correlations from H-9 and H-11 to C-12 (δ 200.2), from H-11, Me-27 (δ 1.38), and H-19 (δ 1.82/1.42) to C-13 (δ 90.0) suggested that a ketone was positioned at C-12, and C-11 and C-13 were oxygenated, respectively. The H_{ax-2} , H_{ax-3} were deduced by their coupling constants ($J = 9.5\text{ Hz}$), and were also evident from ROESY interactions of H-3 (δ 3.05) with Me-23 (δ 1.05). Additional ROESY correlations from Me-25 (δ 1.07) to H-2 (δ 3.71) and H-11, H-2 to Me-24 (δ 0.84), $H\beta-1$ (δ 2.18) to H-2 and H-11, suggested that the hydroxyl group at C-11 was α oriented, which was in agreement with the coupling constant of H-11 with H-9 ($J = 7.5\text{ Hz}$). It should be noted that the signal for C-11 is abnormally up-field shifted, which may be caused by the gauche effects of $H\beta-11$ with both $13\beta\text{-OH}$ and axial Me-25. Thus, the structure of **1** was determined to be $2\alpha,3\beta,11\alpha,13\beta$ -tetrahydroxy-12-keto-oleanan-28-oic acid.

Compound **2** was obtained as a white powder. Its molecular formula $C_{30}H_{44}O_4$ was deduced from the HRESIMS at m/z 491.3129 $[M+Na]^+$ (calcd. for $C_{30}H_{44}O_4Na$ 491.3137). The ^{13}C -NMR (Table 1) and DEPT spectra of **2** showed thirty carbons, including seven methyls, eight methylenes, six methines,

and nine quaternary signals. These data, together with the analysis of ^1H - ^1H COSY and HMBC spectra suggested that **2** possessed an ursane-type skeleton, and was structurally related with ursolic acid [4]. The differences between **2** and ursolic acid were mainly in ring B. A double bond between C-9 and C-11, a ketone group at C-12, and an oxygenated carbon signal at C-13 were observed in **2**, HMBC correlations of Me-25 (δ 1.28), Me-26 (δ 1.37), H-5 (δ 1.04), H-7 (δ 1.76/1.52), and H-11 (δ 6.00) all with C-9, and H-18 (δ 2.42) with C-12 confirmed the positions of the double bond and ketone groups. The UV absorption at 259 nm and IR peak at 1632 cm^{-1} indicated the presence of an α,β -unsaturated ketone. In addition, a lactone ring between C-13 and C-28 inferred from the molecular mass, was also confirmed by the absorption peak at 1780 cm^{-1} in the IR spectrum. The hydroxyl at C-3 was assigned a β orientation by comparison of its ^{13}C -NMR data with literature data [6], and ROESY correlations of H-3 (δ 3.18) with Me-23 (δ 1.04). The *cis*-fusion of D/E rings could be judged from the large coupling constant of Hax-18 ($J=12.0\text{ Hz}$). It was noted that the signal for C-9 appeared at a very abnormally down-field position, and the reasons for this phenomenon still remain unclear. Taken together, the structure of **2** was identified as 3 β -hydroxy-12-keto-9(11)-ursen-28,13 β -olide. The acetate of **2** has previously been described in the literature as a result of chemical transformation [7]. However, as a naturally occurring compound, **2** was isolated for the first time, the ^1H - and ^{13}C -NMR data of **2** have now been unambiguously assigned on the basis of 2D NMR experiments for the first time.

Table 1. ^1H - and ^{13}C -NMR spectra data for compounds **1** and **2** ^a.

Position	1		2	
	$\delta_{\text{H}} (J=\text{Hz})$	δ_{C}	$\delta_{\text{H}} (J=\text{Hz})$	δ_{C}
1	2.18 m; 1.30 m	47.0 t	2.04 m; 1.42 m	37.0 t
2	3.71 m	68.6 d	1.72 m	28.4 t
3	3.05 d (9.5)	83.2 d	3.18 m	77.6 d
4		39.1 s		39.9 s
5	0.91 m	55.0 d	1.04 m	50.8 d
6	1.67 m; 1.55 m	17.4 t	1.74 m	18.0 t
7	1.31 m	31.6 t	1.76 m; 1.52 m	34.5 t
8		41.5 s		46.6 s
9	2.16 d (7.5)	57.7 d		185.1s
10		39.8 s		41.3 s
11	4.31 d (7.5)	56.1 d	6.00 s	122.0 d
12		200.2 s		193.3 s
13		90.0 s		88.4 s
14		41.7 s		42.9 s
15	1.66 m	26.3 t	1.80 m; 1.43 m	26.5 t
16	2.18 m; 1.38 m	21.3 t	2.18 m; 1.38 m	22.7 t
17		44.4 s		45.6 s
18	2.71 dd (13.5, 3.0)	48.0 d	2.42 d (12.0)	55.5 d
19	1.82 m; 1.42 m	37.0 t	1.80 m	37.7 d
20		31.6 s	1.01 m	40.5 d
21	1.33 m	34.2 t	1.58 m	31.2 t
22	1.75 m; 1.31 m	26.8 t	1.54 m	32.1 t
23	1.05 s	28.3 q	1.04 s	28.5 q

Table 1. Cont.

24	0.84 s	16.3 q	0.84 s	16.1 q
25	1.07 s	18.0 q	1.28 s	24.9 q
26	0.98 s	19.7 q	1.37 s	30.8 q
27	1.38 s	19.7 q	1.21 s	20.8 q
28		177.6 s		178.4 s
29	0.90 s	33.2 q	0.76 d (6.4)	18.7 q
30	0.99 s	23.3 q	0.92 d (6.4)	19.4 q

^aThe spectra of **1** were recorded in CDCl₃ and **2** in CD₃COCD₃ (400 MHz for ¹H, 100 MHz for ¹³C)

The cytotoxic activity of compounds **1** and **2** against three human tumor cell lines (A-549, HCT-8, and BEL-7402) were evaluated. However, they exhibited no cytotoxic effects at concentrations up to 5 µg/mL (data not shown).

Experimental

General

Melting points were recorded on an XRC-1 micromelting apparatus. Optical rotations were determined on a JASCO-20C digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained with a Bruker Tensor 27 FT-IR spectrophotometer with KBr pellets. ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded on a Bruker AM-400 spectrometer, with TMS as an internal reference. 2D NMR spectra were measured with a DRX-500 spectrometer. FABMS were recorded on a VG Auto Spec-3000 spectrometer. ESIMS and HRESIMS were carried out with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh and 10-40 µm) for column chromatography and GF₂₅₄ for TLC were obtained from Qingdao Marine Chemical Factory (Qingdao, P. R. China). Sephadex LH-20 was obtained from Amersham Pharmacia Biotech (Sweden).

Plant Material

The dried and matured leaves of *P. serrulata* Lindl. were purchased from Nanjing Pharmaceutical Ltd. Corporation of Jiangsu Province (P. R. China) in March, 2006, and identified by Mrs. Xuedong Geng (Nanjing Pharmaceutical Ltd. Corporation of Jiangsu Province). A voucher specimen (CHYX0392) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The dried and powdered plant materials of *P. serrulata* (15 kg) were extracted three times with 80% EtOH under reflux. The extracts were concentrated and suspended in water followed by

successive partition with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc extracts (866 g) were subjected to column chromatography (CC) over silica gel (200-300 mesh) and eluted with CHCl₃-MeOH (15:1) to afford fractions 1-3. Fraction 2 (160 g) was submitted to CC over silica gel, eluted with CHCl₃-MeOH-EtOAc (6:1:1) to give fractions 2.1-2.2. Fraction 2.2 (145 g) was further fractionated on silica gel eluted with a CHCl₃-MeOH gradient (98:2, 96:4, 95:5) to afford fractions 2.2.1-2.2.5. Fraction 2.2.1 (16 g) was fractionated into four portions by CC over silica gel eluted with gradient CHCl₃-Me₂CO (50:1, 40:1, 30:1, 15:1), i.e. fractions 2.2.1.1-2.2.1.4. Repeated chromatography of fraction 2.2.1.1 (4 g) over silica gel (10-40 μm), and Sephadex LH-20 (CHCl₃-MeOH, 6:4) yielded compounds **1** (3.6 mg), and **2** (3.7 mg).

2α,3β,11α,13β-tetrahydroxy-12-ketooleanan-28-oic acid (1). White powder; mp 218-219°C; $[\alpha]_D^{27.8}$ -30.86° (c 0.14, CHCl₃); UV (CHCl₃) λ_{max} (log ε) nm: 241 (3.24); IR (KBr) ν_{max} : 3430, 2952, 2925, 2855, 1777, 1725, 1632, 1455, 1053 cm⁻¹; ¹H- and ¹³C-NMR data, see Table 1; positive ESIMS *m/z*: 543 [M+Na]⁺; negative ESIMS *m/z*: 555 [M+Cl]⁻, 519 [M-H]⁻.

3β-hydroxy-12-keto-9(11)-ursen-28,13β-olide (2). White powders; mp 237-238°C; $[\alpha]_D^{26.4}$ +5.47° (c 0.31, Me₂CO); UV (MeOH) λ_{max} (log ε) nm: 259 (4.07), 230 (3.89); IR (KBr) ν_{max} : 3440, 2954, 2927, 2871, 1780, 1632, 1455, 1384 cm⁻¹; ¹H- and ¹³C-NMR data, see Table 1; positive FABMS *m/z*: 469 (100) [M+H]⁺; negative ESIMS *m/z*: 503 [M+Cl]⁻, 467 [M-H]⁻; positive ESIMS *m/z*: 507 [M+K]⁺, 491 [M+Na]⁺, 469 [M+H]⁺.

Cytotoxic Assay

Compounds **1** and **2** were tested for their cytotoxic effects against human A-549, HCT-8 and Bel-7402 cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as previously described [8], with fluorouracil (5-FU) as positive control. All the experiments were run in triplicate.

Acknowledgements

This work was financially supported by a “Talent Scholarship for the Youth of Yunnan” (No. 2007PY01-48), “Xi-Bu-Zhi-Guang” Project from the Chinese Academy of Sciences and startup grants from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, and Ministry of Education of the People’s Republic of China.

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Sample Availability: Samples of compounds **1** and **2** are available from the authors.

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