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A NEW *N*-CONTAINING CUCURBITACIN FROM *Hemsleya endecaphylla*

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A new cucurbitacin, endecaphyllacin C, was isolated from the tubers of Hemsleya endecaphylla*. The structure was elucidated as 2β,16α,20β,25-tetrahydroxy-24-acetylaminocucurbita-5-en-3,11,22-trione (**1**) on the basis of extensive 1D and 2D NMR techniques, including COSY, HMBC, HMQC, and NOESY correlations, as well as HR-FAB-MS analysis.*

Keywords: *Hemsleya endecaphylla*, tubers, cucurbitacin, anti-HIV-1.

Hemsleya spp. (Cucurbitaceae) are mainly distributed in the southwest of China, especially in Yunnan and Sichuan Provinces, 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, by local people. The tubers of *H. amabilis* and the representative constituents, hemslecin A (25-acetoxy-23,24-dihydrocucurbitacin F) and hemslecin B (23,24-dihydrocucurbitacin F), firstly isolated from *H. amabilis* [1], have been included in the *Pharmacopoeia of China* in 1977 as antibacterial medicines. To date, nearly 50 new cucurbitacins and cucurbitane glycosides have been isolated from the genus *Hemsleya* [2–12]. In a preceding paper, we had reported the isolation and structural elucidation of two octanorcucurbitacins and their anti-HIV-1 activities [10]. In continuation of this study, we now describe the isolation and structural elucidation of a new *N*-containing cucurbitacin, endecaphyllacin C, obtained from the tubers of *H. endecaphylla* C. Y. Wu.

Compound **1** was obtained as a white powder, mp 190–192°C, $[\alpha]_D^{20}$ +3.5° (*c* 0.31, MeOH). The absorption bands of its IR spectrum suggested the presence of hydroxyl and/or amino (3447 cm^{-1}) and carbonyl (1714 and 1693 cm^{-1}) groups. The positive FAB-MS exhibited an $[M + H]^+$ ion at *m/z* 576. The molecular formula was established as $C_{32}H_{50}NO_8$ by HR-ESI-MS at *m/z* $[M + 1]^+$ 576.3529 (calcd 576.3536 for $C_{32}H_{50}NO_8$). The ^1H NMR spectrum of **1** displayed one amino signal at δ 8.44 (1H, d, J = 8.8 Hz), eight methyl singlets at δ 1.14, 1.08, 1.70, 1.55, 1.54 ($H_3 \times 2$), 1.44, and 1.31, an *N*-acetyl methyl group at δ 2.16, a doublet attributed to a trisubstituted double bond at δ 5.69 (1H, m), and two doublets assignable to an isolated methylene at δ 2.84 (1H, d, J = 14.7 Hz) and 3.35 (1H, d, J = 14.7 Hz), in addition to three methine signals at δ 4.99 (1H, m), 5.05 (1H, m), and 5.02 (1H, m) in the lower field region. The ^{13}C NMR spectrum of **1** showed 32 carbon signals, and the DEPT experiment differentiated them to be nine methyl groups, five methylenes, seven methines, and eleven quaternary carbons. Careful analysis of the NMR data indicated that it is a tetracyclic triterpenoid, which would be a cucurbitacin based on the fact that the compounds isolated thus far from the genus *Hemsleya* are cucurbitane-type compounds. So, compound **1** was tentatively proposed to have a basic skeleton of cucurbitacin. Furthermore, on comparison of the NMR data of **1** with those of 23,24-dihydrocucurbitacin D [13], it was evident that these two compounds are structurally very similar in rings A–D exception of the obvious different chemical shifts at C-23, C-24, and C-25. In the ^{13}C NMR spectrum of **1**, the resonances of C-23, C-24, and C-25 were shifted downfield nearly by 8, 18, and 3 ppm, respectively, which suggested a strong electron-withdrawing group linked at C-24.

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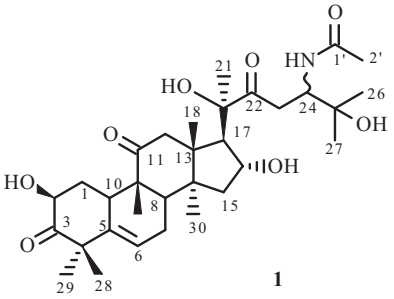
TABLE 1. ^{1}H and ^{13}C NMR Data for Compound **1**

C atom	δ_C	δ_H, J/Hz	C atom	δ_C	δ_H, J/Hz
1	37.0 t	1.59 (1H, overlap);	16	70.9 d	5.05 (1H, m)
		2.61 (1H, m)	17	58.9 d	3.02 (1H, d, J = 7.0)
2	72.5 d	4.99 (1H, m)	18	20.7 q	1.14 (3H, s)
3	213.3 s		19	20.4 q	1.08 (3H, s)
4	51.0 s		20	80.4 s	
5	141.4 s		21	26.0 q	1.70 (3H, s)
6	120.3 d	5.69 (1H, m)	22	214.2 s	
7	24.5 t	1.90 (1H, overlap);	23	38.3 t	3.93 (2H, m)
		2.27 (1H, m)	24	55.1 d	5.02 (1H, m)
8	42.9 d	1.91 (1H, overlap)	25	73.3 s	
9	48.9 s		26	28.5 q	1.55 (3H, s)
10	34.3 d	3.09 (1H, t-like, J = 12.2)	27	26.6 q	1.54 (3H, s)
11	212.9 s		28	22.2 q	1.44 (3H, s)
12	49.4 t	2.84 (1H, d, J = 14.7);	29	29.8 q	1.31 (3H, s)
		3.35 (1H, d, J = 14.7)	30	19.4	1.54 (3H, s)
13	51.2 s		NHCOMe	170.7 s	
14	48.7 s		NHCOMe	23.7 q	2.16 (3H, s)
15	46.4 t	1.72 (1H, overlap);	NHCOMe		8.44 (1H, d, J = 8.8)
		1.91 (1H, overlap)			

The electron-withdrawing group was deduced as an acetamide on the basis of the signals at δ_H 8.44 (1H, d, J = 8.8 Hz) and δ_H 2.16 (3H, s) in the ^{1}H NMR spectrum and at δ_C 170.7 (s) and 23.7 (q) in the DEPT spectrum. This suggestion was confirmed by the observation of HMBC correlations from H-21 (δ_H 1.70, 3H, s) to C-17 (58.9, d), C-20 (δ_C 80.4, s), and C-22 (δ_C 214.2, s), from H-24 (δ_H 5.02, 1H, m) to C-22 (δ_C 214.2, s), C-23 (δ_C 38.3, t), C-25 (δ_C 73.3, s), C-26 (δ_C 28.5, q), and C-27 (δ_C 26.6, q), and from NH (δ_H 8.44, 1H, d, J = 8.8 Hz) to C-24 (δ_C 55.1, d), C′-1 (δ_C 170.7, s), and C′-2 (δ_C 23.7, q). Cucurbitacins possess the biogenetically 19-(10→9β)-abeo-10α-lanost-5-ene (also known as 9β-methyl-19-norlanosta-5-ene) skeleton [14]. The NOESY correlations of H-8 to H-18 and H-19, of H-10 to H-29 and H-30, and of H-17 to H-30 established the stereochemistry of H-8β, H-29α, H-30α, and H-17α. Thus, compound **1** was identified as 2β,16α,20β,25-tetrahydroxy-24-acetylaminocucurbita-5-en-3,11,22-trione, named endecaphyllacin C. To date, nearly 300 cucurbitane compounds have been obtained from plants of different families, even from several mushroom species. To the best of our knowledge, this is the third report of *N*-containing cucurbitane compounds [15, 16] and also the first example of a cucurbitacin containing an *N*-acetyl group.



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The anti-HIV-1 activities of compound **1** in preventing the cytopathic effects of HIV-1 IIIB in C8166 were evaluated using the MTT method as described in the literature [10], and the cytotoxicity was measured in parallel with the determination of antiviral activity using AZT as a positive control. Compound **1** showed no anti-HIV-1 activity, with an EC_{50} value of more than 200 μg/mL.

EXPERIMENTAL

General Experimental Procedures. Melting point was measured on an X-4 micro-melting point apparatus and is uncorrected. Optical rotation was measured with a Horiba SEPA-300 polarimeter, and IR spectra on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were measured in pyridine-d_5 on DRX-500 instruments, and chemical

shifts (δ) are 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 Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered tubers of *H. endecaphylla* (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 (1:0, 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. Fraction A (6 g) was chromatographed repeatedly over silica gel H, developed with petroleum–EtOAc (3:1), to afford compound **1** (8 mg).

Bioassays. The anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), and cytotoxicity assay against C8166 cells (IC_{50}) was assessed using the MTT method as described in the literature [10].

Endecaphyllacin C (1). White powder, $[\alpha]_D^{20}$ +3.5° (*c* 0.31, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3447, 2977, 2931, 1714, 1693, 1657, 1551, 1464, 1375. EI-MS (*m/z*, I_{rel}, %): 540 (5), 236 (10), 194 (25), 170 (35), 154 (30), 127 (100), 112 (60). Positive-ion FAB-MS (*m/z*, I_{rel}, %): 576 $[M + 1]^+$ (2), 558 (2), 540 (5), 80 (100). Positive-ion HR-FAB-MS *m/z*: 576.3529 $[M + 1]^+$ (calcd for $C_{32}H_{50}NO_8$, 576.3536). For ^1H NMR (500 MHz, pyridine-d_5, δ, ppm, J/Hz) and ^{13}C NMR (125 MHz, pyridine-d_5, δ, ppm) data, see Table 1.

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