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Yaoshanenolides A and B: New Spirolactones from the Bark of *Machilus yaoshansis*

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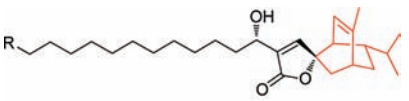
ABSTRACT



1 R = Et
2 R = H

Two novel tricyclic spirolactones bearing long linear alkyl chains, yaoshanenolides A (1) and B (2), formed by Diels–Alder[4 + 2] cycloaddition of a molecule of each butenolide with β-phellandrene, were isolated from the bark of *Machilus yaoshansis*. Their structures and absolute configurations were determined by extensive spectroscopic methods, especially 2D NMR and ECD data analysis. The proposed biosynthetic pathway is discussed. Both compounds exhibited nonselective cytotoxic activities against several human cancer cell lines.

Species of the genus *Machilus* (Lauraceae) are sources of secondary metabolites with interesting chemical structures (lignans, butanolides, sesquiterpenes, alkaloids, and flavonoids) 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 and used as a folk medicine by the ethnic Zhuang in Guangxi province for the treatment of rheumatism. In our previous study, two novel glycosidic triterpene alkaloids with cytotoxic and TNF-α inhibitory activities, machilaminosides A and B,[4] two novel homocucurbitane glycosides, machilusides A and B,[5] and 12 cucurbitane triterpene glucosides[6] were isolated from the H_2O soluble portion of the EtOH extract of the bark or the root of *M. yaoshansis*. Subsequent investigation of the EtOAc-soluble lipophilic portion of the EtOH extract of the bark led to the isolation of two novel butenolide derivatives with unusual 5′*H*-spiro-[bicyclo[2.2.2]oct[2]ene-7,2′-furan]-5′-one moieties and long linear alkyl chains, designated as yaoshanenolides A (**1**) and B (**2**) (Figure 1). They represent the first examples of natural products possessing tricyclic spirolactone structures biosynthesized through the Diels–Alder [4 + 2] cycloaddition of a molecule of each butenolide with β-phellandrene. Herein, we report details of the isolation, structure elucidation, postulated biogenetic pathway, and biological activity of **1** and **2**.[7]

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(7) Plant material, experimental procedures, and physical–chemical properties for compounds **1** and **2**; see: Supporting Information.

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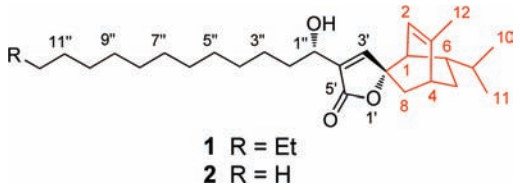

Figure 1. Structures of yaoshanenolides A (**1**) and B (**2**).

Yaoshanenolide A (**1**) was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +18.6 (*c* 0.52, $CHCl_3$). The IR spectrum of **1** showed absorption bands for hydroxy (3443 cm^{-1}) and α,β-unsaturated γ-lactone (1753 cm^{-1}) functionalities.[8,9] The (+)-FABMS of **1** exhibited a quasimolecular ion peak at *m*/*z* 445 $[M + H]^+$. The molecular formula of $C_{29}H_{48}O_3$, with six degrees of unsaturation, was indicated by HR-FABMS at *m*/*z* 445.3705 $[M + H]^+$ (calcd. 445.3682 for $C_{29}H_{49}O_3$) combined with the NMR data (Table 1). The ^1H NMR spectrum of **1** displayed resonances attributable to two trisubstituted double bonds at δ_H 6.86 (brs, H-3′) and 5.58 (brd, J = 6.5 Hz, H-2), an oxymethine at δ_H 4.35 (dd, J = 8.0, 5.0 Hz, H-1″), an olefinic methyl at δ_H 1.74 (brs, H_3-12), and three aliphatic methyls at δ_H 0.81 (t, J = 7.0 Hz, H_3-14″), 0.76 (d, J = 6.5 Hz, H_3-10), and 0.74 (d, J = 6.5 Hz, H_3-11). It also displayed resonances assignable to two olefinic methines at δ_H 2.38 (m, H-4) and 2.32 (J = 6.5 Hz, H-1) and partially overlapped aliphatic methylenes and/or methines between δ_H 1.95 and 0.95. Besides carbon resonances corresponding to the above units, the ^{13}C NMR and DEPT spectra indicated the presence of an oxygen-bearing quaternary carbon at δ_C 90.7 (C-2′) (Table 1). These spectroscopic data suggested that **1** was an unusual α,β-unsaturated γ-lactone, which was further deduced by 2D NMR data analysis. The proton and protonated carbon resonances in the NMR spectra of **1** were assigned by the HMQC experiment. In the $^1H-^1H$ COSY spectrum of **1**, homonuclear coupling correlations of H-4/H_2-5/H-6/H-1/H-2/H_3-12, H-6/H-9/H_3-10, and H_3-11, together with the shifts of these proton resonances, indicated the presence of a 3-methyl-6-isopropyl-cyclohex-2-ene moiety. This was confirmed by two and three bond heteronuclear correlations of H-1/C-2, C-3, C-5, C-6, and C-9; H-2/C-1, C-3, C-4, and C-12; H_3-12/C-2, C-3, and C-4; and H_3-10, H_3-11/C-6, and C-9 in the HMBC spectrum of **1**. In addition, HMBC correlations of H-3′/C-2′, C-1″, C-4′, and C-5′; H-1″/C-3′, C-4′, C-5′, C-2″, and C-3″; and H_3-14″/C-12″ and C-13″, in combination with shifts of these proton and carbon resonances and the remaining overlapped methylene units, confirmed the presence of the butenolide moiety (5′*H*-furan-5′-one) with a 1″-hydroxytetradecyl unit at C-4′ in **1**. In addition, $^1H-^1H$ COSY correlations of H-4/H_2-8 and HMBC correlations of H-1/C-2′, C-3′, and C-8; H-4/C-2′; and H_2-8/C-2′, C-3, C-3′, C-4, and C-5 revealed that C-2′ was connected to C-1 and through C-8 to C-4 to give a spiro structure for **1**, as shown in Figure 2.

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

	1[b]		**2**[b]	
no.	δ_H	δ_C	δ_H	δ_C
1	2.32 d (6.5)	43.4	2.33 d (6.5)	43.4
2	5.58 brd (6.5)	120.5	5.59 brd (6.5)	120.5
3		145.4		145.4
4	2.38 m	36.3	2.39 m	36.3
5a	1.80 m	31.1	1.80 m	31.1
5b	0.94 m		0.95 m	
6	1.93 m	39.7	1.90 m	39.7
8a	1.67 m	33.8	1.65 m	33.8
8b	1.56 m		1.58 m	
9	1.06 m	32.9	1.07 m	32.9
10	0.76 d (6.5)	20.9	0.76 d (6.5)	20.9
11	0.74 d (6.5)	20.2	0.74 d (6.5)	20.2
12	1.74 s	19.9	1.75 s	19.9
2′		90.7		90.7
3′	6.86 brs	154.3	6.87 brs	154.3
4′		132.9		132.9
5′		172.9		172.9
1″	4.35 dd (8.0, 5.0)	67.1	4.35 dd (8.0, 5.0)	67.1
2″a	1.61 m	35.5	1.61 m	35.5
2″b	1.58 m		1.58 m	
3″	1.38 m	25.4	1.38 m	25.4
12″/10″	1.18 m/	31.9/	/1.19 m	/31.9
13″/11″	1.18 m/	22.7/	/1.19 m	/22.7
14″/12″	0.81 t (7.0)/	14.1/	/0.81 t (7.0)	/14.1

[a] NMR data (δ) were measured at 500 MHz for ^1H and at 125 MHz for ^{13}C. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, $^1H-^1H$ gCOSY, gHSQC, and gHMBC experiments of **1**. Resonances of H_2-4″–H_2-11″ in **1** and H_2-4″–H_2-9″ in **2** were overlapped around δ_H 1.18 and 1.19, respectively, and C-4″–C-11″ in **1** and C-4″–C-9″ in **2** were overlapped between δ_C 29.4 and 29.6 and between δ_C 29.3 and 29.6.

The relative configuration of compound **1** was elucidated from NOESY correlations. In the NOESY spectrum of **1**, correlations between H-2 with H-3′ and H-6 revealed that these protons were cofacial, and correlations between H-1 and H_3-10 indicated they were also cofacial. These NOE correlations, in combination with lowest energy conformation analysis (MM2), indicated that the ring moiety of **1** had a relative configuration as depicted in Figure 2.

The absolute configuration at C-1″ in **1** was assigned by using the bulkiness rule for the $Rh_2(OCOCF_3)_4$ induced circular dichroism (CD) data, wherein the E band (around 350 nm) was demonstrated to be useful for determining the

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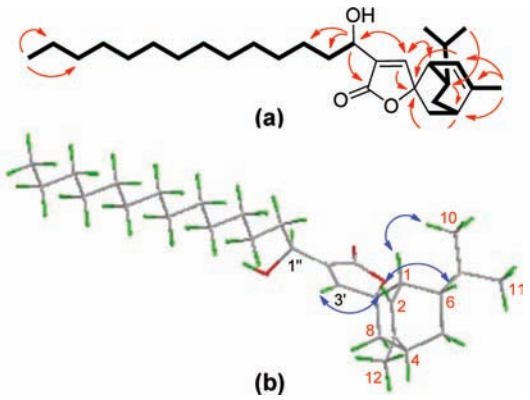

Figure 2. (a) $^1H-^1H$ COSY (thick lines) and main HMBC (red arrows) correlations of **1**. (b) Key NOESY correlations (blue arrows) of **1**.

absolute configuration of chiral secondary and tertiary alcohols.[10] The $Rh_2(OCOCF_3)_4$ induced CD spectrum of **1** displayed a positive Cotton effect at 346 nm (the E band), which predicts the 1″*S* configuration by applying the bulkiness rule. While the presence of the carbonyl group could influence the application of the bulkiness rule,[10] our attempt at preparation of Mosher's derivatives failed to confirm the configuration due to decomposition of **1** under the reaction conditions (DMAP/pyridine). However, the assignment was supported by measurement of the $Rh_2(OCOCF_3)_4$ induced CD spectra of the epimers, (+)-(2*E*,3*R*,4*S*)-2-(dodec-11-ynylidene)-3-hydroxy-4-methylbutanolide and (−)-(2*E*,3*S*, 4*S*)-2-(dodec-11-ynylidene)-3-hydroxy-4-methylbutanolide containing enolide carbonyl and secondary alcohol groups similar to those of **1**, which were isolated from *M. wangchiana*.[11] The $Rh_2(OCOCF_3)_4$ induced CD spectra of the epimers (Supporting Information, Figure S11) showed inverse curves of each other (around 340 nm) predicting the absolute configurations of the secondary alcohols consistent with that determined by chemical transformation and Mosher's methods.[11] This indicates that the enolide carbonyl group does not affect the application of the bulkiness rule for assignment of the absolute configuration of secondary alcohol in these compounds. The absolute configuration at C-2′ in **1** was determined by comparison of the experimental CD spectrum with the electronic circular dichroism (ECD) spectrum predicted from quantum mechanical time dependent density functional theory (TDDFT) calculations, a recent approach increasingly applied for the determination of absolute configurations of natural products (Figure 3).[12] In the ECD calculation, the aliphatic chain was shortened to simplify the computation since the long aliphatic chain

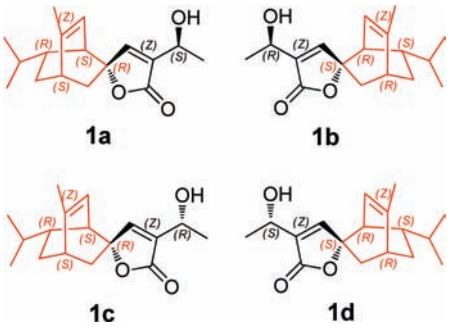

Figure 3. Proposed model compounds in the ECD calculation.

may generate various conformations but have no significant effect on the CD data.[13] Two pairs of enantiomers (**1a**−**1d**) were proposed to be the model compounds according to the NMR data of **1**. Conformational analysis of two (**1a** and **1c**) of the four possible stereoisomers was performed by using the MMFF94 molecular mechanics force field via the MOE software package.[14] The conformers were further optimized with the software package Gaussian 03[15] at the B3LYP/6-31 g(d) level. The polarizable continuum model (PCM) was adopted to consider solvent effects using the dielectric constant of chloroform ($\varepsilon = 4.9$). The 25 lowest electronic transitions were calculated, and rotational strengths of each electronic excitation were given using both dipole length and dipole velocity representations. ECD spectra of different conformers were simulated using a Gaussian function with a half-bandwidth of 0.3 eV, and the final ECD spectra were obtained according to the Boltzmann weighting of each conformer. The theoretical ECD spectra of **1b** and **1d** were similar to the experimental CD spectrum of **1** (Supporting Information, Figures S3 and S5−S7). This combined with the 1″*S* configuration assigned by the $Rh_2(OCOCF_3)_4$ induced CD data indicated that **1** had the same configuration as **1d**. Therefore, compound **1** was determined as (+)-(1*R*,2′*S*,-4*R*,6*S*)-4′-[(*S*)-1″-hydroxytetradecyl]-6-isopropyl-3-methyl-5′*H*-spiro{bicyclo[2.2.2]oct-[2]-ene-7,2′-furan}-5′-one and designated as yaoshanenolide A.

Compound **2** was obtained as a white amorphous solid with $[\alpha]_D^{20}$ +19.6 (*c* 0.48, $CHCl_3$). The spectroscopic data of **2** (Table 1 and Supporting Information) were extremely similar to those of **1**. However, HR-FABMS at *m*/*z* 417.3343 $[M + H]^+$ indicated that **2** had the molecular formula $C_{27}H_{44}O_3$ (calcd 417.3368 for $C_{27}H_{45}O_3$) with two fewer CH_2 units than **1**. This demonstrated that **2** was a homologue of **1** with a 1″-hydroxydodecyl side chain. The positive specific rotation indicated that **2** had the same configuration as **1**. Therefore, compound **2** was determined

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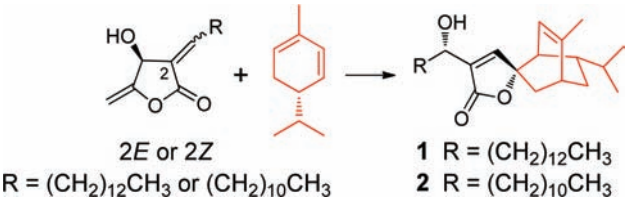
as (+)-(1*R*,2′*S*,4*R*,6*S*)-4′-[(*S*)-1″-hydroxydodecyl]-6-isopropyl-3-methyl-5′*H*-spiro{bicyclo[2.2.2]oct-[2]-ene-7,2′-furan}-5′-one and named as yaoshanenolide B.

Now, we can conclude that the structures of **1** and **2** are characterized by a tricyclic spirolactone nucleus bearing linear alkyl chains at C-4′. In nature the spiro{bicyclo[2.2.2]oct-[2]-ene-7,2′-furan}-5′-one core in yaoshanenolides A (**1**) and B (**2**) is very unusual, though [3-(2-hydroxy-5-methyl-7-isopropylbicyclo[2.2.2]oct-5-en-yl)-propanoic acid 1,4-lactone with a similar core was synthesized previously by Alonso et al.[16] A plausible pathway for **1**−**2** is proposed in Scheme 1. The biosynthetic precursors of **1** and **2** are proposed to be the co-occurring obtusilactone A and/or isoobtusilactone A[17] and dihydroisoobtusilactone.[18] They were isolated from species of the *Machilus* genus, and obtusilactone A was also obtained from *M. yaoshansis*. Compounds **1**−**2** would be biosynthesized through an enzyme-catalyzed Diels−Alder [4 + 2] cycloaddition of a molecule of the precursors with a molecule of *β*-phellandrene which was also reported to exist in species of the *Machilus* genus,[19] followed by a simultaneous or sequential allylic hydroxy rearrangement.

The tricyclic spirolactone substructure is encountered in several bioactive natural products from terrestrial and marine organisms, such as the dimeric phthalides from Umbelliferae plants,[20] lambertellols from the filamentous fungi *Lambertella* sp.,[21] abyssomincins from a marine *Verrucosispora* strain,[22] and ircinianin from a marine sponge of the genus *Ircinia*.[23] This, together with the ethnic use of the plant, prompted us to test the bioactivities of compounds **1** and **2**. In a cytotoxic assay against human (A549) by using an MTT method, compounds **1** and **2** showed nonselective cytotoxicity to the tested cell lines with IC_{50} values of 5.1−6.6 μM. The positive control, camptothecin, gave IC_{50} values of 0.16−11.3 μM. In addition, they were also assessed for inhibitory activities of tumor necrotic factor α (TNA α) secretion of mouse peritoneal macrophages and PTP1B (protein tyrosine phosphatase 1B) but were inactive at 10 μM.

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



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Supporting Information Available. Plant material, experimental procedures, and physical−chemical properties for compounds **1** and **2**, calculated ECD spectra for **1a**−**1d**, CD spectra and the $Rh_2(OCOCF_3)_4$ induced CD spectra of (+)-(2*E*,3*R*,4*S*)-2-(dodec-11-ynylidene)-3-hydroxy-4-methylbutanolide and (−)-(2*E*,3*S*,4*S*)-2-(dodec-11-ynylidene)-3-hydroxy-4-methylbutanolide, and copies of CD, MS, HRMS, IR, and 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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The authors declare no competing financial interest.