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Machilusides A and B: Structurally Unprecedented Homocucurbitane Glycosides from the Stem Bark of *Machilus yaoshansis*

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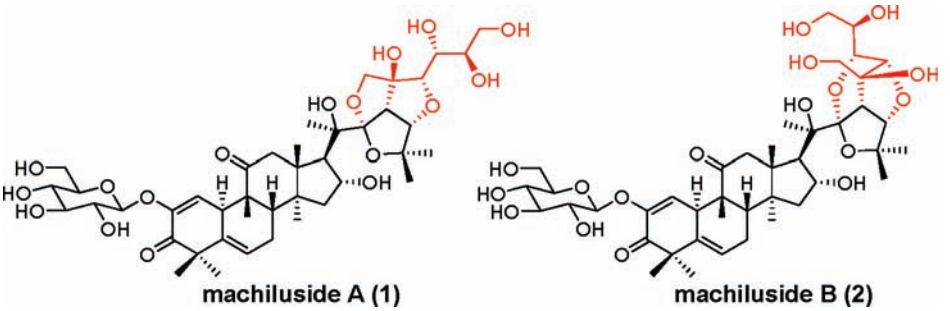
ABSTRACT



Two structurally novel homocucurbitane triterpenoid glycosides, machilusides A (1) and B (2), possessing an unprecedented C_{36} skeleton with a D-fructose moiety incorporated into a cucurbitane nucleus forming unique cage-like tricyclic ring moieties, were isolated from the stem bark of *Machilus yaoshansis*. Their structures were determined by spectroscopic methods. Both compounds exhibited nonselective cytotoxic activities against several human cancer cell lines. The biosynthetic pathway of 1 and 2 was postulated.

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 investigated the stem barks of *Machilus yaoshansis* S. Lee et F. N. Wei that is widely distributed in the south of China. In our previous study, two novel glycosidic triterpene alkaloids with cytotoxic and TNF-α inhibitory activities, machilaminosides A and B,[4] were isolated from the H_2O soluble portion of the

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(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.

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EtOH extract of the stem bark of *Machilus yaoshansis*. Continuing examination of the H_2O soluble portion of this material led to the identification of two novel homocucurbitane glycosides, machilusides A (**1**) and B (**2**) (Figure 1). They represent the first examples of C_{36} homocucurbitane triterpenoid nuclei with unprecedented skeletons featuring D-fructose moieties fused into unique cage-like tricyclic ring systems in their side chain. Herein, we present the isolation, structural elucidation, postulated biogenetic formation, and biological activity of **1** and **2**.[5]

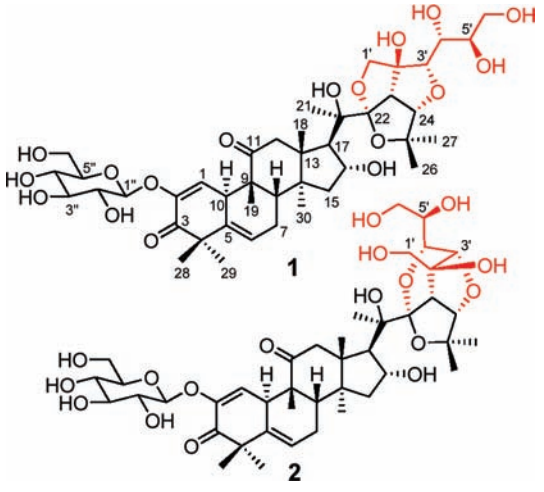

Figure 1. Structures of machilusides A (**1**) and B (**2**).

Machiluside A (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −38.6 (*c* 0.05, MeOH). IR absorptions at 3410, 1687, and 1640 cm^{-1} implied the existence of hydroxy, carbonyl, and double bond functional groups, respectively. The (+)-ESIMS of **1** exhibited quasi-molecular ion peak at *m*/*z* 861. The molecular formula of $C_{42}H_{62}O_{17}$ was determined by HRESIMS at *m*/*z* 861.3861 $[M+Na]^+$ with 12 degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1 and Supporting Information Table S1) in DMSO-d_6 showed two olefinic methines and ten exchangeable hydroxy signals in the lower field region. In addition, it displayed eight methyl singlets, three methylenes, and four methines, together with several overlapping multiplets attributed to oxymethines and oxymethylenes, in addition to characteristic signals due to a β-D-glucopyranosyl moiety (Table 1). The presence of the β-D-glucopyranosyl moiety was supported by the ^{13}C NMR data (Table 1) and further confirmed by enzymatic hydrolysis of **1** using the same methods as described in the literature.[3c] The ^{13}C NMR spectrum of **1** showed 42 carbon signals, and the DEPT experiment differentiated them to be 8 × CH_3, 6 × CH_2, 16 × CH, and 12 × C. (Table 1). On the basis of the chemical shift values, the 12 quaternary carbons were assigned to be two carbonyls, two sp^2 carbons (one oxygen bearing, δ > 145 ppm), and eight sp^3 carbons (four oxygen-bearing, δ > 74 ppm). All the above spectroscopic data suggested that **1** is a highly oxygenated and unusual cucurbitane glycoside.[4,6]

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

	1[b]		**2**	
no.	δ_H	δ_C	δ_H	δ_C
1	5.76 brs	120.6 d	5.76 brs	120.8 d
2		145.0 s		145.0 s
3		196.1 s		196.1 s
4		48.7 s		48.7 s
5		136.3 s		136.3 s
6	5.70 brs	120.0 d	5.73 brs	120.0 d
7α	1.97 brd (19.8)	23.3 t	1.99 brd (17.0)	23.3 t
7β	2.27 dd (19.8, 9.0)		2.30 dd (17.0, 9.0)	
8	1.92 brd (9.0)	41.1 d	1.93 brd (9.0)	41.0 d
9		48.0 s		48.0 s
10	3.61 brs	34.3 d	3.66 brs	34.3 d
11		214.6 s		214.5 s
12α	3.24 d (15.0)	49.1 t	3.31 d (15.0)	49.5 t
12β	2.54 d (15.0)		2.48 d (15.0)	
13		51.3 s		51.6 s
14		47.2 s		47.0 s
15α	1.37 d (12.0)	43.8 t	1.39 d (12.0)	43.8 t
15β	1.71 dd (12.0, 10.2)		1.72 dd (12.0, 10.0)	
16	4.45 m	70.3 d	4.46 m	70.0 d
17	2.36 d (6.0)	56.3 d	2.62 d (6.0)	54.5 d
18	0.83 s	19.4 q	0.86 s	19.2 q
19	0.84 s	19.6 q	0.86 s	19.6 q
20		74.8 s		75.2 s
21	1.25 s	24.4 q	1.27 s	24.8 q
22		122.7 s		114.1 s
23	3.45 d (8.4)	62.7 d	3.17 d (2.5)	46.6 d
24	4.03 d (8.4)	85.2 d	3.87 d (2.5)	84.8 d
25		84.9 s		83.4 s
26	1.22 s	28.0 q	1.10 s	26.4 q
27	1.25 s	23.8 q	1.31 s	22.6 q
28	1.16 s	20.4 q	1.19 s	20.4 q
29	1.16 s	27.1 q	1.19 s	27.2 q
30	1.29 s	17.2 q	1.33 s	17.2 q
1′a	4.19 d (10.2)	74.3 t	3.57 d (12.0)	66.2 t
1′b	3.68 d (10.2)		3.48 d (12.0)	
2′		88.0 s		76.5 s
3′	3.79 d (2.4)	83.2 d	3. 97 brs	74.3 d
4′	3.41 ddd (8.0, 6.0, 2.4)	72.0 d	3.72 (8.0)	77.0 d
5′	3.42 ddd (8.0, 6.0, 3.0)	69.0 d	3.79 ddd (8.0,6.0,3.0)	69.5 d
6′a	3.61 dd (12.0, 3.0)	63.2 t	3.71 dd (11.0, 3.0)	63.2 t
6′b	3.40 dd (12.0, 6.0)		3.41 dd (11.0, 6.0)	
1″	4.55 d (7.8)	98.6 d	4.55 d (8.0)	98.6 d
2″	3.11 ddd (8.4, 7.8, 4.8)	72.7 d	3.13 dd (8.0, 8.5)	72.7 d
3″	3.18 dd (8.4, 8.4)	76.8 d	3.20 dd (8.0, 8.5)	76.8 d
4″	3.26 dd (8.4, 8.4)	68.9 d	3.28 dd (8.0, 8.5)	68.9 d
5″	3.07 ddd (8.4, 4.0, 2.0)	76.9 d	3.08 ddd (8.5, 4.0, 2.0)	76.9 d
6″a	3.70 ddd (12.0, 5.4, 2.0)	60.0 t	3.72 dd (12.0, 2.0)	60.0 t
6″b	3.60 ddd (12.0,5.4, 4.0)		3.61 dd (12.0, 4.0)	

[a] Data were recorded in DMSO-d_6 at 600 MHz (^1H) and 150 MHz (^{13}C) for **1** and at 500 MHz (^1H) and 125 MHz (^{13}C) for **2**, respectively. Coupling constants (*J*) in Hz were given in parentheses. The multiplicity of ^{13}C resonances was determined by the DEPT experiment. The assignments were based on $^1H-^1H$ COSY, HSQC, and HMBC. [b] Data for hydroxy protons of **1**: δ_H 4.87 brs (O*H*-16), 4.53 s (O*H*-20), 5.35 s (O*H*-2′), 4.46 d (6.0 Hz, O*H*-4′), 4.77 brs (O*H*-5′), 4.35 brs (O*H*-6′), 5.17 d (4.8 Hz, O*H*-2″), 5.00 brs (O*H*-3″), 4.87 brs (O*H*-4″), 4.33 t (5.4 Hz, O*H*-6″). For data for **1** in MeOH-d_4, see Supporting Information Table S1.

(5) For plant material, experimental procedures, and physical–chemical properties for compounds **1** and **2**, see: Supporting Information.

The structure of **1** was finally established by 2D NMR experiments. The proton and protonated carbon signals in the NMR spectra of **1** were unambiguously assigned by the HSQC experiment. Extensive analysis of the $^{1}H-^{1}H$ COSY and HMBC spectroscopic data of **1** respectively in MeOH-d_4 and DMSO-d_6 allowed the planar structure of the nucleus with the tetracyclic ring system to be defined as shown (Figure 2), which revealed unequivocally a cucurbita-1,5-diene-3,11-dione nucleus for **1** identical to that of machilaminosides A and B isolated from the same material before.[4] In addition, HMBC correlations of C-16, C-20, C-2′, C-4′, C-5′, and C-6′ with respective hydroxy protons, together with the chemical shift values of these carbons, located a hydroxy group at each of the six carbons, respectively. The connection of a hexose moiety (C-1′−C-6′) to C-23 via the quaternary carbon C-2′ was clearly demonstrated by the HMBC correlations from H-1′a and H-1′b to C-2′ and C-3′; from H-3′ to C-1′, C-2′, C-4′, C-5′, and C-23; from O*H*-2′ to C-1′, C-2′, and C-23; and from H-23 to C-1′ and C-2′. Meanwhile, the key correlations of H-3′ and C-24, H-24 and C-3′, and H-1′a/H-1′b and C-22, along with the chemical shifts of C-1′ (δ 74.3), C-2′ (δ 88.0), C-3′ (δ 83.2), and C-24 (δ 85.2), unequivocally established the linkage of C-3′ and C-24, and C-1′ and C-22 via an oxygen atom to form two fused five-membered rings in the side chain of the nucleus. Furthermore, the linkage of C-22 and C-25 via an oxygen atom to form another five-membered ring was suggested by the chemical shifts of C-22 (δ 122.7) and C-25 (δ 84.9) and the accurate assignment of the ten exchangeable hydroxy signals, together with the absence of O*H*-25 and O*H*-22. This was confirmed by the molecular formula of **1**. Moreover, an HMBC correlation from H-1″ to C-2 indicated that the β-D-glucopyranosyl moiety was located at C-2 of the nucleus. Accordingly, the planar structure of **1** was determined as depicted in Figure 2 a.

The configuration of **1** was elucidated from NOESY correlations and *J*-based configurational analysis combined with circular dichroism (CD) data. NOE correlations of H-7β/H_3-19, H-8/H-15β, H-16, H_3-18, and H_3-19, and H-16/H-15β and H_3-18 indicated that these protons were oriented on the same side of the nucleus, whereas NOE correlations of H-10/H_3-29 and H_3-30, H-15α/H-7α and H_3-30, and H-12α/H-17 and H_3-30 revealed that they were oriented on the other side of the ring system. These data suggested that the configuration of the tetracyclic ring system of **1** was identical to those of cucurbitane derivatives.[4,6,7] In the CD spectrum of **1**, negative Cotton effects at 335 ($\Delta\varepsilon$ −4.14) and 242 ($\Delta\varepsilon$ −3.85) nm and positive Cotton effects at 298 ($\Delta\varepsilon$ +4.48) and 275 ($\Delta\varepsilon$ +6.68) nm indicated that the configuration of the nucleus moiety was identical to that of the reported cucurbitane analogues.[4,7b]

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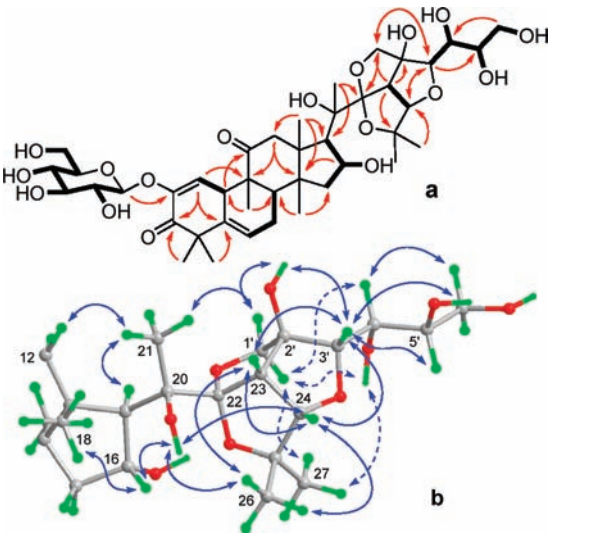


Figure 2. (a) $^{1}H-^{1}H$ COSY (thick lines) and main HMBC (red arrows) correlations of **1**. (b) Key NOESY correlations (blue arrows) for the side chain moiety of **1**.

In addition, the presence of NOE correlations of O*H*-20/H-16 and H_3-18 and H_3-21/H-12β and H-17 and the absence of an NOE correlation of H-16/H_3-21 indicated that free rotation of the single bond between C-17 and C-20 was limited and possessed a major conformation in the solution state as shown in Figure 2 b, supporting the 20*R* configuration in **1**. This is also consistent with that of all the reported cucurbitane derivatives.[6,7]

The NOE correlations of H-3′/H-23, H-24, and O*H*-2′, H-23/H-24, and H_3-26 and H-1′b/O*H*-2′ unambiguously revealed that these protons were cofacial and arbitrarily defined as having a β-orientation. H_3-27 and H-1′a were assigned to be α-configuration judging from the NOE correlations of H-1′a/H_3-27, H-4′, and O*H*-4′ and H_3-27/O*H*-4′. On the basis of the 20*R* configuration in **1**, the crucial NOE correlations of O*H*-20/H-23, H-24, and H_3-26, and H_3-21/H-1′b and H-12β and the absence of an NOE correlation of O*H*-20/H-1′b suggested that the flexibility of the unique tricyclic ring system in the side chain was severely limited and possessed a major conformation in the solution state as shown in Figure 2 b, which indicated a 22*R*,23*S*,24*S*,2′*S*,3′*S* configuration for **1**. The lowest-energy 3D conformation, obtained by Monte Carlo searching with the MMFF molecular mechanics force field using the SPARTAN 04 program,[8] was consistent with that assigned by the NOESY data (Supporting Information, Figure S2). In the 4′,5′,6′-triolyl unit, the 3′,4′-*threo*-4′,5′-*erythro* configuration was assigned by the magnitude of small $^{3}J_{H\text{-}3',H\text{-}4'}$ (2.4 Hz) and large $^{3}J_{H\text{-}4',H\text{-}5'}$ (8.0 Hz) coupling constants[9] and the NOE correlations of H-1′a/H-4′ and O*H*-4′, H-3′/H-5′ and OH-5′, and H-4′/H_2-6′ (for detailed *J*-based configuration analysis, see Supporting Information Figure S3).[10] This, combined with the 3′*S*

(8) *Spartan '04*; Wavefunction, Inc.: Irvine, CA

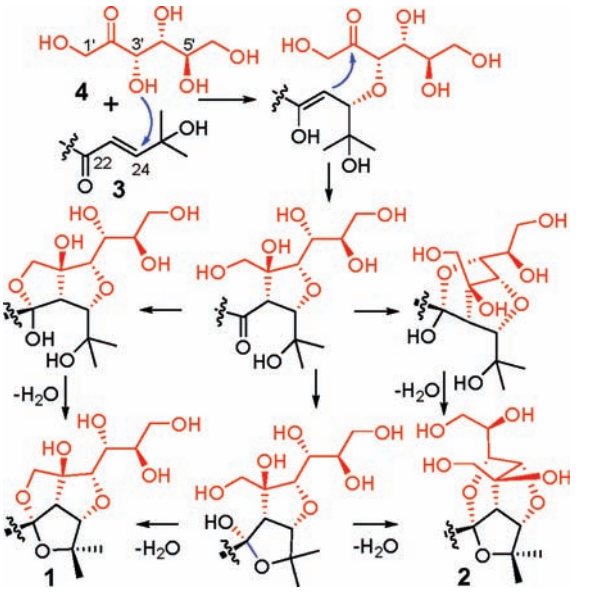
configuration as elucidated above, demonstrated that **1** had a 4′*R*,5′*R*-configuration. Therefore, the structure of compound **1** was determined and designated as machiluside A.

The spectroscopic data of machiluside B (**2**) (Table 1 and Supporting Information) indicated that it was an isomer of **1**. 2D NMR data (^{1}H−^{1}H COSY, HSQC, and HMBC) analysis revealed that **2** differed from **1** only in the replacement of the oxygen-bridged linkage of C-22 and C-1′ in **1** by that of C-22 and C-4′ in **2**. In particular, this was confirmed by the presence of a correlation from H-4′ to C-22 and the absence of the correlation from H_2-1′ to C-22 in the HMBC spectrum (Surpporting Information, Figure S1). The similarity of the coupling constants and CD data of **2** and **1** (Table 1 and Supporting Information) suggested that the two compounds had the same configuration. Therefore, the structure of machiluside B (**2**) was determined as shown in Figure 1.

The plausible biosynthetic pathways of machilusides A (**1**) and B (**2**) are postulated in Scheme 1. They may be biosynthesized from an enzymatic catalyzed coupling of a molecule of the co-occurring 2-*O*-*β*-D-glucopyranosyl-cucurbitacin I (**3**, an important precursor of several cucurbitane derivatives[4,6a,11]) with a molecule of D-fructose (**4**, a common natural product). The precursors would be transformed into key intermediates sequentially or simultaneously via enzymatically catalyzed intermolecular nucleophilic addition followed by intramolecular nucleophilic addition and then dehydration to yield **1** and **2**. The assigned configurations of **1** and **2** are supported by the biosynthetic postulation.

The most structurally intriguing part of machilusides A (**1**) and B (**2**) is the triterpenoid nucleus that possesses a C_{36} skeleton with a D-fructose moiety incorporated into a cucurbitane nucleus forming unique cagelike tricyclic ring moieties (three tetrahydrofuran rings *syn*-fused into a contracted cagelike structure in **1** and two tetrahydrofuran rings with one tetrahydropyran ring *syn*-fused into a contracted cagelike structure in **2**) in the side chain, which has never been described before in a natural product.

Scheme 1. Plausible Biosynthetic Pathways of **1** and **2**



In a cytotoxic assay against human cancer cell lines including the ovary (A2780), colon (HCT-8), hepatoma (Bel-7402), stomach (BGC-823), and lung (A549) by using an MTT method,[3a] compounds **1** and **2** showed nonselective cytotoxic activities against the above cell lines with IC_{50} values of 0.40−6.52 μM, while the positive control camptothecin gave IC_{50} values of 0.28−12.5 μM.

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Supporting Information Available. Plant material, experimental procedures, and physical−chemical properties for compounds **1** and **2**; and copies of MS, HRMS, CD, IR, 1D and 2D NMR spectra of compounds **1** and **2**. Detailed *J*-based configuration analysis for C-3′−C-5′ of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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