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Two Novel Glycosidic Triterpene Alkaloids from the Stem Barks of *Machilus yaoshansis*

Ming-Tao Liu, Sheng Lin, Ying-Hong Wang, Wen-Yi He, Shuai Li, Su-Juan Wang, Yong-Chun Yang, and Jian-Gong Shi*

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education), Beijing 100050, People's Republic of China

shijg@imm.ac.cn

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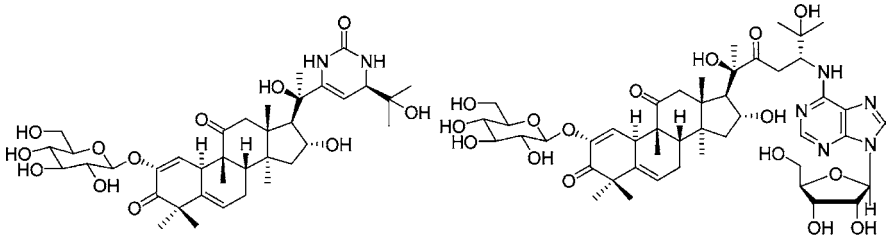
ABSTRACT



machilaminoside A (1) **machilaminoside B (2)**

Two unusual glycosidic triterpene alkaloids, machilaminosides A (1) and B (2), have been isolated from the stem barks of *Machilus yaoshansis*. Their structures were elucidated by detailed spectroscopic analysis. A possible biogenetic origin of 1 and 2 mediated by the coupling of 2-*O*-β-D-glucopyranosyl-cucurbitacin I, respectively, with urea and adenosine was postulated. 1 and 2 showed nonselective cytotoxic activities against several human cancer cell lines as well as TNF-α secretion inhibitory activities.

Species of the genus *Machilus* (Lauraceae) are sources of secondary metabolites with interesting chemical structures and significant bioactivities.[1] Several plants of this genus have long been used for the treatment of various diseases including edema, abdominal distension, pain, and inflammation in China.[2] As part of a program to assess the chemical and biological diversity of several traditional Chinese medicines,[3] we carried out the investigation of the stem barks of *Machilus yaoshansis* that is widely distributed in southern China. From the H_2O-soluble portion of the EtOH extract of this material, two unusual glycosidic triterpene alkaloids with cytotoxic and TNF-α inhibitory activities, designated as machilaminosides A (**1**) and B (**2**), have been isolated. We report herein the isolation, structural elucidation, postulated biogenetic formation, and biological activity of **1** and **2**. To our knowledge, this is the first isolation of glycosidic triterpene alkaloids derived from cucurbitane derivatives.

The stem bark of *Machilus yaoshansis* (5 kg) was collected in the Dayao mountain, Guangxi province, China. The air-dried plant material was extracted with 95% EtOH at room temperature. The residue (320 g) obtained by concentrating the EtOH extract in vacuo was partitioned between H_2O and EtOAc, and then the H_2O phase was chromatographed by a

* To whom correspondence should be addressed. Tel: 86-10-83154789. Fax: 86-10-63017757.

(1) For some examples, see: (a) Giang, P. M.; Son, P. T.; Matsunami, K.; Otsuka, H. *Chem. Pharm. Bull.* **2006**, *54*, 308. (b) Cheng, M.-J.; Tsai, I.-L.; Lee, S.-J.; Jayaprakasam, B.; Chen, I.-S. *Phytochemistry* **2005**, *66*, 1180. (c) Park, E. Y.; Shin, S. M.; Ma, C. J.; Kim, Y. C.; Kim, S. G. *Planta Med.* **2005**, *71*, 393.

(2) *Jiangsu New Medical College Dictionary of Traditional Chinese Medicine*; Shanghai Science and Technology Publishing House: Shanghai, 1977; pp 114, 1009, and 1423.

(3) (a) Wang, Y.; Wang, S. J.; Mo, S. Y.; Li, S.; Yang, Y. C.; Shi, J. G. *Org. Lett.* **2005**, *7*, 4733. (b) Xue, Z.; Li, S.; Wang, S. J.; Wang, Y. H.; Yang, Y. C. *J. Nat. Prod.* **2006**, *69*, 907.

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macroporous adsorbent resin (HPD-100) column eluted successively with H_2O, 30% EtOH, 70% EtOH, and 95% EtOH to yield four corresponding fractions (A_1-A_4). The fraction A_3 (5.8 g) showing cytotoxic (IC_{50} < 15 μg/mL) and TNF-α inhibitory (86% inhibition at 10 μg/mL) activities was fractionated via reversed-phase medium-pressure liquid chromatography (C-18) by eluting with a gradient of increasing MeOH (0−100) in H_2O to give three major fractions (A_3-1, A_3-2, and A_3-3). The fraction A_3-2 (1.6 g) with cytotoxic (IC_{50} < 5 μg/mL) and TNF-α inhibitory (96% inhibition at 2.5 μg/mL) activities was further chromatographed over Sephadex LH-20 with $CHCl_3$−MeOH (1:1) as eluent to afford four fractions (A_3-2-1−A_3-2-4). The fraction A_3-2-4 was further purified by reversed-phase semipreparative HPLC by using MeOH−H_2O (50:50) as the mobile phase to afford compounds **1** (4.5 mg) and **2** (7.2 mg) (Figure 1).

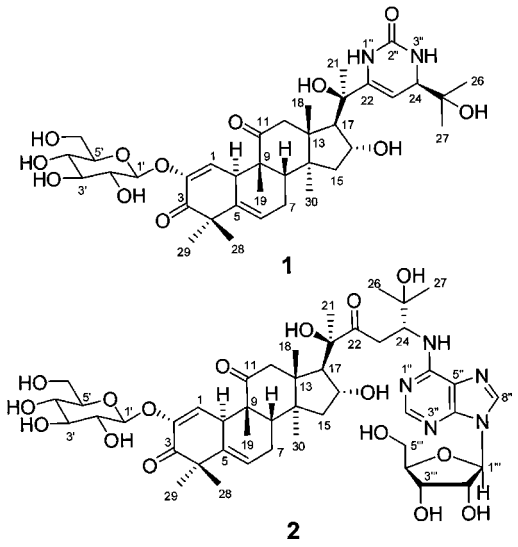


Figure 1. Structures of machilaminosides A (**1**) and B (**2**).

Compound **1** was obtained as a white amorphous powder, $[\alpha]^{20}_D$ −48.8 (*c* 0.06, MeOH). The absorption bands of its IR spectrum suggested the presence of a hydroxyl and/or an amino (3383 cm^{-1}) and conjugated carbonyl (1685 and 1666 cm^{-1}) functional groups. The negative and positive ESIMSs of **1** exhibited quasimolecular ion peaks at *m*/*z* 717.0 $[M - H]^-$ and 741.1 $[M + Na]^+$, respectively. The molecular formula $C_{37}H_{54}N_2O_{12}$ was indicated by the positive HR-ESIMS at *m*/*z* 741.3588 $[M + Na]^+$ (calcd 741.3574 for $C_{37}H_{54}N_2O_{12}Na$). The ^1H NMR spectrum of **1** in DMSO-d_6 showed two exchangeable amino signals at δ 7.66 (1H, brs, N*H*-1″) and 6.34 (1H, brs, N*H*-3″) and seven exchangeable hydroxy signals at δ 5.21 (1H, d, J = 5.1 Hz, O*H*-2′), 5.05 (1H, d, J = 3.9 Hz, O*H*-3′), 4.92 (1H, d, J = 5.1 Hz, O*H*-4′), 4.67 (1H, s, O*H*-20), 4.63 (1H, s, O*H*-25), 4.38 (1H, d, J = 4.2 Hz, O*H*-16), and 4.37 (1H, t, J = 5.4 Hz, O*H*-6′). In the higher field region, the ^1H NMR spectrum of **1** in DMSO-d_6 + D_2O displayed eight methyl singlets at δ 0.78 (H_3-18), 0.83 (H_3-19), 1.00 (H_3-26), 1.01 (H_3-27), 1.14 (H_3-28), 1.15 (H_3-29), 1.27 (H_3-30), and 1.34 (H_3-21) and several partially overlapped multiplets attributed to methylenes and methines between δ 1.25 and 2.40 ppm. Meanwhile, in the relative lower field region, it showed two broadened singlets and a doublet attributed to three trisubstituted double bonds at δ 5.82 (1H, br s, H-1), 5.72 (1H, br s, H-6), and 4.75 (1H, d, J = 5.0 Hz, H-23), two doublets assignable to an isolated methylene at δ 2.35 (1H, d, J = 15.0 Hz, H-12β) and 3.24 (1H, d, J = 15.0 Hz, H-12α), and three methine signals at δ 4.35 (1H, dd, J = 7.0 and 6.0 Hz, H-16), 3.51 (1H, br s, H-10), and 3.54 (1H, d, J = 5.0 Hz, H-24), in addition to characteristic signals due to a β-D-glucopyranosyl moiety (Table 1).[4] The presence of the β-D-glucopyranosyl moiety was supported by the ^{13}C NMR data (Table 2) and further confirmed by the acidic hydrolysis of **1** in 2 N HCl at 80 °C for 6 h, which resulted in a release of glucose identified by PC and TLC comparisons of the hydrolyzate with the authentic sugar sample. The ^{13}C NMR spectrum of **1** showed 37 carbon signals, and the DEPT experiment differentiated them to be 8 × CH_3, 4 × CH_2, 13 × CH, and 12 × C. (Table 2). On the basis of the chemical shift values, the 12 quaternary carbons were assigned to be two carbonyls, four sp^2 carbons (three oxygen- and/or nitrogen-bearing, δ > 145 ppm), and six sp^3 carbons (two oxygen-bearing, δ >

Table 1. ^1H NMR Data for Machilaminosides A (**1**) and B (**2**)[a]

	1	**2**		**1**	**2**
no.	δ_H, mult.	δ_H, mult.	no.	δ_H, mult.	δ_H, mult.
1	5.82 s	5.80 brd	27	1.01 s	1.11 s
6	5.72 brs	5.71 m	28	1.14 s	1.15 s
7α	1.94 d	1.93 d	29	1.15 s	1.15 s
7β	2.26 dd	2.28 dd	30	1.27 s	1.25 s
8	1.91 d	1.90 d	1′	4.55 d	4.54 d
10	3.51 brs	3.58 brs	2′	3.14 dd	3.13 dd
12α	3.23 d	3.34 d	3′	3.22 dd	3.20 dd
12β	2.35 d	2.40 d	4′	3.28 t	3.27 t
15α	1.28 d	1.28 d	5′	3.12 m	3.09 m
15β	1.72 dd	1.67 dd	6′a	3.61 brd	3.60 brd
16	4.35 dd	4.31 dd	6′b	3.72 brd	3.68 brd
17	2.31 d	2.30 d	2″		8.17 s
18	0.78 s	0.75 s	8″		8.29 s
19	0.83 s	0.83 s	1‴		5.84 d
21	1.34 s	1.19 s	2‴		4.60 dd
23a	4.75 d	2.97 dd	3‴		4.11 dd
23b		3.18 dd	4‴		3.95 m
24	3.54 d	4.76 t	5‴a		3.52 brd
26	1.00 s	1.07 s	5‴b		3.61 brd

[a] Data were recorded in DMSO-d_6 + D_2O at 500 MHz for the proton. $J_{1,10} \approx 0.0$, $J_{7\alpha,7\beta} = 15.5$, $J_{7\alpha,8} = 7.5$, $J_{7\beta,8} \approx 0.0$, $J_{12\alpha,12\beta} = 15.0$, $J_{15\alpha,15\beta} = 12.5$, $J_{15\alpha,16} = 7.0$, $J_{15\beta,16} \approx 0.0$, $J_{16,17} = 6.0$, $J_{1',2'} = 8.0$, $J_{2',3'} = 8.0$, $J_{3',4'} = 9.0$, $J_{4',5'} = 9.0$, $J_{5',6'a} \approx 3.5$, $J_{5',6'b} \approx 1.0$, $J_{6'a,6'b} = 12.0$ Hz, for both **1** and **2**; $J_{23,24} = 5.0$ Hz for **1**; $J_{23a,23b} = 16.5$, $J_{23a,24} = 10.0$, $J_{1''',2'''} = 6.5$, $J_{2''',3'''} = 3.0$, $J_{3''',4'''} = 4.5$, $J_{4''',5'''a} = 2.5$, $J_{4''',5'''b} = 2.0$, $J_{5'''a,\ 5'''b} = 9.5$ Hz for **2**.

(4) The configuration of the glucopyranosyl was assigned as β-D- on the basis of the coupling constant of the anomeric proton and of the abundance of the β-D-glucopyranosyl unit in natural products.

Table 2. ^{13}C NMR Data for Machilaminosides A (**1**) and B (**2**)[a]

	1	**2**		**1**	**2**
no.	δ_C, mult.	δ_C, mult.	no.	δ_C, mult.	δ_C, mult.
1	120.5 d	120.6 d	24	59.8 d	53.8 d
2	145.3 s	145.1 s	25	72.7 s	72.1 s
3	196.0 s	196.1 s	26	25.3 q	26.5 q
4	48.6 s	48.7 s	27	25.4 q	27.6 q
5	136.1 s	136.3 s	28	20.3 q	20.4 q
6	120.3 d	120.0 d	29	26.9 q	27.1 q
7	23.3 t	23.3 t	30	17.5 q	17.7 q
8	41.0 d	41.0 d	1′	98.6 d	98.7 d
9	48.1 s	48.2 s	2′	72.8 d	72.7 d
10	34.6 d	34.3 d	3′	76.8 d	76.9 d
11	214.1 s	213.8 s	4′	68.9 d	68.9 d
12	48.7 t	49.1 t	5′	76.9 d	77.0 d
13	50.0 s	50.0 s	6′	60.0 t	60.0 t
14	47.5 s	47.6 s	2″	154.2 s	152.1 d
15	44.9 t	45.7 t	4″		148.2 s
16	70.1 d	69.1 d	5″		119.6 s
17	56.6 d	57.8 d	6″		154.9 s
18	19.8 q	19.9 q	8″		139.9 d
19	19.8 q	19.7 q	1‴		88.0 d
20	72.3 s	79.1 s	2‴		73.3 d
21	25.9 q	25.0 q	3‴		70.8 d
22	145.1 s	213.3 s	4‴		86.0 d
23	90.2 d	38.0 t	5‴		61.8 t

[a] Data were recorded in DMSO-d_6 at 125 MHz, and the multiplicity was determined by the DEPT experiment.

70 ppm). All above spectroscopic data suggested that **1** is a highly oxygenated unusual glycosidic triterpene alkaloid.

The structure of **1** was finally established by a careful analysis of its 2D NMR spectroscopic data. The proton and protonated carbon signals in the NMR spectra of **1** were unambiguously assigned by the HMQC experiment. In the $^1H-^1H$ COSY spectrum in DMSO-d_6, homonuclear coupling correlations between H-1 and H-10, from H-6−H_2-7 to H-8, from H_2-15−H-16 to H-17, from H-23−H-24 to N*H*-3″, and from H-1′−H-5′ to H_2-6′, as well as correlations of the hydroxyl protons with their vicinal protons, indicated unambiguously the presence of partial structural units in **1** (Figure 2, thick line units). In the HMBC spectrum of **1** in DMSO-d_6, a series of two- and three-bond correlations from

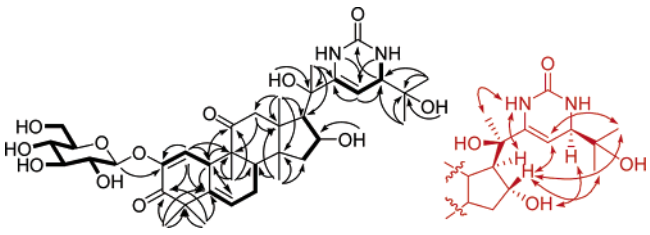


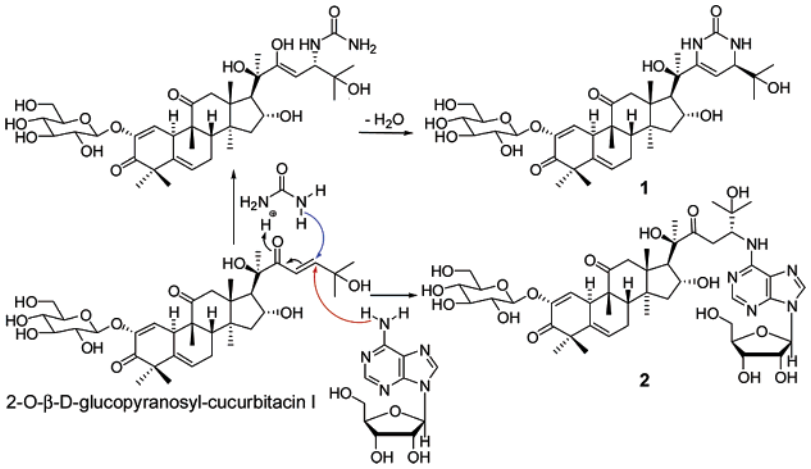
Figure 2. Main $^1H-^1H$ COSY (thick lines), HMBC (arrows), and NOESY (double arrows in the red fragment) correlations of machilaminosides A (**1**).

H-1 to C-2, C-3, C-5, and C-9, from both H_3-28 and H_3-29 to C-3, C-4, and C-5, from H_3-19 to C-8, C-9, C-10, and C-11, from H-10 and H_2-12 to C-11, from H_3-30 to C-8, C-13, C-14, and C-15, from H_3-18 to C-12, C-13, C-14, and C-17, from H_3-21 to C-17, C-20, and C-22, from both H_3-26 and H_3-27 to C-24 and C-25, from H-23 to C-24, and from H-24 to C-22 and C-23 (Figure 2, arrows), in combination with chemical shift values of these protons and carbons, revealed unequivocally a 9-methyl-19-norlanosta-1,5,22-trien-3,11-dione nucleus for **1**. Meanwhile, HMBC correlations of C-16, C-20, and C-25 with respective hydroxyl protons, together with the chemical shift values of these carbons, located a hydroxyl group at each of the three carbons, respectively. In addition, HMBC correlations from both N*H*-1″ and N*H*-3″ to C-23 and from H-24 to the remaining quaternary carbon at δ 154.2 (C-2″), together with the molecular composition of **1**, indicated unequivocally the presence of an ureido unit coupling through N and N′ with C-22 and C-24 to form a 3,4-dihydro-1*H*-pyrimidin-2-one ring in the side chain of the nucleus. This was further confirmed by long-range W-type homonuclear coupling correlations among N*H*-1″, N*H*-3″, and H-23 in the $^1H-^1H$ COSY spectrum of **1** in DMSO-d_6. Furthermore, a HMBC correlation from H-1′ to C-2 indicated that the β-D-glucopyranosyl moiety was located at C-2 of the nucleus. Therefore, **1** was deduced to be an unusual 2-*O*-β-D-glucopyranosyl-9-methyl-19-norlanosta-1,5,22-trien-3,11-dione triterpene alkaloid with a 3,4-dihydro-1*H*-pyrimidin-2-one ring in the side chain.

The stereochemistry of **1** was elucidated from its NOESY and CD spectroscopic data. NOE correlations between H-1 and H_3-19, between H-7β with H-15β and H_3-19, between H-8 with H-12β, H-16, H_3-18, and H_3-19, and between H-16 with H-15β and H_3-18 indicated that these protons are oriented on the same side of the nucleus, whereas NOE correlations between H-10 with H_3-29 and H_3-30 and between both H-12α and H-17 and H_3-30 revealed that they are oriented on another side of the ring system. These data suggested that the relative stereochemistry of the tetracyclic ring system and the chiral center at C-20 of **1** is identical to those of cucurbitane derivatives[5] with the 9-methyl-19-norlanosta-1,5-diene nucleus. On the basis of the octant rule for the cyclohexenone,[6] in the CD spectrum of **1**, the negative Cotton effect at 331 nm ($\Delta\epsilon_{max}$ −1.25) for $n\rightarrow\pi^*$ suggested a 10*R* configuration for **1**, identical to that of cucurbitane derivatives.[7] In addition, NOE correlations between N*H*-1′ and both H-17 and H_3-21 suggested that **1** possessed a major conformation of which the 3,4-dihydro-1*H*-pyrimidin-2-one ring is perpendicular to the plane consisting of C-17, C-20, and C-21, in the solution state. Meanwhile, NOE correlations between OH-16 and H-24 and between H-16 and H-23 which in turn correlated to H_3-26 and H_3-27 (Figure 2, red fragment) suggested a 24*R* configuration for **1**. Accordingly, the

(5) Chen, J. C.; Chiu, M. H.; Nie, R. L.; Cordell, G. A.; Qiu, S. X. *Nat. Prod. Rep.* **2005**, *22*, 386.
(6) (a) Ye, X. L. *Stereochemistry*; Beijing University Express: Beijing, 1999; pp 257−259. (b) Snatzke, G. *Tetrahedron* **1965**, *21*, 413.
(7) Lavie, D.; Shvo, Y.; Gottlieb, O. R.; Glotter, E. *J. Org. Chem.* **1963**, *28*, 1790.

Scheme 1. Proposed Biogenesis of Machilaminosides A (**1**) and B (**2**)



structure of **1** was determined as (−)-(16*R*,20*R*,24*R*)-16,-20,25-trihydroxy-22*N*,24*N*′-ureido-19-(10→9*β*)-abeo-10α-norlanosta-1,5,22-trien-3,11-dione 2-*O*-*β*-D-glucopyranoside, named as machilaminoside A.

Compound **2** was obtained as a white amorphous powder (MeOH), $[\alpha]^{20}_{D}$ −90.3 (*c* 0.06, MeOH). The positive ESIMS exhibited $[M + H]^+$ at *m*/*z* 944.4. The molecular formula $C_{46}H_{65}N_5O_{16}$ was established by HR-ESIMS at *m*/*z* 944.4514 $[M + H]^+$ (calcd 944.4505 for $C_{46}H_{66}N_5O_{16}$). The IR and NMR spectral features of **2** were similar to those of **1**, except for that the NMR signals due to the ureido unit of **1** were replaced by signals attributable to an adenosine moiety in the NMR spectra of **2** in DMSO-d_6 + D_2O (Tables 1 and 2). In addition, the signals due to the double bond between C-22 and C-23 of **1** disappeared in the NMR spectra of **2**; instead, resonances were attributed to a carbonyl carbon at δ_C 213.3 (C-22), a methylene at δ_H 2.97 (1H, dd, J = 16.5 and 9.5 Hz, H-23a) and 3.18 (1H, d, J = 16.5 Hz, H-23b), and δ_C 38.0 (C-23). Meanwhile, C-20 of **2** was downfield shifted by $\Delta\delta_C$ 6.8 ppm, and C-24 of **2** was upfield shifted by $\Delta\delta_C$ 6.0 ppm, compared to those of **1**. These data indicated that **2** is another unusual cucurbitane triterpene alkaloid with an adenosine moiety coupling through its amino group with C-24 of 2-*O*-*β*-D-glucopyranosyl-cucurbitacin L.[8] This was confirmed by 2D NMR experiments of **2** which enabled the assignment of the NMR data of **2** (Tables 1 and 2). In the HMBC spectrum of **2**, a long-range correlation from H-24 to C-6″, along with chemical shifts of C-24, revealed that the amino group at C-6″ of the adenosine moiety was connected to C-24 of the triterpene nucleus in **2**. This was confirmed by a strong homonuclear vicinal coupling correlation between N*H*-6″ and H-24 in the ^{1}H−^{1}H COSY of **2** in DMSO-d_6. Though the NOESY spectrum of **2** did not give any useful information for the assignment of the C-24 configuration, a postulated biogenetic formation of **1** and **2** catalyzed by the same enzyme suggested a 24*R* configuration for **2**. From a biogenetic point of view, compounds **1** and **2** may be biosynthesized from enzyme-catalyzed coupling of a molecule of 2-*O*-*β*-D-glucopyranosyl-cucurbitacin I[8] with a molecule of urea or adenosine (Scheme 1), respectively. Therefore, the structure of **2** was determined as (−)-(16*R*,20*R*,24*R*)-24*N*-(adenosine-6-amino)-16,20,25-trihydroxy-19-(10→9*β*)-abeo-10α-norlanosta-1,5-dien-3,11-dione 2-*O*-*β*-D-glucopyranoside, named as machilaminoside B.

In the in vitro bioactive assays, machilaminosides A (**1**) and B (**2**) showed nonselective cytotoxic activities toward several human cancer cell lines including the human ovary cancer cell line (A 2780), the colon cancer cell line (HCT-8), the hepatoma cell line (Bel-7402), the stomach cancer cell line (BGC-823), and the lung cancer cell line (A549) with IC_{50} values of 0.3−0.8 μM. They also showed TNF-α secretion inhibitory activities of mouse peritoneal macrophages with IC_{50} values of 0.5 and 0.1 μM, respectively.

Though more than 200 cucurbitane triterpenoids with a variety of biological activities, either nonglycosylated or glycosylated, have been isolated from plants of different families, as well as from several genera of mushroom,[5,9] this is the first report not only of glycosidic triterpene alkaloids derived from cucurbitane derivatives but also of cucurbitane derivatives from the family Lauraceae.

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Supporting Information Available: MS, HRMS, IR, 1D, and 2D NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Hatam, N. A. R.; Whiting, D. A.; Yousif, N. J. *Phytochemistry* **1989**, *28*, 1268.

(9) Seer, C.; Sturm, S.; Haslinger, E.; Stuppner, H. *Org. Lett.* **2004**, *6*, 633.