Octanorcucurbitane and Cucurbitane Triterpenoids from the Tubers of *Hemsleya endecaphylla* with HIV-1 Inhibitory Activity

Jian-Chao Chen,[†] Gao-Hong Zhang,[‡] Zhong-Quan Zhang,[†] Ming-Hua Qiu,*[,†] Yong-Tang Zheng,[‡] Liu-Meng Yang,[‡] and Kai-Bei Yu[§]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China, Key Laboratory of Animal Models and Human Disease Mechanisms and Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, People's Republic of China, and Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, Sichuan, People's Republic of China

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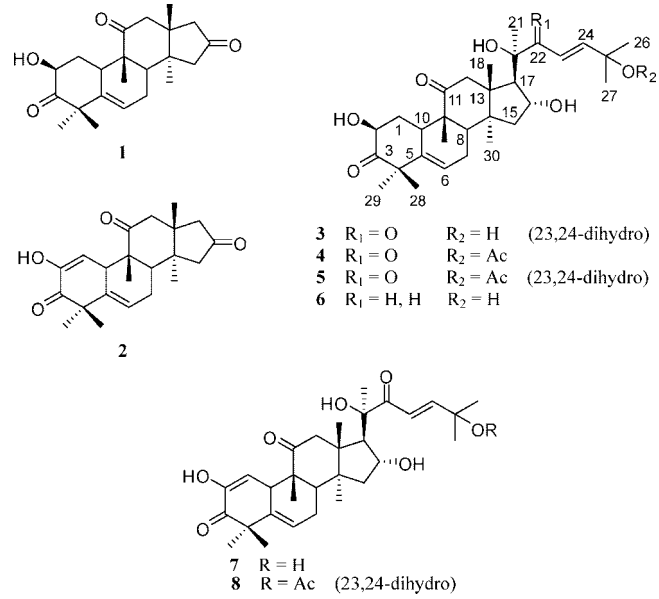
Two new cucurbitacins, endecaphyllacins A (**1**) and B (**2**), together with six known analogues (**3**−**8**), were isolated from the tubers of *Hemsleya endecaphylla*. The structures of **1** and **2** were elucidated by NMR and MS spectroscopic analysis. The relative stereochemistry of **1** was determined by single-crystal X-ray diffraction. Compound **4** (cucurbitacin B) showed potent anti-HIV-1 in C8166 cells (EC = 0.09 μg/mL) with a selectivity index of 16.7.

A number of plants of the genus *Hemsleya* (Cucurbitaceae) grow abundantly in Yunnan and Sichuan Provinces, People's Republic of China, while *H. endecaphylla* C. Y. Wu is a rare plant indigenous to Lijiang prefecture in northwestern Yunnan. The tubers of some *Hemsleya* species have been used for the treatment of a variety of ailments and symptoms, such as fever, pain, and inflammation.[1,2] Up to the present, more than 40 new cucurbitacins and cucurbitane glycosides have been isolated from the genus *Hemsleya*.[3] The compounds hemslecins A (25-acetoxy-23,24-dihydrocucurbitacin F) and B (23,24-dihydrocucurbitacin F), isolated initially from the genus *Hemsleya*, possess antibacterial activities, and the effectiveness of hemslecin A against infectious diseases, such as enteritis, bronchitis, acute tonsillitis, and bacillary dysentery, has been established in clinical trials.[4] Aimed at finding potential bioactive cucurbitacins from this genus, we investigated the tubers of *H. endecaphylla*, which led to the isolation of two new 20,21,22,23,24,25,26,27-octanorcucurbitacins, endecaphyllacins A (**1**) and B (**2**), along with six known cucurbitane derivatives, 23,24-dihydrocucurbitacin D (**3**),[5] cucurbitacin B (**4**),[6] 23,24-dihydrocucurbitacin B (**5**),[7] 22-deoxocucurbitacin D (**6**),[8] cucurbitacin I (**7**),[5] and 22,23-dihydrocucurbitacin E (**8**).[6] Herein, we describe the isolation and structural elucidation of the new octanorcucurbitacins **1** and **2** and the anti-HIV-1 activities of compounds **1**–**8**.



Endecaphyllacin A (**1**) was assigned as $C_{22}H_{30}O_4$ by HRESIMS ($[M + Na]^+$, *m/z* 381.2041) and ^{13}C NMR data. Its IR spectrum showed absorptions for hydroxyl (3484 cm^{-1}) and carbonyl (1725 and 1689 cm^{-1}) groups. The UV spectrum showed no conjugated group based on the absence of absorption from 230 to 350 nm. Obvious signals in the ^1H NMR spectrum were five methyl singlets at δ_H 0.91 (3H, s), 1.14 (3H, s), 1.19 (3H, s), 1.28 (3H, s), and 1.43 (3H, s), as well as three pairs of AB doublets at δ_H 1.93 (1H, d, J = 17.6 Hz) and 2.58 (1H, d, J = 17.6 Hz), δ_H 2.11 (1H, d, J = 17.5 Hz) and 2.28 (1H, d, J = 17.5 Hz), and δ_H 2.31 (1H, d, J = 14.6 Hz) and 3.36 (1H, d, J = 14.6 Hz). The ^{13}C NMR spectrum of **1** displayed resonances for five tertiary methyl groups, five methylenes, four methines, and eight quaternary carbons. Considering the fact that the tetracyclic triterpenoids isolated thus far from the genus *Hemsleya* are cucurbitane-type compounds, compound **1** was tentatively proposed to have a basic skeleton of 20,21,22,23,24,25,26,27-octanorcucurbitacin. Furthermore, on comparison of the NMR data of **1** with those of **3**,[5] it was evident that these two compounds are structurally very similar in rings A−C and that there was one more carbonyl group in ring D and a lack of eight signals of the side chain in compound **1**. Detailed comparison of the ^{13}C NMR data of these two compounds disclosed that a methine group (C-17) at δ_C 57.8 and an oxymethine group (C-16) at δ_C 70.9 in **3** were replaced by a methylene group at δ_C 49.4 and a carbonyl group at δ_C 215.7, respectively, in **1**. The HMBC correlation observed from H-18 (δ_H 0.91, 3H, s) to C-17 (δ_C 49.4, t) confirmed this proposal. The geminal coupling constants of H-17 (J = 17.6 Hz) and H-15 (J = 17.5 Hz) substantiated that the carbonyl group (δ_C 215.7) was located at C-16 (Figure 1). Thus, compound **1** was established as 2β-hydroxy-20,21,22,23,24,25,26,27-octanorcucurbita-5-ene-3,11,16-trione. The single-crystal X-ray crystallographic results of **1** confirmed the proposed structure (Figure 2).

Endecaphyllacin B (**2**) gave the molecular formula $C_{22}H_{28}O_4$, on the basis of the HRESIMS. The ^{13}C NMR spectrum displayed signals for five tertiary methyl groups (δ_C 18.4, 20.0, 20.9, 24.2, and 27.8), four methylenes (δ_C 24.1, 46.3, 49.4, and 50.4), four methines (δ_C 35.2, 42.2, 115.6, and 120.0), and nine quaternary carbons (δ_C 44.5, 45.5, 48.6, 50.6, 137.6, 147.4, 198.8, 211.7, and 215.6). Comparison of the ^1H and ^{13}C NMR spectroscopic data of **2** with those of **1** showed similarities except for an enol structure

* To whom correspondence should be addressed. Tel: +86-871-5223327. Fax: +86-871-5223255. E-mail: mhchiu@mail.kib.ac.cn.
[†] Kunming Institute of Botany.
[‡] Kunming Institute of Zoology.
[§] Chengdu Institute of Organic Chemistry.

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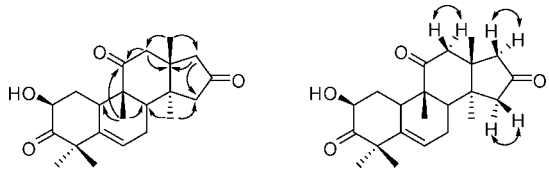

Figure 1. Key HMBC and COSY correlations for **1**.

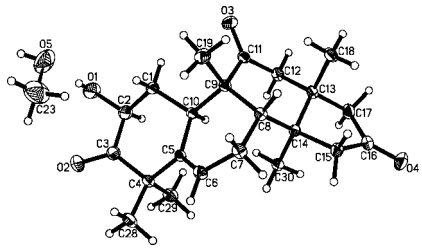

Figure 2. X-ray structure of **1** showing the relative configuration.

in **2** instead of a methylene at C-1 and an oxymethine at C-2 in **1**. The base peak at *m/z* 164 $[C_{10}H_{12}O_2]^+$, due to the loss of rings C and D, by the cleavage of C-7/C-8 and C-9/C-10, implied that ring A of **2** has a Δ^1-2-ol-3-one unit. In the HMBC spectrum of **2**, long-range correlations observed from H-10 (δ_H 3.80, 1H, s) to C-1 (δ_C 115.6, d), and from H-1 (δ_H 6.26, 1H, d, J = 2.4 Hz) to C-3 (δ_C 198.8, s), also supported the presence of this ring A functionality. Therefore, the structure of **2** was elucidated as 2-hydroxy-20,21,22,23,24,25,26,27-octanorcucurbita-1,5-dien-3,11,16-trione. Compound **2** is the aglycon of khekadaengoside L, previously isolated from *Trichosanthes tricuspidata*.[9]

The anti-HIV-1 activities of compounds **1**–**8** were evaluated in preventing the cytopathic effects of HIV-1 in C8166 cells, and cytotoxicity was measured in parallel with the determination of antiviral activity. Compounds **3**, **4**, and **7** exerted significant anti-HIV-1 activity with EC_{50} values of 0.13, 0.09, and 0.70 μg/mL, individually (Table 2). Compounds **4**, **5**, **7**, and **8** have been subjected to evaluation on HIV replication in H9 lymphocyte cells previously, but potent activity was not reported.[6] The present study is the first to show anti-HIV-1 activity of the very common cucurbitane compounds, especially cucurbitacin B (**4**). The biological testing procedure used herein may be particularly susceptible to the anti-HIV-1 activities of cucurbitacin B (**4**) and its analogues.

Experimental Section

General Experimental Procedures. Melting points were measured on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer, and IR spectra on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra experiments were measured in pyridine-d_5 on Bruker AM-400 and (or) DRX-500 instruments, and chemical shifts (δ) were expressed in ppm with reference to the solvent signals. MS data were obtained on a VG Autospec-3000 mass spectrometer. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), silica gel H (10−40 μm, Qingdao Marine Chemical Inc.), or RP-18 gel (40−63 μm, Merck, Darmstadt, Germany). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 15% H_2SO_4 in H_2O.

Plant Material. The tubers were collected from Diandong Village of Yulong County, Yunnan Province, People's Republic of China, in October 2005, and authenticated by Dr. Hongtao Li at the Kunming Institute of Botany. A voucher specimen (No. KIB 2005-10-7) has been 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 air-dried and powdered tubers (72 g) were extracted with methanol (3 × 500 mL) at 60 °C. After removal of the solvent under vacuum, the methanol extract (17 g) was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ part was evaporated to afford 16 g of residue, which was subjected to silica gel column chromatography eluted with $CHCl_3$−MeOH (0:1, 30:1, 20:1) to yield fractions I−III. Fraction II (13 g) was chromatographed over silica gel H, developed with petroleum−EtOAc (from 5:1 to 1:1), to furnish fractions A and B. Compounds **3** (80 mg), **6** (14 mg), and **8** (9 mg) were purified from fraction B (3 g), using petroleum−EtOAc (2:1) as eluent. Fraction A (6 g) was chromatographed on LH-20 eluted with MeOH, and fractions 1 and 2 were obtained. Fration 1 (3 g) was chromatographed repeatedly over silica gel H, developed with petroleum−EtOAc (3:1), to afford compounds **1** (150 mg) and **2** (20 mg). In the same way, compounds **4** (62 mg), **5** (49 mg), and **7** (10 mg) were isolated from fraction 2 (2 g) eluted with petroleum−EtOAc (3:1).

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
1	37.0, CH_2	2.63, 1.58, m	115.6, CH	6.26, d (2.4)
2	72.3, CH	4.84, m	147.4, qC	
3	213.1, qC		198.8, qC	
4	51.0, qC		48.6, qC	
5	141.2, qC		137.6, qC	
6	119.6, CH	6.30, br s	120.0, CH	5.64, t-like (2.5)
7	24.4, CH_2	2.30, 1.82, m	24.1, CH_2	2.29, 1.86, m
8	43.0, CH	2.08, m	42.2, CH	2.14, br s
9	50.2, qC		50.6, qC	
10	34.2, CH	3.13, d (12.7)	35.2, CH	3.80, br s
11	210.9, qC		211.7, qC	
12	46.1, CH_2	3.36, d (14.6)	46.3, CH_2	3.39, d (14.5)
		2.31, d (14.6)		2.31, d (14.5)
13	44.4, qC		44.5, qC	
14	45.3, qC		45.5, qC	
15	50.2, CH_2	2.28, d (17.5)	50.4, CH_2	2.28, d (17.2)
		2.11, d (17.5)		2.12, d (17.2)
16	215.7, qC		215.6, qC	
17	49.4, CH_2	2.58, d (17.6)	49.4, CH_2	2.58, d (17.6)
		1.93, d (17.6)		1.94, d (17.6)
18	24.2, CH_3	0.91, s	24.2, CH_3	0.86, s
19	20.0, CH_3	1.14, s	20.0, CH_3	1.14, s
28	21.8, CH_3	1.43, s	20.9, CH_3	1.44, s
29	29.2, CH_3	1.28, s	27.8, CH_3	1.25, s
30	19.1, CH_3	1.19, s	18.4, CH_3	1.22, s

[a] Spectra were recorded in C_5D_5N; chemical shifts (δ) are in ppm with J values in Hz.

Table 2. Summary of Cytotoxicities and Anti-HIV-1 Activities of Compounds **1**–**8**

compound	anti-HIV-1 activity, EC_{50} (μg/mL)	cytotoxicity, CC_{50} (μg/mL)	selectivity index (SI), CC_{50}/EC_{50}
1	31.6	>200	6.3
2	12.0	62	5.0
3	0.13	6.5	50.0
4	0.09	1.5	16.7
5	4.20	25.5	6.1
6	15.8	120.9	7.7
7	0.70	14.4	20.6
8	2.4	21.6	9.2
AZT	0.0034	>200	>50 000

Endecaphyllacin A (1): colorless prism crystals; mp 207−209 °C; $[\alpha]^{24}_D$ +2.9 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (2.6) nm; IR (KBr) ν_{max} 3484, 2968, 1725, 1689, 1469, 1434, 1208, 978 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* $[M]^+$ 358 (35), 343 (40), 340 (42), 325 (100), 297 (40), 193 (60), 105 (83), 91 (79); HRESIMS *m/z* $[M + Na]^+$ 381.2041 (calcd for $C_{22}H_{30}O_4Na$, 381.2050).

X-ray Crystallographic Data of 1. $C_{23}H_{34}O_5$, MW = 390.50 (in this case, there was a hydrogen bond between CH_3OH and 2β-hydroxy of **1** in the crystal); orthorhombic, space group $P2_12_12_1$; a = 7.9551(1) Å, b = 12.1239(2) Å, c = 21.4220(4) Å, V = 2066.08(6) Å^3, Z = 4, D_{calc} = 1.255 g/cm^3; Mo Kα (λ = 0.71073 Å). The data were collected on a MAC DIP-2030K diffractometer, with graphite-monochromated Mo Kα radiation using a colorless crystal of dimensions of 0.68 × 0.64 × 0.58 mm^3, maximum 2θ value of 54.96°, independent reflections

20 254, observed number of reflections 4733 ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure of **1** was solved by direct methods with SHELXS-86[10] and expanded using different Fourier techniques, refined by the program and method NOMCSDP[11] and full-matrix, least-squares calculations. The final indices were $R = 0.035$, $R_w = 0.103$. Crystallographic data for the structure of **1** has been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 626587). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Endecaphyllacin B (2): colorless prism crystals; mp 229−231 °C; $[\alpha]^{24}_D$ −17.1 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (2.4), 328 (1.8) nm; IR (KBr) ν_{max} 3450, 2970, 1738, 1695, 1658, 1409, 1228, 1048 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* $[M]^+$ 356 (20), 341 (8), 299 (10), 228 (14), 189 (27), 164 (100), 136 (36), 122 (61), 121 (37); HRESIMS *m/z* $[M + Na]^+$ 379.1887 (calcd for $C_{22}H_{28}O_4Na$, 379.1885).

Anti-HIV-1 Assay. Each tested compound was dissolved in DMSO to a concentration of 25 mg/mL and diluted to the required concentrations with the medium. Cytotoxicity was measured by the MTT method as described previously.[12] Briefly, C8166 cells were seeded in the absence or presence of various concentrations of compounds in triplicate for 3−7 days. The percentage of viable cells was quantified at 595/630 nm ($A_{595/630}$) in an ELISA reader. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from a dose–response curve.

The cytopathic effect in the same cell lines was measured by counting the number of syncytia (multinucleated giant cells) in each well under an inverted microscope.[13] Different concentrations of compound were added in a 96-well microtiter plate. C8166 cells were seeded and inoculated with 100 TCID50 HIV-1 and then incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 72 h. Control assays were performed without the test compounds in HIV-1-infected and uninfected cultures. AZT was used as a positive control. The number of syncytia in each well was counted under an inverted microscope. The percentage inhibition of syncytial cell formation was calculated by percentage of syncytial cell numbers in compound-treated cultures to that of infected control culture. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC_{50}) was determined from the dose−response curve. The selectivity index (SI) was calculated from the ratio of CC_{50}/EC_{50}.

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