Notes

Protolimonoids from *Melia toosendan*

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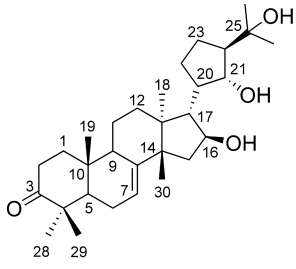
Toosendanone A (**1**), a new euphane (tirucallane)-type triterpene bearing a five-membered ring in the side chain and the first cyclopentanyl protolimonoid, was isolated from the bark of *Melia toosendan*, along with two new tirucallanes, toosendanic acids A (**2**) and B (**3**). The structure and absolute configuration of compound **1** was elucidated by spectroscopic data interpretation and X-ray diffraction analysis. Compounds **1**−**3** were evaluated for cytotoxicity against a small panel of cancer cell lines.

Melia toosendan Sieb. & Zucc. (Meliaceae), distributed in the southwest region of mainland China, has been used as an anthelmintic for the treatment of roundworm since ancient times. Plants in the genus *Melia* contain highly functionalized limonoids along with their biogenetic precursor protolimonoids that possess euphane or tirucallane skeletons.[1] Since some *Melia* limonoids have been reported to have antineoplastic activity,[2] we have reinvestigated the limonoid-related constituents of the title plant. This led to the characterization of a new euphane (tirucallane)-type triterpene bearing a pentacyclic partial structure in the side chain, named toosendanone A (**1**), along with toosendanic acids A (**2**) and B (**3**). These three compounds were tested for cytotoxicity against three human cancer cell lines (HL-60, HT-29, and MCF-7).



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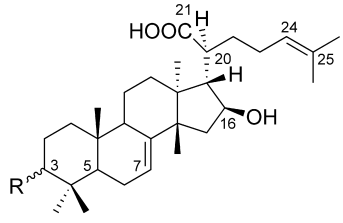

2 R = β-OH
3 R = α-OH

Toosendanone A (**1**) was obtained as a white, amorphous powder. Its HRESIMS displayed a quasi-molecular ion at *m*/*z* 495.3467 [M + Na]$^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445), indicating that the molecular formula is $C_{30}H_{48}O_4$. Its IR absorption bands at 3352 and 1709 cm^{-1} suggested the presence of hydroxyl and carbonyl groups in the molecule. The correlative analysis of its ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra (Table 1) of **1** revealed that the molecule is constructed by five sp^3 quaternary carbons (one oxygenated), seven sp^3 methines (two oxygenated), eight sp^3 methylenes, and seven methyls, in addition to a carbonyl (δ_C 216.8) and a trisubstituted vinyl group resonating at δ_H 5.32 and at δ_C 118.4 and 145.1.

A steroidal nucleus-like substructure (covering C-1→ C-19, C-28 → C-30) with a 3-ketone and a 7,8-double bond was established for **1** by comparing its ^{1}H and ^{13}C NMR spectroscopic data with those of protolimonoids with euphane and tirucallane skeletons reported previously.[3−9] As implied by the molecular formula, the remaining two methyls, three methylenes, two methines, and one quaternary carbon could be incorporated into the molecule and represent the rest of the double-bond equivalents. With this in mind, the oxygenated methine proton (H-21) at δ 3.82, showing clear HMBC correlations with C-17, C-20, C-24, and C-25, suggested that C-21 is linked directly to C-20 and C-24, to afford a five-membered ring E anchored at C-17. This side unit location was also evidenced from the HMBC correlations of H-17 with C-20, C-21, and C-22 (Figure 1). This led to a planar structure for **1**, which was reinforced by the EIMS, with ion peaks at *m*/*z* 421 (base peak), 295, 163, and 107.

The relative configuration of compound **1**, assigned originally from the ROESY spectrum, was confirmed by its single-crystal X-ray diffraction data[10] (Figure 2). To establish its absolute configuration, the CD spectrum of **1** was acquired, revealing a negative Cotton effect at 296 nm, very similar to those of compounds with a 4,4-dimethyl-5-hydrogen-10-methylcyclehexane-3-one skeleton.[11] Thus, compound **1** was assigned with a 5*R*,10*R*-configuration. Unlike the reported protolimonoids with C-17 carrying typically a tetrahydrofuran ring, compound **1** bears an unprecedented cyclopentanyl moiety at this position.

Toosendanic acid A (**2**), isolated as an amorphous powder, displayed a pseudomolecular ion peak at *m*/*z* 477.3335 [M − H_2O + Na]$^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3339) in the HRESIMS, consistent with a molecular formula of $C_{30}H_{48}O_4$. Its IR absorption bands at 3437 and 1779 cm^{-1} indicated the presence of hydroxyl and carboxyl groups. The ^{13}C NMR data of **2** exhibited a total of 30 resonances attributable to one carboxyl, two sp^2 quaternary carbons, four sp^3 quaternary carbons, two sp^2 methines, six sp^3 methines (two oxygenated), eight sp^3 methylenes, and seven methyl carbons. Furthermore, the presence of a 7,8-double bond was evident from characteristic olefinic carbon signals at δ_C 118.6 and 143.3 in the ^{13}C NMR spectrum (Table 1). These data suggested that compound **2** shares the same carbon skeleton with 16-hydroxybutyrospermol (tirucallane-7,24-diene-3β,16β-diol).[9,12]

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1**−**3** in $CDCl_3$[a]

	1		**2**		**3**	
position	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1a	1.43 (1H, m)	38.4	1.10−1.16 (1H, m)	36.9	1.34 (1H, dt, 3.41, 13.12)	31.1
1b	1.96 (1H, m)		1.64 (1H, m)		1.52 (1H, m)	
2a	2.25 (1H, dt, 3.77, 13.76)	34.9	1.98 (1H, m)	23.9	1.61 (1H, m)	25.4
2b	2.75 (1H, td, 5.52, 14.51)		2.18 (1H, m)		1.90 (1H, m)	
3		216.8	3.24 (1H, dd, 4.14, 11.48)	79.1	3.47 (1H, t, 2.74)	76.1
4		47.9		39.0		37.4
5	1.71 (1H, m)	52.5	1.33 (1H, dd, 5.79, 12.01)	50.9	1.81 (1H, dd, 5.78, 12.12)	44.8
6a	2.10 (1H, m)	24.4	1.60 (1H, m)	27.6	1.97 (1H, m)	23.9
6b	2.10 (1H, m)		1.66 (1H, m)		2.06 (1H, m)	
7	5.32 (1H, q, 3.28)	118.4	5.29 (1H, dd, 3.14, 6.66)	118.6	5.28 (1H, dd, 3.08, 6.60)	118.6
8		145.1		143.3		143.6
9	2.32 (1H, m)	47.9	2.42 (1H, m)	48.3	2.55 (1H, m)	48.0
10		35.1		35.5		35.3
11a	1.58 (1H, m)	17.9	1.58 (1H, m)	16.7	1.57 (1H, m)	16.6
11b	1.58 (1H, m)		1.69 (1H, m)		1.72 (1H, m)	
12a	1.78 (1H, m)	32.4	1.69 (1H, m)	29.8	1.70 (1H, m)	29.8
12b	1.48 (1H, m)		1.76 (1H, m)		1.75 (1H, m)	
13		45.7		39.6		39.6
14		49.1		55.2		55.3
15a	2.18 (1H, m)	44.6	1.69 (1H, m)	35.6	1.68 (1H, dd, 7.58, 13.59)	35.6
15b	1.61 (1H, dd, 1.76, 13.66)		2.27 (1H, dd, 10.18, 13.59)		2.27 (1H, dd, 10.18, 13.58)	
16	4.22 (1H, tdd, 1.76, 1.85, 8.04)	77.3	4.15 (1H, ddd, 1.05, 3.12, 9.92)	82.6	4.15 (1H, ddd, 2.7, 10.26, 18.79)	82.6
17	1.68 (1H, m)	61.0	2.12 (1H, m)	58.2	2.12 (1H, t, 11.61)	58.2
18	0.83 (3H, s)	22.9	0.96 (3H, s)	21.5	0.96 (3H, s)	21.5
19	1.01 (3H, s)	12.7	0.76 (3H, s)	12.7	0.78 (3H, s)	12.7
20	1.81 (1H, m)	47.7	2.41 (1H, m)	45.5	2.40 (1H, m)	45.5
21	3.82 (1H, t, 9.20)	80.4		180.7		180.7
22a	1.27 (1H, m)	23.2	1.47 (1H, m)	29.3	1.47 (1H, m)	29.3
22b	1.77 (1H, m)		1.95 (1H, m)		1.96 (1H, m)	
23a	1.25 (1H, m)	25.7	2.04 (1H, m)	26.1	2.04 (1H, m)	26.1
23b	1.78 (1H, m)		2.12 (1H, m)		2.11 (1H, m)	
24	1.99 (1H, m)	54.2	5.11 (1H, tt, 1.31, 5.57)	123.5	5.11 (1H, tt, 1.33, 5.67)	123.5
25		74.1		132.6		132.5
26	1.23 (3H, s)	23.6	1.62 (3H, s)	17.9	1.62 (3H, s)	17.9
27	1.22 (3H, s)	30.5	1.69 (3H, s)	25.7	1.70 (3H, s)	25.7
28	1.04 (3H, s)	24.5	0.98 (3H, s)	27.6	0.94 (3H, s)	27.7
29	1.12 (3H, s)	21.5	0.86 (3H, s)	14.6	0.92 (3H, s)	21.5
30	1.24 (3H, s)	28.4	1.21 (3H, s)	32.1	1.21 (3H, s)	32.1

[a] Assigned by a combination of ^{1}H−^{1}H COSY, ROESY, HSQC, and HMBC experiments.

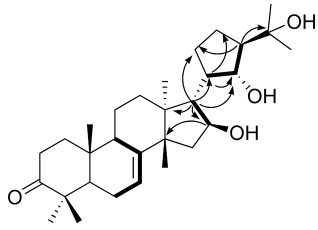

Figure 1. Key ^{1}H−^{1}H COSY (—) and HMBC (H→C) correlations of **1**.

However, the 21-methyl carbon signal discernible for the latter compound was missing and was replaced by a quaternary carbon resonance at δ_C 180.7. This observation, along with the HMBC correlations of this carbonyl signal to H-17, H-20, H-22a, and H-22b, indicated the presence of a C-21 carboxylic acid unit. This was substantiated by 2D NMR (COSY, ROESY, HSQC, and HMBC) experiments (Figure 3). The relative configuration was established by analysis of the ROESY spectrum of **2**. In particular, the ROESY correlations between H-3/CH_3-28 and H-16/CH_3-18 showed that H-3 and H-16 could be assigned as α-oriented. The characteristic splitting pattern of H-3 (dd, 4.14, 11.48) confirmed that the hydroxyl at C-3 is *β*-oriented.[13−15]

The molecular formula of toosendanic acid B (**3**), afforded as an amorphous powder, was shown to be identical with that of **2**, as evidenced from the signals in its HRESIMS at *m/z* 477.3344 [M − H_2O + Na]$^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3345) and 455.3520 [M − H_2O + H]$^+$ (calcd for $C_{30}H_{46}O_3H$, 455.3525). The ^{1}H and ^{13}C NMR data of **3**, when compared with those of compound **2**, revealed that both compounds share the same structure, with the only difference being the configuration at C-3. The proton signal at δ_H 3.47 (1H, t, 2.74, H-3) in the ^{1}H NMR spectrum and the methine carbon signal at δ_C 76.1 in the ^{13}C NMR spectrum were characteristic of an α-hydroxyl at C-3. Thus, compound **3** was assigned as the C-3 epimer of **2**.

Compounds **1**−**3** were evaluated against the three human cancer cell lines HL-60 (promyelocytic leukemia), HT-29 (colon cancer), and MCF-7 (breast cancer), but none were substantially cytotoxic (IC_{50} < 10 *μ*M).

Experimental Section

General Experimental Procedures. Melting points were measured on a SGWX-4 melting instrument and are uncorrected. Optical rotations were determined on a JASCO P1020 digital polarimeter. The CD spectrum was recorded on a JASCO J-810 spectrometer. IR spectra were recorded on a Nicolet Impact 410 spectrometer. NMR spectra were recorded with TMS as internal reference on a Bruker AV-500 spectrometer. ESIMS, EIMS, and HRESIMS were run in the positive-ion mode on an Agilent LC/TOF MS spectrometer, a Shimadzu QP-2010 GCMS spectrometer, and a Mariner ESITOF spectrometer, respectively. All solvents used were of analytical grade (Nanjing Chemical Reagents Company, Ltd.). Silica gel (100−200, 200−300 mesh, Qingdao Haiyang Chemical Co. Ltd.), C_{18} reversed-phase silica gel (150−200 mesh, Merck), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. Preparative HPLC was performed with a Waters 600 instrument and a Waters dual *λ* absorbance 2487 detector, on a 19 mm × 300 mm i.d., 6 *μ*m, Prep Nova-Pak HR C_{18} column

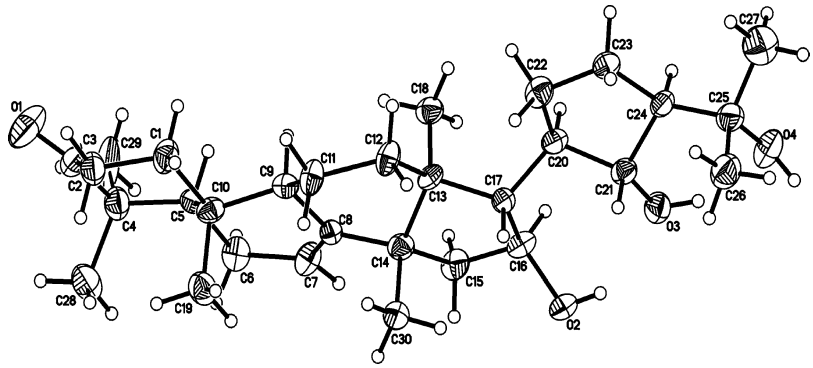

Figure 2. Single-crystal X-ray structural diagram of **1**.

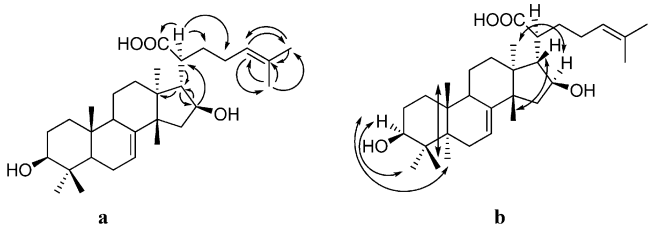

Figure 3. (a) Key HMBC (H→C) correlations of **2**. (b) Selected ROESY correlations of **2**.

(Waters). The flow rate and detected wavelength were adjusted to 15 mL/min and 210 nm, respectively.

Plant Material. The bark of *Melia toosendan* was purchased in Ding town, Kai County, Sichuan Province, People's Republic of China in June 2006. The plant was identified by Professor Jian-Wei Chen of Nanjing University of Traditional Chinese Medicine. A voucher specimen (SIM-060606) was deposited in the botany herbarium at Jiangsu Simcere Pharmaceutical R&D Company, Ltd.

Extraction and Isolation. The air-dried bark (30 kg) was cut into small pieces and extracted with 95% EtOH (30 L × 3) under reflux for 1 week. The solvent was evaporated in vacuo, and the resulting residue (1.9 kg) was suspended in H_2O (2 L). Then, the mixture was partitioned between petroleum ether (600 mL × 3), ethyl acetate (600 mL × 3), and *n*-butanol (600 mL × 3), respectively. The ethyl acetate fraction (250 g) was divided into 11 fractions by chromatography on silica gel (100−200 mesh, 1500 g), eluted with 0−100% ethyl acetate−petroleum ether. The sixth fraction (3.5 g) was again purified by passage over silica gel, eluted with (80:1) $CHCl_3$−CH_3OH, and then further purified by reversed-phase silica gel (90% MeOH−H_2O), as well as Sephadex LH-20 [(1:1) $CHCl_3$−CH_3OH], column chromatography. This fraction was subjected to preparative HPLC to give 29 mg of **1** (t_R 32.0 min when eluted by the isocratic mode with 55% MeCN−H_2O).

The petroleum ether fraction (325 g) was fractionated to 15 fractions using chromatography on silica gel eluted with 0−100% EtOAc−petroleum ether. The fourth fraction (10 g) was subjected to further chromatography on silica gel, with (5:1) petroleum ether−EtOAc as the eluting system. Then, this fraction was purified by reversed-phase ODS and Sephadex LH-20 (1:1 $CHCl_3$−CH_3OH) chromatography. Finally, the fraction was subjected to preparative HPLC to give 7.6 mg of **2** (t_R 14.5 min when eluted by the isocratic mode with 89% MeCN−H_2O) and 38 mg of **3** (t_R 24.0 min when eluted by the isocratic mode with 83% MeCN−H_2O). The second and third fractions (17.4 g) were also eluted with petroleum ether−EtOAc (1:0 and 10:1) as an eluting system, on silica gel chromatography. These were purified by reversed-phase ODS and Sephadex LH-20 (1:1 $CHCl_3$−CH_3OH) chromatography and then subjected to preparative HPLC to give 39 mg of 16-hydroxybutyrospermol[9,12] (t_R 30.0 min when eluted by the isocratic mode with 80% MeCN−H_2O).

Toosendanone A (1): colorless, monoclinic crystals from $CHCl_3$−CH_3OH (1:1); mp 225−226 °C; $[\alpha]^{20}_D$ −31.1 (*c* 0.065, $CHCl_3$); CD (*c* 0.2, $CHCl_3$) 296 nm (−0.59); IR (KBr) ν_{max} 3352, 2962, 1709, 1382, 1165 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 421 (base peak), 295, 163, 107; HRESIMS *m*/*z* 495.3467 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

Toosendanic acid A (2): amorphous powder; $[\alpha]^{16}_D$ −28.9 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 3437, 2951, 1779, 1456, 1384, 1028, 737 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/*z* 477.3335 $[M - H_2O + Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3339).

Toosendanic acid B (3): amorphous powder; $[\alpha]^{16}_D$ −27.5 (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max} 3437, 2950, 1780, 1383, 1028 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 477.3344 $[M - H_2O + Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3345), 455.3520 $[M - H_2O + H]^+$ (calcd for $C_{30}H_{46}O_3H$, 455.3525).

Cytotoxicity Testing. A sulforhodamine B assay (SRB) was used for the cytotoxicity test. The activity of the compounds **1**−**3** was tested at several concentrations against three cultured human tumor cell lines in vitro: HL-60 (promylocytic leukemia), HT-29 (colon cancer), and MCF-7 (breast cancer). Adriamycin was used as a positive control with IC_{50} values of 0.016, 0.33, and 0.12 μM against HL-60, HT-29, and MCF-7 cell lines, respectively.

X-ray Crystallographic Analysis of Toosendanone A (1). Crystals suitable for X-ray structure analysis were obtained by recrystallization in $CHCl_3$−CH_3OH (1:1). The X-ray data were collected on a Nonius CAD4 diffractometer using Mo Kα radiation at room temperature. Crystal data: $C_{30}H_{48}O_4$, $M = 472.68$, monoclinic, space group $P2_1$, $a = 7.6910(15)$ Å, $b = 10.688(2)$ Å, $c = 16.499(3)$ Å, $V = 1335.8(5)$ $Å^3$, $Z = 2$, $T = 293(2)$ K, $D_c = 1.175$ g/cm^3, $\lambda = 0.71073$ Å, $R_1 = 0.0844$ for 2544 observations with $I > 2\sigma(I)$, $R_1 = 0.1295$ ($wR_2 = 0.2631$) for all data.

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Supporting Information Available: Physical and spectroscopic data of **1**−**3** and crystal data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Crystallographic data for toosendanone A (**1**) have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-684135. Copies of the data can be obtained, free of charge, on application to the Director, 12 Union Road,

Cambridge CB2 1EZ, UK (fax +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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