ORGANIC
LETTERS

2012
Vol. 14, No. 2
460–463

Bicunningines A and B, Two New Dimeric Diterpenes from *Cunninghamia lanceolata*

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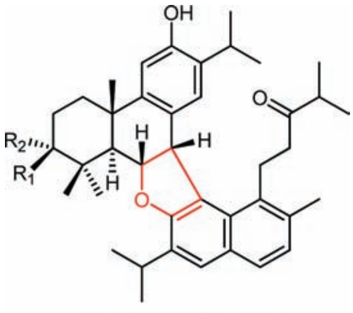
Received October 31, 2011

ABSTRACT



bicunningine A R_1=R_2=O
bicunningine B R_1=OH, R_2=H

Two unprecedented dimeric diterpenoids, with a 2,3-dihydrofuran ring fusing an abietane and a 4,5-*seco*-abietane diterpene, were isolated from *Cunninghamia lanceolata*. Their structures were elucidated by spectroscopic measurements, and their absolute configurations were determined by quantum chemical TDDFT ECD calculations, chemical transformations, and Mosher's method. The Mosher method carried out with MPA and MTPA esters of the sterically hindered *sec*-hydroxyl group gave contradictory results, while MPA afforded the correct absolute configuration.

The genus *Cunninghamia* (Taxodiaceae) consists of two species and two variants, which are all distributed in China.[1] *Cunninghamia lanceolata* (Lamb.) Hook. is a traditional Chinese medicine used for the treatment of hernia, arthritis, and strangury. Previous investigations resulted in the identification of bislabdane,[2−4] lignans,[5,6] diterpenoids,[4] and bisflavonoids[7] from its heartwoods, roots, and leaves. As a continuation of our efforts to explore new structures, we performed a study on this plant and separated two new dimeric diterpenoids, bicunningines A (**1**) and B (**2**) (Figure 1), which featured an abietane and a 4,5-*seco*-abietane diterpene moiety fused by a 2,3-dihydrofuran ring. In this paper, the isolation and structural elucidation of these two compounds are reported.

The air-dried barks of *C. lanceolata* (13.5 kg)[8] were ground into powder and percolated with acetone at room temperature 3 times (7 days each). The combined

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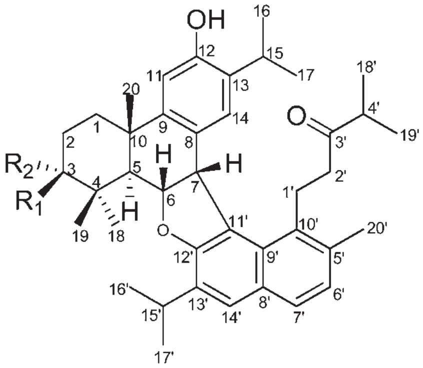
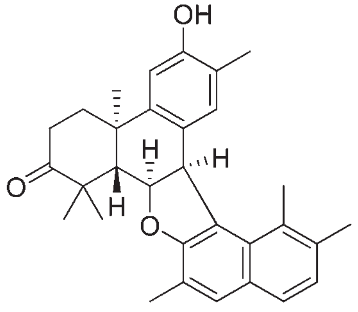
(8) The barks of *C. lanceolata* were collected in December, 2009, in Gongcheng County of Suining, Hunan province, China, and identified by Professor Jingui Shen from Shanghai Institute of Materia Medica. A voucher (2001024) was deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

10.1021/ol202915r

percolates were concentrated under reduced pressure, affording a brown syrup. The syrup was then suspended in hot water and partitioned successively with petroleum ether (PE), CH_2Cl_2, and EtOAc to give PE (15 g), CH_2Cl_2 (160 g), and EtOAc (470 g) extracts, respectively. The CH_2Cl_2 extract was subjected to column chromatography (CC) (100−200 mesh) over silica gel and eluted with a gradient of PE/acetone to yield fractions 1−10. Fraction 3 was separated over an MCI gel column eluted with MeOH/H_2O (40%-95%), affording subfractions A−E. Subfraction E was subjected to CC over silica gel (200−300 mesh) eluted gradiently with $CHCl_3$/MeOH (from 100:1 to 20:1), and then Sephadex LH-20 ($CHCl_3$/MeOH 1:1), and finally purified by preparative HPLC to give **1** (5.6 mg). In a similar way, compound **2** (14 mg) was isolated from fraction 10 by CC over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) as solvent system and then preparative HPLC.



bicunningine A (**1**): R_1=R_2=O
bicunningine B (**2**): R_1=OH, R_2=H



3: truncated model compound of **1** with (5*S*,6*R*,7*S*,10*R*) absolute configuration

Figure 1. Structures of compounds **1** and **2** and the truncated model compound of **1** used for the TDDFT ECD calculation.

Compound **1** was obtained as a white powder: $[\alpha]_D^{23}$ = +272 (*c* = 0.09, MeOH).[9] It had a molecular formula of $C_{40}H_{50}O_4$ as determined by HREIMS at *m*/*z* 594.3732 $[M]^+$ (calcd 594.3709), indicating 16 degrees of unsaturation. The IR spectrum shows absorption bands of hydroxyl (3432 cm^{-1}) and carbonyl (1709 cm^{-1}) groups. The ^{13}C and DEPT NMR spectra (Table 1) revealed 40 resonances ascribed to 10 methyls, 4 methylenes, 11 methines, and 15 quaternary carbons. The ^1H NMR spectrum (Table 1) displayed signals of five aromatic protons at δ_H 7.17 (d, 8.1 Hz, 1H), 7.61 (d, 8.1 Hz, 1H), 7.51 (s, 1H), 6.63 (s, 1H), 6.55 (s, 1H), a benzylic angular methyl at 2.50 (s, 3H), and three isopropyl groups at 3.05 (sept, 6.9 Hz, 1H), 1.27 (d, 6.9 Hz, 6H); 2.52 (sept, 6.9 Hz, 1H), 1.03 (d, 6.9 Hz, 3H), 1.04 (d, 6.9 Hz, 3H); and 2.88 (sept, 6.9 Hz, 1H), 0.87 (d, 6.9 Hz, 3H), 0.69 (d, 6.9 Hz, 3H). These data, together with additional analyses of 2D NMR spectra, revealed the presence of two abietane-type diterpenoid moieties.

In one moiety, characteristic signals of a tetra-substituted benzyl [δ_H 6.63 (s, 1H), 6.55 (s, 1H), δ_C 109.3, 125.9, 127.6, 131.5, 151.5], an isopropyl group [δ_H 2.88, 0.87, 0.69, δ_C 26.3, 22.0, 22.1], a carbonyl (δ_C 215.2) and an oxygenated methine (δ_H 5.29, dd, 7.4, 8.1 Hz, 1H; δ_C 83.1) suggested the presence of a typical abietane-type diterpenoid skeleton. HMBC correlations (Figure 2) from two geminal methyls to the carbonyl carbon indicated that the carbonyl was assigned as C-3; correlations from the oxygenated methine to two quaternary carbons (one aromatic at δ_C 127.6, one connected with two geminal methyls at δ_C 48.2) revealed that the oxygenated position was at C-6.

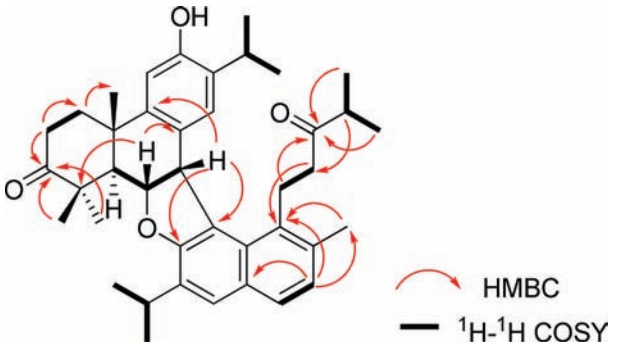


Figure 2. Selected HMBC and ^1H−^1H COSY for compound **1**.

Other resonances, including a penta-substituted naphthyl [δ_H 7.17 (d, 8.1 Hz, 1H), 7.61 (d, 8.1 Hz, 1H), 7.51 (s, 1H), δ_C 118.0, 126.3, 126.8, 127.5, 129.9, 130.1, 132.0, 132.3, 134.1, 157.1], two isopropyl groups [δ_H 2.52 (sept, 6.9 Hz, 1H), 1.03(d, 6.9 Hz, 3H), 1.04(d, 6.9 Hz, 3H), δ_C 41.1, 18.0, 18.3], [δ_H 3.05 (sept, 6.9 Hz, 1H), 1.27 (d, 6.9 Hz), 1.27 (d, 6.9 Hz), δ_C 28.6, 21.4, 22.5], a carbonyl (δ_C 214.1), and an aromatic methyl [δ_H 2.50, s, 3H; δ_C 20.7], constructed a 4,5-*seco*-abietane moiety. The side chain $-CH_2-CH_2-CO-CH(CH_3)_2$, assigned to C-10′, was interpreted by its COSY and HMBC correlations (Figure 2). The aromatic methyl was attached to C-5′

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(9) Bicunningdione A (**1**): white powder, $[\alpha]_D^{23}$ = +272 (*c* = 0.09, MeOH), CD (MeOH, λ [nm] ($\Delta\varepsilon$), $c = 1.34 \times 10^{-3}$); 342 (−3.09), 326sh (−1.72), 308sh (1.39), 292sh (2.58), 279sh (3.91), 248 (67.67), 240sh (48.58), 218 (30.36), 199 (−70.26); IR (film) ν_{max} 3432, 2871, 1709, 1622, 1504, 1464, 1414 cm^{-1}; UV (MeOH) λ_{max} (log ε) 342 (3.31), 328 (3.26), 304 (3.50), 281 (3.63), 245 (4.49) nm; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; EIMS *m*/*z* 594 $[M]^+$; HREIMS *m*/*z* 594.3732 $[M]^+$ (calcd for $C_{40}H_{50}O_4$, 594.3709).

according to the HMBC correlation from H-6′ to C-10′ and C-20′.

Table 1. NMR Data of Compounds **1** and **2** (δ in ppm, *J* in Hz)

	1		**2**	
no.	δ_H	δ_C	δ_H	δ_C
1	α, 2.06, dd (13.8, 3.4)	37.6	α, 1.79, m	36.9
	β, 2.51, m		β, 2.19, m	
2	α, 2.54, m	34.4	α, 1.93, m	27.4
	β, 3.03, m		β, 3.11, m	
3		215.2	3.41, dd (9.5, 6.0)	78.7
4		48.2		39.8
5	2.15, d (7.4)	54.5	1.76, d (8.3)	52.8
6	5.29, dd (7.4, 8.1)	83.1	5.23, dd (8.3, 7.8)	84.1
7	4.91,d (8.1)	46.3	4.76, d (7.8)	46.2
8		127.6		127.6
9		145.7		147.0
10		36.7		36.9
11	6.63, s	109.3	6.63, s	109.3
12		151.5		151.5
13		131.5		131.2
14	6.55, s	125.9	6.50, s	125.7
15	2.88, sept (6.9)	26.3	2.89, sept (6.8)	26.3
16	0.87, d (6.9)	22.0	0.86, d (6.8)	22.1
17	0.69, d (6.9)	22.1	0.69, d (6.8)	22.2
18	1.35, s	25.6	1.28, s	28.3
19	1.42, s	22.6	1.17, s	16.2
20	1.55, s	19.9	1.30, s	20.9
1′	2.67, m	41.1	2.66, m	41.1
2′	α, 3.12, m	24.1	α, 3.11, m	24.0
	β, 3.37, m		β, 3.35, m	
3′		214.1		214.1
4′	2.52, sept (6.9)	41.1	2.51, sept (6.9)	41.0
5′		134.1		134.0
6′	7.17, d (8.1)	126.3	7.15, d (8.2)	126.2
7′	7.61, d (8.1)	127.5	7.60, d (8.2)	127.4
8′		132.3		132.4
9′		130.1		130.2
10′		129.9		129.8
11′		118.0		118.5
12′		157.1		157.3
13′		132.0		132.0
14′	7.51, s	126.8	7.50, s	126.6
15′	3.05, sept (6.9)	28.6	3.11, sept (6.9)	28.6
16′	1.27, d (6.9)	21.4	1.27, d (6.9)	21.3
17′	1.27, d (6.9)	22.5	1.28, d (6.9)	22.7
18′	1.04, d (6.9)	18.3	1.01, d (6.9)	18.4
19′	1.03, d (6.9)	18.0	1.03, d (6.9)	18.0
20′	2.50, s	20.7	2.48, s	20.7

Thus, two moieties were fully established with a total of 15 degrees of unsaturation. Considering the overall formula of **1**, the missing one degree of unsaturation should be ascribed to the linkage between these two subunits creating an additional ring. HMBC correlations from H-7 to C-11′ and C-12′ confirmed a C–C linkage between C-7 and C-11′. The remaining oxygenated methine at C-6 and the oxygenated aromatic quaternary carbon of C-12′ suggested that there must be an oxygen bridge between C-6 and C-12′, which constructed a 2,3-dihydrofuran moiety as a connection between the two diterpenoid subunits. HMBC correlations from H-15′ to C-12′ rather than C-11′ further supported such a proposed structure. The relative configuration of **1** was inferred from the ROESY experiment, which showed effects for H-6/H_3-20, H-7/H_3-20, and H-5/H_3-18, suggesting that H-6, H-7, and H_3-20 are oriented on the same face, while H-5 and H_3-18 on the other face of the molecule. Accordingly, the structure of **1** was established as shown in Figure 1 and named bicunningine A.

Compound **2**, orange powder,[10] $[\alpha]_D^{23}$ = +320 (*c* = 0.14, MeOH), possessed a molecular formula of $C_{40}H_{52}O_4$ as determined by HRESIMS at *m*/*z* 595.3799 $[M - H]^-$ (calcd 595.3787), corresponding to 15 degrees of unsaturation. Detailed analyses of 1D and 2D NMR data of **2** (Table 1) revealed a high similarity between the structures of **1** and **2**. In comparison with **1**, **2** showed two additional protons in the molecular formula, which implied one degree of unsaturation less for compound **2**. This corroborated the fact that, in the ^1H and ^{13}C NMR spectra of **2**, one oxygenated methine (δ_H 3.41, δ_C 78.7) instead of a carbonyl (δ_C 215.2) of **1** was observed, suggesting that an oxygenated methine took place at C-3 in the molecule of **2**. HMBC correlations from H-3 (δ_H 3.41) to the C-1 (δ_C 36.9), C-18 (δ_C 28.3), and C-19 (δ_C 16.2) confirmed the hydoxyl located at C-3. The ROESY correlations of H-3/H_3-18 and H-3/H-5 further indicated that H-3 was on the same face with H-5 and H_3-18. Thus, compound **2** was deduced to be a 3-hydroxyl analogue of **1**. This structure was confirmed by the chemical transformation of **2** to **1** by Jones reagent (see details in the Supporting Information). Therefore, the structure of **2** was established and named bicunningine B.

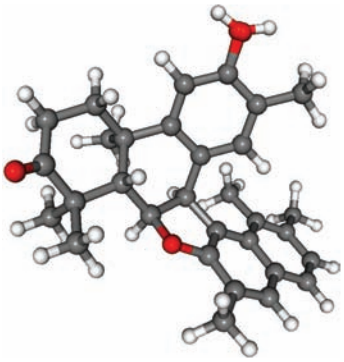

Figure 3. Two overlapped solution conformers of the truncated model compound of bicunningines A (**1**) with Boltzmann populations of 58.4 and 40.8% indicating that they differ only in the orientation of the phenolic proton.

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(10) Bicunningdione B (**2**): light yellow powder, $[\alpha]_D^{23}$ = +320 (*c* = 0.14, MeOH), CD (MeOH, λ [nm] (Δε), *c* = 1.34 × 10^{-3}); 342 (−2.67), 326sh (−1.18), 307sh (1.76), 292sh (3.44), 281 (4.77), 248 (63.18), 240sh (44.26), 219 (28.59), 199 (−73.97); IR (film) ν_{max} 3430, 2962, 2931, 2871, 1704, 1623, 1461 cm^{-1}; UV (MeOH) λ_{max} (log ε) 342 (3.22), 329 (3.17), 280 (3.65), 246 (4.43) nm; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; ESIMS *m*/*z* 597.2 $[M + H]^+$, 619.2 $[M + Na]^+$, 595.3 $[M - H]^-$; HRESIMS *m*/*z* 595.3799 $[M - H]^-$ (calcd for $C_{40}H_{51}O_4$, 595.3787).

In order to determine the absolute configurations of these two compounds, electronic circular dichroism (ECD) spectra of **1** and **2** were measured, which were almost identical.[9,10] For **1**, weak negative Cotton effect (CE) was observed at 342 nm, an intense positive one at 248 nm with positive shoulders on both sides, and a negative CE at 199 nm in methanol. For the conformational analysis of **1**, its alkyl side chains were truncated to methyls and the model compound **3** with (5*S*,6*R*,7*S*,10*R*) absolute configuration was analyzed. The MMFF conformational search of **3** followed by DFT reoptimizations afforded two slightly different conformers (Figure 3) in chloroform, the ECD calculations of which were performed with different functionals (B3LYP, BH&HLYP, PBE0) and TZVP basis set. All the functionals gave consistently mirror image ECD curves of the experimental ECD spectrum, with PBE0 showing the best agreement (Figure 4), which allowed determining the absolute configuration as (5*R*,6*S*,7*R*,10*S*). Since compound **2** showed nearly the same ECD spectrum and they share the same chromophoric system, its absolute configuration was determined as (3*S*,5*R*,6*R*,7*R*,10*S*) based on the known relative configuration of C-3. It has to be noted that, regarding the corresponding chirality centers, **2** is homochiral with **1** and thus its (6*R*) configuration is only due to the change in the priority orders.

Parallel with ECD calculations, diastereomeric MTPA (α-methoxy-α-trifluoromethylphenylacetic acid) esters of **2** were prepared to use the Mosher's method for the configurational assignment of C-3. Although the Mosher's esters showed consistently positive and negative chemical shift difference values for the protons flanking the C-3 chirality center (see Figure S1 in the Supporting Information), surprisingly their values suggested opposite absolute configuration to that determined by ECD. It was supposed that higher-energy conformers of MTPA esters had a larger contribution to the chemical shift difference due to the presence of the adjacent two methyl groups, which ultimately led to the wrong assignment. Then diastereomeric MPA (methoxyphenylacetic acid) esters of **2** were also prepared,[11,12] the results of which were in accordance with the absolute configuration obtained by ECD (Figure S2). The present case confirms that the widely used MTPA Mosher esters may lead to wrong assignment on sterically crowded chirality centers and MPA esters are superior to them as also suggested by Riguera and co-workers.[13]

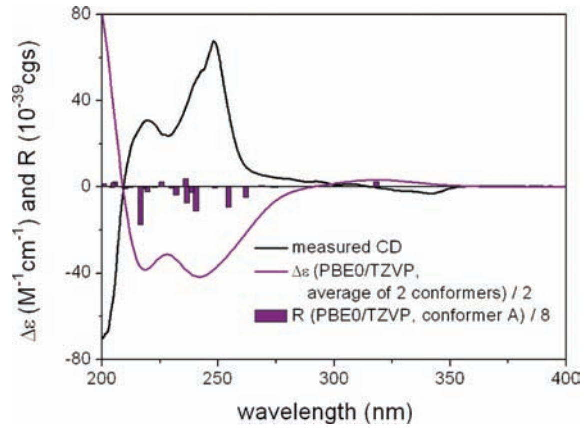

Figure 4. Solution ECD spectrum of bicunningine A (**1**) in methanol compared with the PBE0/TZVP computed Boltzmann-weighted ECD spectrum of the truncated model compound, (5*S*,6*R*,7*S*,10*R*)-**3**. Bars represent the rotatory strength of the low-energy conformer.

To the best of our knowledge, this is the first report of dimeric diterpenes containing a 2,3-dihydrofuranyl linkage between the two halves. Previous reports disclosed the presence of bislabdanes from this plant, which represented compounds containing two labdane diterpenoids coupled by a Diels–Alder reaction.[2–4] Other diterpenoid dimers were also reported from other species of Taxodiaceae, such as sugikurojins B and C and sugikurojin J from *Cryptomeria japonica*.[14,15] The recent finding reveals a new type of linkage of two diterpenoid moieties and expands our knowledge on the possible connection between diterpenoids in this family.

Acknowledgment. Y.Y. thanks the financial support of the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program" (No. 2009ZX09301-001). Y.Y.'s thanks are also given to the National Natural Science Funds for Distinguished Young Scholar (No. 30925043) and grants from the Ministry of Science and Technology (2010DFA30980) and the Shanghai Commission of Science and Technology (10DZ1972700). T.K. thanks the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 and National Information Infrastructure Development Institute (NIIFI 10038).

Supporting Information Available. Experimental section and NMR spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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