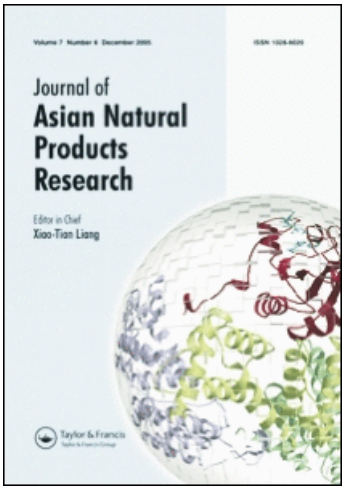

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 27 July 2010

To cite this Article Zhang, Tong , Zhang, Ya-Zhong and Tao, Jian-Sheng(2007) 'Antibacterial constituents from *Stemona sessilifolia*', Journal of Asian Natural Products Research, 9: 5, 479 — 485
To link to this Article: DOI: 10.1080/10286020701189286
URL: http://dx.doi.org/10.1080/10286020701189286

Journal of Asian Natural Products Research, Vol. 9, No. 5, July–August 2007, 479–485

Antibacterial constituents from *Stemona sessilifolia*

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(Received 6 July 2006; revised 2 November 2006; in final form 16 November 2006)

Bioassay-guided fractionation led to the isolation of eight compounds from *Stemona sessilifolia*. Of the eight isolates, three new bibenzyls, stilbostemins M—O (**1**–**3**), and a new tocopherol, 6-methoxy-3,4-dehydro-δ-tocopherol (**4**) were revealed together with four known compounds 3,5-dihydroxy-2′-methoxy bibenzyl (**5**), 3,5-dihydroxy bibenzyl (**6**), β-tocopherol (**7**), and γ-tocopherol (**8**). Compounds **5**, **6**, and **8** exhibited strong antibacterial activities against *Staphylococcus aureus* and *S. epidermidis*.

Keywords: *Stemona sessilifolia*; Stilbenoids; Tocopherols; Antibacterial activities

1. Introduction

Stemona sessilifolia, known as "Baibu" in traditional Chinese medicine, belongs to the Stemonaceae family [1,2]. It is widely distributed in the warm areas of east Asia, such as China, Japan, and the Korean Peninsula [1,2]. Its roots were used in the indigenous system for the treatment of respiratory disorders, including pulmonary tuberculosis and bronchitis, and externally used to kill insect pests [3]. The previous chemical investigations revealed the presence of alkaloids, stilbenoids, lignans, sterols, and glycerin monoesters [4–6]. In our searching for the antimicrobial fractions, the $CHCl_3$-soluble fraction of the MeOH extract prepared from the roots of *S. sessilifolia* showed antibacterial activities against *Staphylococcus aureus* and *S. epidermidis*. Bioassay-directed fractionation led to the purification of four new compounds stilbostemins M—O (**1**–**3**) and 6-methoxy-3,4-dehydro-δ-tocopherol (**4**), as well as four known compounds 3,5-dihydroxy-2′-methoxy bibenzyl (**5**), 3,5-dihydroxy bibenzyl (**6**), β-tocopherol (**7**), and γ-tocopherol (**8**). The above isolates were subjected to antimicrobial test against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, and *Candida albicans*. Compounds **5**, **6**, and **8** exhibited strong antibacterial activities against *S. aureus* and *S. epidermidis*.

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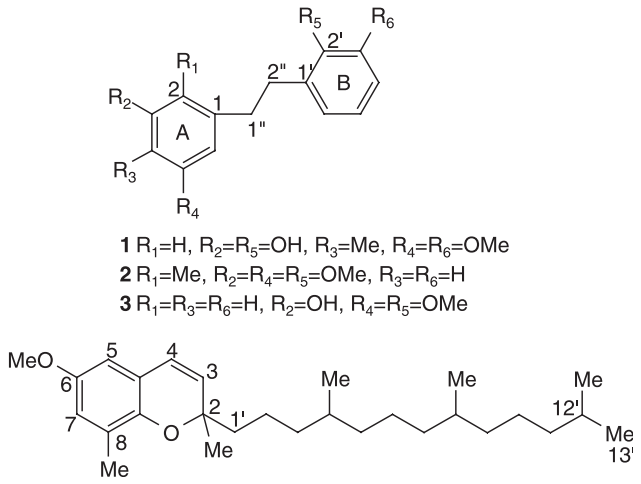
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020701189286

2. Results and discussion

Stilbostemin M (**1**) was obtained as colourless oil. The molecular formula of **1** was deduced as $C_{17}H_{20}O_4$ from a molecular ion peak at *m/z* 288.1369 in the HREI-MS spectrum, which was consistent with the EI-MS and ^{13}C NMR spectral data. UV absorptions at 219 and 275 nm revealed the presence of benzyl moieties. The ^1H NMR spectrum showed representative proton signals of bibenzyls (2 × CH_2, δ 2.84–2.90, *m*) [7], suggesting that **1** was a bibenzyl type compound. The ^1H NMR spectrum also displayed one 1,2,3-trisubstituted benzene [δ 6.85 (1H, *dd*, $J = 8.1$, 1.8 Hz), 6.95 (1H, *dd*, $J = 8.1$, 7.7 Hz) and 6.75 (1H, *dd*, $J = 7.7$, 1.8 Hz)], two *meta*-coupling protons [δ 6.37 (1H, *br s*), 6.31 (1H, *br s*)], one methyl group (δ 2.08, 3H, *s*) and two methoxyl groups (δ 3..77 and 3.79, each 3H, *s*). NOESY experiments were carried out to determine the positions of substituted groups. NOE correlations between H-6 and H-1″ and between H-6 and the methoxyl group at δ 3.79 revealed that the aromatic proton was located at C-6 and the methoxyl group was attached to C-5. Strong NOE correlation between 5-methoxy and the methyl group at δ 2.08 suggested the presence of 4-methyl. The *meta*-coupling between the aromatic protons at δ 6.37 (H-2) and H-6 confirmed the proton was in position 2. The remaining position 3 in ring A should be substituted by one hydroxyl group. Considering the 1,2,3-trisubstituted pattern and the typical ion fragments at *m/z* 137 ($C_8H_9O_2$) in the EI-MS spectrum, it was suggested that a methoxyl group and a hydroxyl group should be assigned at C-2′ or C-3′ in ring B. The NOESY correlation was observed between the methoxyl group at δ 3.77 and H-4′, implied that the methoxyl group was attached to C-3′. The remaining position 2′ was substituted by the other hydroxyl group. HMBC experiments provided further evidence for the substituted patterns. The correlations were observed between H-2 and C-3, C-4, and H-6 and C-4, C-5, suggesting a 3-hydroxy-5-methoxy-4-methyl substitution in ring A. The HMBC cross peaks were also found between H-6′ and C-2′, H-2″ and C-2′, H-4′ and C-3′, and H-5′ and C-3′, confirming a 2′-hydroxy-3′-methoxy substitution in ring B. Thus, **1** was identified as 3,2′-dihydroxy-5,3′-dimethoxy-4-methyl bibenzyl (figure 1).

Stilbostemin N (**2**) was isolated as an oil and possessed the molecular formula of $C_{18}H_{22}O_3$, which was deduced from its HREI-MS spectral data. The ^1H NMR spectrum



1 R_1=H, R_2=R_5=OH, R_3=Me, R_4=R_6=OMe
2 R_1=Me, R_2=R_4=R_5=OMe, R_3=R_6=H
3 R_1=R_3=R_6=H, R_2=OH, R_4=R_5=OMe

Figure 1. Structures of **1**–**4**.

Table 1. ^{1}H NMR (400 MHz) data for compounds **1**–**3** in $CDCl_3$.

No.	***1***	***2***	***3***
2	6.37 *br s*		6.32 *t* (2.4, 1.2)
4		6.35 *br s*	6.27 *t* (2.4, 2.0)
6	6.31 *br s*	6.36 *br s*	6.36 *t* (2.0, 1.2)
3′		6.91 *dd* (7.6, 2.2)	6.87 *dd* (7.5, 1.9)
4′	6.85 *dd* (8.1, 1.8)	7.24 *ddd* (7.6, 7.7, 1.7)	7.21 *ddd* (7.9, 7.5, 1.7)
5′	6.95 *dd* (8.1, 7.7)	6.92 *ddd* (7.7, 7.3, 2.2)	6.89 *ddd* (7.9, 7.6, 1.9)
6′	6.75 *dd* (7.7, 1.8)	7.17 *dd* (7.3, 1.7)	7.17 *dd* (7.6, 1.7)
1″	2.84 *m*	2.86 *br s*	2.94 *m*
2″	2.90 *m*	2.86 *br s*	2.84 *m*
2-Me		2.19 *s*	
4-Me	2.08 *s*		
3-OMe		3.82 *s*	
5-OMe	3.79 *s*	3.86 *s*	3.79 *s*
2′-OMe		3.79 *s*	3.87 *s*
3′-OMe	3.77 *s*		

(table 1) disclosed signals for one 1,2-disubstituted benzene ring [δ 6.91 (1H, *dd*, $J = 7.6$, 2.2 Hz), 7.24 (1H, *ddd*, $J = 7.6$, 7.7, 1.7 Hz), 6.92 (1H, *ddd*, $J = 7.7$, 7.3, 2.2 Hz), and 7.17 (1H, *dd*, $J = 7.3$, 1.7 Hz)], two broad singlets [δ 6.36 (1H, *br s*), 6.35 (1H, *s*)], three methoxyl groups [δ 3.79 (3H, *s*), 3.82 (3H, *s*), 3.86 (3H, *s*)] and a methyl group [δ 2.19 (3H, *s*)], as well as two methylenes [δ 2.86 (4H, *br s*)], which indicated **2** could be a tetrasubstituted bibenzyl. The location of functional groups in **2** was determined by spectral methods as following. In the NOESY spectrum, the correlations between H-1″ and 2-Me, 2-Me and 3-OMe, 3-OMe and H-4, and H-4 and 5-OMe confirmed the 3,5-dimethoxy-2-methyl substitution in ring A. Additionally, the cross peaks between the methoxyl group at δ 3.79 and H-2″, H-3′ showed the substituted group was attached to C-2′ in ring B. HMBC experiment provided evidence to elucidate the structure of **2** (figure 3). These findings led to a conclusion for the structure of 2 as 3,5,2′-trimethoxy-2-methyl bibenzyl.

Stilbostemin O (**3**) was obtained as colourless oil and assigned the molecular formula $C_{16}H_{18}O_3$ by its HREI-MS and ^{13}C NMR spectra. The ^{1}H NMR spectrum (table 1) showed signals attributable to four aromatic protons in one 1,2-disubstituted benzene moiety, three *meta*-coupling triplets in the other benzene moiety, two methoxyl groups, and two methylenes, indicating **3** to be a trisubstituted bibenzyl. The special fragment ions at *m/z* 137 ($C_8H_9O_2$) and 121 (C_8H_9O) disclosed ring A was substituted by a hydroxyl and a methoxyl group, and ring B was substituted by a methoxyl group. The positions of the functional groups in rings A and B were confirmed by the NOESY spectrum. The NOE correlations between H-4 and 5-OCH_3, 5-OCH_3 and H-6, H-6 and H-1″, and H-1″ and H-2 established the 3-hydroxy, 5-methoxy substitution model in ring A, while NOE correlations between the methoxyl group at δ 3.87 and H-3′, H-2″ revealed that the substituted group was in position 2′. Furthermore, HMBC experiments confirmed this substitution pattern and also allowed the assignment of the ^{13}C resonances (figure 3). Therefore, the structure of **3** was established as 3-hydroxy-5,2′-dimethoxy bibenzyl.

Compound **4** was obtained as optically active oil with the molecular formula $C_{28}H_{46}O_2$, derived from the HREI-MS data. The ^{1}H NMR spectrum exhibited proton signals for an olefinic AB system at δ 6.24 (1H, d, $J = 9.7$ Hz) and 5.59 (1H, d, $J = 9.7$ Hz), two *meta*-coupling protons at δ 6.47 (1H, d, $J = 2.9$ Hz) and 6.32 (1H, d, $J = 2.9$ Hz), a methoxy group at δ 3.87, an aromatic methyl group at δ 2.13, an aliphatic methyl group at δ 1.35, and a saturated terpenoid side chain characterised by two methyl singlets at δ 0.87, two methyl

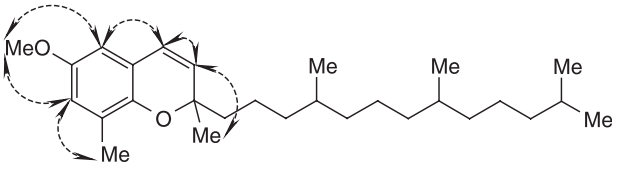


Figure 2. Key NOESY correlations for **4**.

doublets at δ 0.84, a multiplet at δ 1.62 (2H), and a multiplet at 1.53 (1H), very close to those of 3,4-dehydro-δ-tocopherol except for a methoxyl group at C-6, instead of a hydroxyl group in 3,4-dehydro-δ-tocopherol [8]. Moreover, the ^{13}C NMR spectrum showed 28 carbon signals including five quaternary carbon atoms, seven methines, nine methylenes, six methyls, and a methoxy. A detailed 2D NMR analysis (^{1}H—^{1}H COSY, NOESY, HSQC and HMBC) confirmed this structure. NOESY correlations proved the aromatic substitution pattern, and HSQC and HMBC spectra allowed a complete assignment of all ^{13}C resonances (figures 2 and 3). With respect to the stereochemistry of **4**, this 6-methoxy-3,4-dehydro-δ-tocopherol was revealed as the diastereomeric mixture of a ratio *ca*. 52:48 by the analysis of several twin signals in the ^{13}C NMR spectrum in $CDCl_3$. Since Brem *et al.* [8] had determined the absolute configuration of several diastereomeric 3,4-dehydrotocopherols according to the Newman's "Rule of Six" [9], the above method was also applied to confirm the absolute configuration of **4**. The measurements for ^{13}C NMR of **4** in $CDCl_3$ gave the diastereomeric shift differences $\Delta(\delta)$ (C-3 $= -0.02$, C-1′ $= +0.04$, C-2′ $= +0.04$, and 4′-Me $= +0.04$). The $\Delta(\delta)$ values were calculated by subtracting the chemical shift value of the minor diastereomer from the corresponding dominating one. The diastereomeric shift differences of **4** agreed well with those of the diastereomeric mixture (2*S*,4′*R*,8′*R*:2*R*,4′*R*,8′*R*, *ca*. 60:40) [8], suggesting that the 2*S*,4′*R*,8′*R* configuration predominated in **4**. Thus, **4** was elucidated as a diastereomeric mixture of ~52% (2*S*,4′*R*,8′*R*) diastereomer with ~48% (2*R*,4′*R*,8′*R*) one.

By comparison with the previously published data [7,10–12], four known compounds were identified as 3,5-dihydroxy-2′-methoxy bibenzyl (**5**), 3,5-dihydroxy bibenzyl (**6**), β-tocopherol (**7**), and γ-tocopherol (**8**).

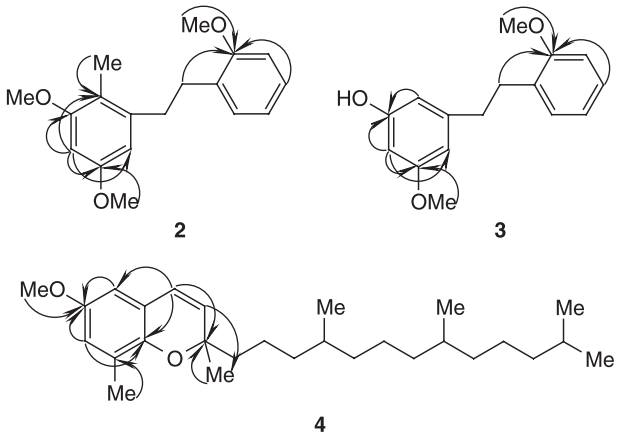


Figure 3. Key HMBC correlations for **2**–**4**.

The isolates were tested against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 26555) *in vitro* by a microdilution assay [13]. Two most potential natural antibacterial agents, bakuchiol [14] and magnolol [15], were used as positive controls in the test. Of the isolates, compounds **5**, **6**, and **8** showed strong antibacterial activities against two hospital pathogenic Gram-positive bacteria *S. aureus* and *S. epidermidis* at the level of MICs 12.5–25 μg/ml (table 3). In comparison with the positive controls, other compounds exhibited more weak antibacterial and antifungal activities against *E. coli* and *C. albicans*.

3. Experimental

3.1 General experimental procedures

The UV spectra were detected on a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrophotometer. All MS data were obtained with MAT-95 mass spectrometer. NMR spectra were recorded on a Bruker AM-400 instrument with TMS as internal standard; the chemical shift values are reported in unit δ and coupling constants J are given in Hz. Silica gel for column chromatography (CC) (100–200, 200–300 mesh) and for preparative TLC (GF_{254}) precoated plates were produced by Qingdao haiyang Chemical Group Co., Qingdao, China.

3.2 Plant material

The roots of *Stemona sessilifolia* (Stemonaceae) were collected in March 2003 in Anji County (Zhejiang Province, China) and identified by Dr. Yazhong Zhang, Shanghai University of Traditional Chinese Medicine, where a voucher specimen (No. 2003/SS001/TCM) is deposited.

3.3 Extraction and isolation

Air-dried roots of *S. sessilifolia* (3.5 kg) were powdered and then percolated with 95% EtOH. The extract (200 g) was suspended in H_2O (1.0 L) and partitioned successively with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$-soluble fraction (19 g) was subjected to CC over silica gel eluting with petroleum ether/acetone gradients (5:1–1:2) to yield 6 fractions. Fr. 1 was subjected to repeated CC on silica gel with 10% EtOAc in hexane, which afforded **4** (15 mg), **7** (50 mg), and **8** (6 mg). Fr. 3 was purified by CC on silica gel with 25% EtOAc in hexane to afford **1** (4 mg) and **2** (13 mg), respectively. Fr. 4 (300 mg) was separated with Sephadex LH-20 (MeOH) to afford crude **3** (25 mg) and **5** (45 mg). Then **3** (11 mg) was obtained by prep. TLC (hexane/EtOAc 100:30), and **5** (26 mg) was gained by recrystallisation in acetone. Fr. 5 (240 mg) was purified by prep. TLC ($CHCl_3$/MeOH, 100:1) to yield **6** (17 mg).

3.4 Antimicrobial bioassay

Bioassay on antimicrobial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida albicans in vitro* were carried out according to the protocols described in the literature [13].

Table 2. ^{13}C NMR (100 MHz) data for compounds **1**–**3** in $CDCl_3$.

No.	***1***	***2***	***3***
1	141.4 *s*	143.3 *s*	145.2 *s*
2	108.6 *d*	115.0 *s*	108.0 *s*
3	156.2 *s*	157.8 *s*	156.5 *s*
4	110.2 *s*	99.8 *s*	98.9 *s*
5	159.4 *s*	158.3 *s*	160.7 *s*
6	104.1 *d*	107.4 *d*	106.7 *d*
1′	135.6 *s*	130.3 *s*	130.1 *s*
2′	143.2 *s*	157.3 *s*	157.5 *s*
3′	153.2 *s*	110.2 *d*	110.3 *d*
4′	114.2 *d*	127.4 *d*	127.2 *d*
5′	125.6 *d*	120.3 *s*	120.4 *s*
6′	122.1 *d*	129.6 *d*	129.8 *d*
1″	36.9 *t*	37.4 *t*	36.3 *t*
2″	31.7 *t*	31.7 t	32.2 *t*
2-Me		8.1 *q*	
4-Me	8.7 *q*		
3-OMe		54.7 *q*	
5-OMe	55.6 *q*	55.2 *q*	55.4 *q*
2′-OMe		55.2 *q*	55.4 *q*
3′-OMe	55.8 *q*		

3.5 Identification

3.5.1 Stilbostemin M (1). Colourless oil; UV (MeOH) λ_{max} (log ε) (nm): 275 (3.43), 219 (4.21); IR (KBr) ν_{max} (cm^{-1}): 3288, 2929, 1619, 1594, 1502, 1456, 1220, 1195, 1159, 958, 811, 744; EI-MS *m/z*: 288 $[M]^+$(35), 271 (25), 257 (30), 240 (32), 211 (15), 183 (8), 165 (13), 151 (100), 137 (32), 109 (31), 83 (38), 78 (25); HREI-MS *m/z*: 288.1369 (calcd for $C_{17}H_{20}O_4$, 288.1362); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data are shown in tables 1 and 2, respectively.

3.5.2 Stilbostemin N (2). Colourless oil; UV (MeOH) λ_{max} (log ε) (nm): 280 (3.18), 221 (4.51); IR (KBr) ν_{max} (cm^{-1}): 3134, 2956, 2923, 1609, 1502, 1458, 1278, 1219, 838, 756; EI-MS *m/z*: 286 $[M]^+$(18), 271 (11), 240 (25), 209 (13), 178 (21), 165 (32), 134 (29), 121 (100), 91 (14), 74 (15); HREI-MS *m/z*: 286.1564 (calcd for $C_{18}H_{22}O_3$, 286.1569); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data are shown in tables 1 and 2, respectively.

Table 3. Antimicrobial activities of compounds **1**–**8** in MIC† values (μg/ml).

Fraction/compound	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*
$CHCl_3$	25	50	>50	>50
1	50	>50	>50	>50
2	>50	>50	>50	>50
3	50	>50	>50	>50
4	>50	>50	>50	>50
5	12.5	12.5–25.0	>50	>50
6	12.5	12.5	>50	>50
7	50	50	>50	>50
8	25	25	>50	>50
Bakuchiol‡	25	12.5	50	25
Magnolol‡	25	12.5	50	50

† MIC was defined as the lowest concentration that inhibited visible growth.
‡ Bakuchiol and Magnolol were used as positive control agents.

3.5.3 Stilbostemin O (3). Colourless oil; UV (MeOH) λ_{max} (log ε) (nm): 278 (3.09), 214 (4.34); IR (KBr) ν_{max} (cm^{-1}): 3388, 2937, 2837, 2854, 1605, 1597, 1494, 1463, 1348, 1242, 1195, 1147, 1060, 833, 754; EI-MS *m/z*: 258 $[M]^+$(46), 227 (7), 137 (5), 122 (8), 121 (100), 91 (35), 65 (3); HREI-MS *m/z*: 258.1252 (calcd for $C_{16}H_{18}O_3$, 258.1256); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data are shown in tables 1 and 2, respectively.

3.5.4 6-Methoxy-3,4-dehydro-δ-tocopherol (4) (2S,4′*R*,8′*R*:2*R*,4′*R*,8′*R* *ca*. 52:48). Light yellow oil; $[\alpha]_D^{25}$ + 8.0 (EtOH, *c* 0.50); UV (MeOH) λ_{max} (log ε) (nm): 332 (2.09), 264 (3.12), 230 (4.02); IR (KBr) ν_{max} (cm^{-1}): 3136, 2958, 2932, 2869, 1469, 1382, 1364, 1321, 1237, 1145, 1132, 989, 931, 858; EI-MS *m/z*: 414 $[M]^+$(12), 399 (8), 383 (15), 189 (100), 175 (35); HREI-MS *m/z*: 414.3501 (calcd for $C_{28}H_{46}O_2$, 414.3498); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.47 (1H, *d*, *J* = 2.9 Hz, 7-H), 6.32 (1H, *d*, *J* = 2.9 Hz, 5-H), 6.24 (1H, *d*, *J* = 9.7 Hz, 4-H), 5.59 (1H, *d*, *J* = 9.7 Hz, 3-H), 3.87 (3H, *s*, 6-OMe), 2.13 (3H, *s*, 8-Me), 1.62 (2H, *m*, 1′-H), 1.53 (1H, *m*, 12′-H), 1.35 (3H, *s*, 2-Me), 1.1–1.5 (18H, *m*, 2′-H—11′-H), 0.87 (6H, *d*, *J* = 6.7 Hz, 12′, 13′-Me), 0.84 (6H, *d*, *J* = 6.7 Hz, 4′, 8′-Me); ^{13}C NMR ($CDCl_3$, 100 MHz): 152.90 (C-6), 145.02 (C-8a), 130.89/130.87 (C-3), 126.21 (C-8), 122.92 (C-4), 121.23 (C-4a), 116.01 (C-7), 108.80 (C-5), 77.92 (C-2), 55.64 (6-OMe), 41.10/41.06 (C-1′), 39.41 (C-11′), 37.54 (C-3′), 37.45 (C-5′, 7′), 37.35 (C-9′), 32.86 (C-8′), 32.69 (C-4′), 28.06 (C-12′), 25.83 (2-Me), 24.81 (C-10′), 24.52 (C-6′), 22.71 (12′-Me), 22.64 (C-13′), 21.37/21.33 (C-2′), 19.76 (8′-Me), 19.70/19.66 (4′-Me), 15.62 (8-Me).

Acknowledgements

This work was supported by a grant from Hi-Tech Research and Development Program of China (2004AA2Z3302).

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