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Dibenzocyclooctadiene lignans from *Kadsura philippinensis*

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ABSTRACT

Lignans with the dibenzocyclooctadiene skeleton, kadsuphilols I–L, and one C_{19}-homolignan, kadsuphilol M, were isolated by chromatographic fractionation of an ethyl acetate extract of the aerial parts of *Kadsura philippinensis*. Their structures were elucidated through extensive spectroscopic methods, including HRESIMS and 2D NMR experiments (HMQC, COSY and HMBC). The stereochemistry at the chiral centers and the biphenyl moist, were determined using NOESY, as well as analysis of CD spectra, respectively. The relative configuration of heteroclitin J was confirmed by single crystal X-ray crystallographic analysis. The *in vitro* radical-scavenging activities of these compounds by using DPPH were evaluated.

1. Introduction

Plants from the Schisandraceae have yielded numerous lignans of various pharmacological activities, including those having antitumor (Hausott et al., 2003), cytotoxic (Kuo et al., 1997; Wu et al., 2004), anti-HIV (Chen et al., 1997), antihepatitis (Kuo et al., 2001), hepatoprotective (Tang et al., 2003) and antioxidative (Chiu et al., 2002) effects. The genus *Kadsura* (Schisandraceae) is a rich source of lignans commonly used in traditional Chinese medicine for their healing properties, sometimes as a substitute for *Schisandra chinensis* Baill (Liu and Li, 1993; Ookawa et al., 1995; Li, 1998). Motivated by the search for bioactive metabolites from *Kadsura* (Shen et al., 2006; Shen et al., 2007), a re-investigation of the lignan content of *Kadsura philippinensis* Elmer (Schisandraceae) was carried out. Herein, we report the results of a phytochemical study that led to isolation of five new lignans, kadsuphilols I–M (**1**–**5**). Four of the isolated lignans **1**–**4** possessed a C_{18}-dibenzocyclooctadiene skeleton, while lignan **5** was a C_{19}-homolignan with a spirobenzofuranoid skeleton (Ayres and Loike, 1990; Li and Xue, 1990). Three known lignans, kadsulignan E (**6**) (Gao et al, 2008), heteroclitin J (**7**) (Xu et al, 2007), kadsuphilin K (**8**) (Shen et al., 2008) were also isolated and their structures determined. These lignans were tested for the antioxidative activity using the DPPH radical-scavenging method.

2. Results and discussion

2.1. Isolation, structure determinations

Kadsuphilols I–M (**1**–**5**) and compounds **6**–**8** were obtained from the ethyl acetate extract of the aerial parts of *K. philippinensis* by column chromatography using normal and reversed phase HPLC. Their structures were determined by analysis of spectroscopic data including 2D NMR (HMQC, COSY, HMBC and NOESY), CD spectra, as well as by X-ray analysis.

Compound **1** possessed a molecular formula $C_{25}H_{32}O_9$, as deduced from its HRESIMS (*m/z* 499.1942 $[M + Na]^+$). The UV absorption (λ_{max} 222, 254 and 287 nm), and IR bands (3503, 1734, 1598 cm^{-1}) suggested that **1** was a C_{18}-dibenzocyclooctadiene lignan with both hydroxyl group(s) and ester substitution (Liu and Li, 1993; Ookawa et al., 1995). The ^1H NMR spectrum of **1** (Table 1) showed two aromatic singlets of a biphenyl moiety at δ_H 6.57 and 6.43 (H-4 and H-11), as well as five singlets of methoxyl groups at δ_H 3.92, 3.90, 3.89 (6H) and δ_H 3.75. The cyclooctadiene ring was evident from two secondary methyl doublets at δ_H 1.09 and 0.92 (H-18 and H-17), two methines at δ_H 2.09 and 2.05 (H-8 and H-7), and two benzylic oxymethines at δ_H 5.71 and 4.71 (H-6 and H-9), implying acylation at the former oxymethine. An

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Table 1
^{1}H NMR spectroscopic data ($CDCl_3$, 300 MHz) of **1**–**5**.[a]

Position	1	2	3	4	5
4	6.57 s	6.65 s	6.68 s	6.94 s	6.48 s
6	5.71 d (7.7)	5.73 s	5.91 s	5.68 s	5.67 s
7	2.05 m				
8	2.09 m	2.18 q (7.2)	2.18 q (7.6)	2.24 q (7.2)	2.10q (7.2)
9	4.71 d (5.1)	5.70 s	4.95 s	5.91 s	5.79 s
11	6.43 s	6.54 s	6.42 s	6.49 s	6.37 s
17	0.92 d (6.9)	1.33 s	1.35 s	1.36 s	1.24 s
18	1.09 d (7.2)	1.30 d (7.2)	1.43 d (7.6)	1.29 d (7.2)	1.29 d (7.2)
19			5.80 d (1.0)	6.03 d (1.0)	5.96 d (0.9)
			5.64 d (1.0)	6.00 d (1.0)	5.91 d (0.9)
20					4.73 d (9.0)
					4.09 d (9.0)
1-OCH_3				3.47 s	
2-OCH_3	3.89 s	3.92 s	3.92 s	3.70 s	3.76 s
3-OCH_3	3.92 s	3.93 s	3.95 s	3.99 s	4.06 s
12-OCH_3	3.89 s	3.86 s			
13-OCH_3	3.90 s	3.81 s			
14-OCH_3	3.75 s	3.53 s	3.37 s		
2′				5.71 d (15.7)	
3′		5.93 q (6.6)	7.52 d (8.0)	7.08 d (15.7)	6.10 q (6.9)
4′		1.81 d (6.6)	7.31 t (8.0)		1.89 dd (6.9, 1.2)
5′		1.29 s	7.45 t (8.0)	7.37 br s	1.28 s
6′			7.31 t (8.0)	7.37 br s	
7′			7.52 d (8.0)	7.37 br s	
8′,9′				7.37 br s	
6-OAc	1.76 s			1.63 s	
9-OAc		1.54 s			1.85 s

[a] *J* values (Hz) in parentheses.

acetate methyl was observed at δ_H 1.76 and this was supported by both C-13 signals (δ_C 21.0, 170.1) and an EIMS fragment ion (*m/z* 416 [M-AcOH]$^+$). The ^{13}C NMR spectrum (Table 2) indicated presence of 10 quaternary aromatic signals, as well as two aromatic upfield methines (δ_C 108.0 and 106.8) adjacent to two oxygenated carbons with hydroxyl groups and five methoxyl groups (δ_C 61.1–56.0). The positions of the methoxyl, hydroxyl, and acetate groups were determined by comparison of ^{1}H and ^{13}C NMR spectroscopic data with those of closely related compounds (Yang et al., 1992; Chen et al., 1998, 2002) as well as a meticulous inspection of the HMBC (Fig. 1), ^{13}C NMR, and NOESY (Fig. 2) spectra. The aromatic proton at δ_H 6.57 (H-4) showed long-range correlations to C-3, C-2, C-16, C-1 and C-6, whereas the aromatic proton at δ_H 6.43 (H-11) correlated to C-12, C-13, C-15, C-14 and C-9. The five methoxyls were located at C-2, C-3, C-12, C-13 and C-14 as a result of the HMBC correlation of the previous carbons and the attached methoxyl singlets. The correlations of the oxymethine at δ_H 5.71 (H-6) to C-4, C-16 and C-8, as well as the acetate carbonyl at δ_C 170.1, located the acetyloxy group at C-6. An hydroxyl proton observed at δ_H 1.26 had a COSY correlation to δ_H 4.71 (H-9), while the latter proton showed HMBC correlations to the aromatic carbon signals at δ_C 106.8 (C-11) and 119.5 (C-15), and the signal at 16.9 (C-18). The structure of the cyclooctadiene ring was also established by COSY connectivities between H-6/H-7/H-8/H-9; H-7/H-17; and H-8/H-18. It was concluded that **1** was a dibenzocyclooctadiene lignan with hydroxyl and acetoxyl substitutions at C-1 and C-9, respectively, and methoxyl substitutions at C-2, C-3, C-12, C-13 and C-14. The CD curve of **1** showed a negative cotton effect around 249 nm and a positive one around 224 nm favoring the *S*-biphenyl configuration (Liu and Li, 1993; Ookawa et al., 1995). The relative stereochemistry of **1** was determined through inspection of a molecular model, as well as the NOESY spectrum (Fig. 2) that demonstrated correlations between H-4/H-6, H-17; H-6/H-17 indicating the α-configuration of H_{eq}-6 and H_{ax}-17. An NOE interaction between H_{eq}-9/H-11 and H_{ax}-8 indicated a β-orientation of H-8 and H-9 (Chen et al., 2001). Based on these findings, structure **1** was identified as kadsuphilol I.

Table 2
^{13}C NMR spectroscopic data ($CDCl_3$, 75 MHz) of **1**–**5**.[a]

Position	1	2	3	4	5
1	147.7 s	147.0 s	146.5 s	149.8 s	196.2 s
2	135.6 s	135.2 s	134.7 s	142.0 s	131.9 s
3	151.4 s	150.6 s	150.5 s	152.2 s	155.7 s
4	108.0 d	107.5 d	107.8 d	112.4 d	123.0 d
5	131.5 s	130.1 s	132.8 s	131.7 s	142.0 s
6	81.3 d	84.8 d	85.7 d	84.6 d	82.3 d
7	38.4 d	74.1 s	74.1 s	73.5 s	75.1 s
8	41.0 d	42.8 d	43.4 d	43.0 d	44.0 d
9	80.8 d	83.8 d	84.0 d	83.2 d	81.5 d
10	138.4 s	134.7 s	136.3 s	133.6 s	129.2 s
11	106.8 d	107.1 d	101.9 d	101.7 d	101.2 d
12	153.2 s	153.3 s	148.8 s	148.1 s	150.2 s
13	141.0 s	141.2 s	135.7 s	134.7 s	130.1 s
14	152.2 s	151.0 s	140.0 s	136.9 s	143.5 s
15	119.5 s	119.8 s	119.1 s	117.8 s	119.8 s
16	115.3 s	116.1 s	115.0 s	120.0 s	63.3 s
17	13.4 q	28.9 q	29.6 q	28.5 q	28.5 q
18	16.9 q	17.2 q	17.8 q	16.7 q	17.8 q
19			100.9 t	101.8 t	101.9 t
20					78.7 t
1-OCH_3				61.2 q	
2-OCH_3	61.1 q	60.8 q	60.9 q	60.8 q	58.8 q
3-OCH_3	56.1 q	55.9 q	55.7 q	56.3 q	58.9 q
12-OCH_3	56.0 q	56.1 q			
13-OCH_3	60.9 q	60.5 q			
14-OCH_3	60.9 q	60.8 q	59.1 q		
1′		165.8 s	164.8 s	164.8 s	166.3 s
2′		126.6 s	130.0 s	116.6 d	126.0 s
3′		141.3 d	129.4 d	145.7 d	142.0 d
4′		15.6 q	127.8 d	131.7 s	15.7 q
5′		19.8 q	132.8 d	129.0 d	19.2 q
6′			127.8 d	128.2 d	
7′			129.4 d	133.6 d	
8′				128.2 d	
9′				129.0 d	
OAc-6	170.1 s			169.5 s	
	21.0 q			20.1 q	
OAc-9		168.9 s			168.6 s
		20.1 q			20.3 q

[a] Assignments were aided by HMQC and HMBC experiments.

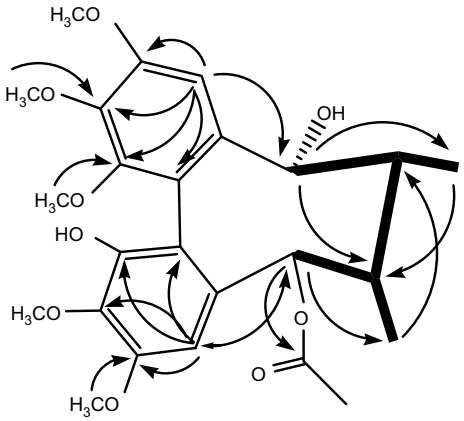


Fig. 1. Selected HMBC (arrows) and COSY (bold line) correlations of **1**.

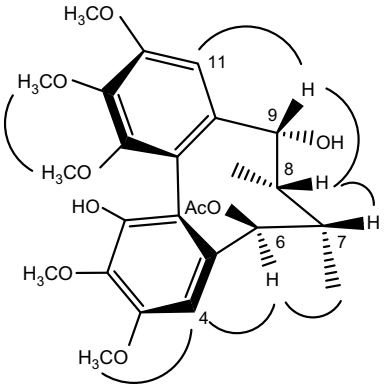


Fig. 2. Selected NOESY correlations of **1**.

The molecular formula of **2** was calculated as $C_{30}H_{38}O_{11}$ from its HRESIMS. The UV and IR spectra of **2** suggested a dibenzocyclooctadiene lignan as for **1**. The NMR spectroscopic data (Tables 1 and 2) displayed aromatic signals of a biphenyl moiety similar to those of **1**. This also established presence of two carbonyl (δ_C 165.8 and 168.9), two oxymethines (δ_C 84.8 and 83.8), a methine (δ_C 42.8) and a quaternary oxygenated carbon (δ_C 74.0) that might suggest a di-substitution at C-6 and C-9 with two acyloxy and a hydroxyl substitutions at either C-7 or C-8. The methyl singlet at δ_H 1.54 (δ_C 20.1) correlated to a carbonyl at δ_C 168.9, and both were attributed to an acetate ester while the proton signals at δ_H 5.93 (H-3′), δ_H 1.81 (H-4′) and $\delta\delta_H$ 1.29 (H-5′) were assignable to angeloyl ester. This was further confirmed by carbon resonances at δ_C 165.8, 126.6, 141.3, 15.6 and 19.8 (Chen et al., 2002; Yang et al., 1992). The methine proton singlet at δ_H 5.73, assigned to H-6, correlated to carbon signals at δ_C 107.5 (C-4), 42.8 (C-8), a tertiary methyl at δ_C 28.9 (C-17), and the angeloyl carbonyl (C-1′) at δ_C 165.8. The oxymethine at δ_H 5.70 (H-9) correlated with C-11 (δ_C 107.1), a secondary methyl (δ_C 17.2, C-18), the oxy-quaternary (δ_C 74.1) and the acetate carbonyl (δ_C 168.9) moieties. These data pointed to substitution of the angeloyloxy at C-6, the acetoxy at C-9, and an hydroxyl at C-7. The hydroxyl substitution at C-7 was further confirmed by the relative low-field shift of C-17 (δ_C 28.9) compared to δ_C 13.4 in **1** and δ_C 15.4 in **2** (Wu et al., 2003).[18] The relative stereochemistry was determined by NOESY correlations between H-4/H-6; H-8/H-9, H-17; H-9/H-11. These data were consistent with the α-orientation of H-6, the hydroxyl at C-7, the methyl at C-8 and the acetoxy at C-9 along with a β-orientation of the angeloyloxy at C-6, H-8, H-9 and the methyl at C-7. Accordingly, lignan **2** was identified as kadsuphilol J.

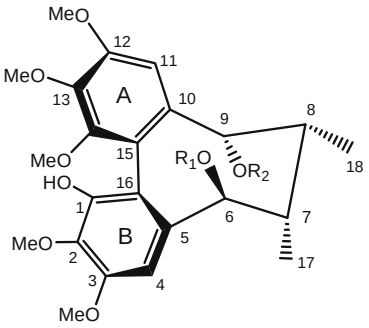
Compound **3** possessed a molecular formula $C_{29}H_{30}O_{10}$ ([M + Na$^+$] at *m/z* 561.1738 in the HRESIMS). Its NMR spectroscopic data (Tables 1 and 2) established the presence of a three methoxyl substitution in the biphenyl moiety at C-2, C-3 and C-14, in addition to a C-12/C-13 methylenedioxy group (δ_H 5.80, 5.64 and δ_C 100.9). The HMBC correlations between the methylenedioxy protons and the carbon signals at 148.8 (C-12) and δ_C 135.7 (C-13) confirmed the position of the methylenedioxy group. The cyclooctadiene moiety disclosed two oxymethines (δ_H 5.91, 4.95 and δ_C 85.7, 84.0), a quaternary oxygenated carbon (δ_C 74.1) and one benzoyl ester (δ_C 164.8, 130.0, 129.4, 127.8 and 132.8), respectively, suggesting substitution with two hydroxyl and one benzoyloxy groups. The oxymethine proton at δ_H 5.91 (H-6) correlated to an aromatic methine at δ_C 107.8 (C-4), a benzoyl carbonyl (δ_C 164.8), an oxy-quaternary (δ_C 74.1) and the C-17 (δ_C 29.6) indicating the attachment of the benzoyloxy group to C-6 and the hydroxyl to C-7. Another hydroxyl was attached to C-9 as deduced from the correlation between H-9 (δ_H 4.95) and C-11 (δ_C 101.9). The proposed structure of **3** was further confirmed by NOESY correlations between H-6/H-4; H-17/H-8 and H-9/H-8; H-11 implying the same configuration as in **3**. The CD spectrum showed strong negative Cotton effect at 245 nm, indicating a *S*-biphenyl configuration. Based on these findings, the structure of **3** was assigned to kadsuphilol K.

Lignan **4** had a molecular formula $C_{33}H_{34}O_{11}$ ([M + Na]$^+$ at *m/z* 629.1996 in the HRESIMS). Its ^{1}H NMR spectroscopic data indicated the presence of three methoxyls and one methylenedioxy substitution in the biphenyl moiety. Comparison of the ^{13}C NMR data (Table 2) with those of **3** established a relative low-field shift in C-2 (+7.3 ppm) and C-4 (+4.6 ppm) with an high-field shift of C-14 (−3.1 ppm) that was attributed to the exchange of methoxyl and hydroxyl substituents at positions C-14 and C-1. The HMBC correlations between the three methoxyl groups at δ_H 3.47, 3.70 and 3.99 with C-1, C-2 and C-3, respectively, together with correlations between the methylenedioxy protons at δ_H 6.03 and 6.00 to C-12 and C-13, confirmed the substitution pattern in the biphenyl moiety. The NMR spectroscopic data also pointed to di-ester and hydroxyl substitutions in the cyclooctadiene ring. An acetate moiety was detected at δ_H 1.63 (δ_C 169.5, 20.1) and a cinnamoyl ester at δ_H 5.71, 7.08 and 7.37 and carbon signals at δ_C 164.8 (carbonyl), 116.6, 145.7, 131.7, 129.0, 128.2 and 133.6. The oxymethine at δ_H 5.68 (H-6) correlated to C-4 (δ_C 112.4), C-17 (δ_C 28.5) and an oxy-quaternary carbon (δ_C 73.5), as well as the acetate carbonyl (δ_C 169.5), thereby positioning the acetoxy at C-6. The other oxymethine at δ_H 5.91 was assigned to position C-9, to which a cinnamoloxy group was attached, as a result of correlations of H-9 to C-11, C-15 and C-18, and NOESY correlation of H-9/H-11. The NOESY correlations were the same as the case in **2** and **3** implying the same configuration of cyclooctadiene moiety. The J values between H-9 and H-8 in compounds **2–4** are zero indicated that the dihedral angles among H-9/C-9/C-8/H-8 of them are nearly 90 degree. Based on these findings, the structure of **4** was determined.

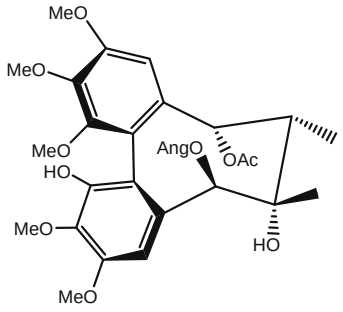
The high-resolution ESIMS of **5** established M + Na$^+$ at *m/z* 579.1842 corresponding to the molecular formula $C_{29}H_{32}O_{11}$. The NMR spectroscopic data of the cycooctadiene ring displayed the same pattern of substitution as in the case of **2**, with angeloyloxy, hydroxyl and acetoxy substitutions at C-6, C-7 and C-9, respectively, that was confirmed by inspection of HMBC and NOESY correlations of H-6 and H-9. In addition to two methoxyl (δ_C 58.9 and 58.8) and a methylenedioxy (δ_C 101.9) groups, the ^{13}C NMR spectrum of **5** displayed a carbonyl signal at δ_C 196.2, an oxymethylene at δ_C 78.7 and a quaternary at δ_C 63.3. This was associated with the absence of the normal chemical shift

values for C-1 and C-16. The HMBC correlations of the methylenedioxy protons (δ_H 5.96 and 5.91) and two methyls at δ_H 4.06 and 3.76 suggested the substitution of two methoxyls at C-2 and C-3 and methylenedioxy at C-12/C-13. Two oxymethylene protons resonating at δ_H 4.73 and 4.09, directly attached to the oxymethylene carbon at δ_C 78.7, correlated to carbon signals at δ_C 119.8 (C-14) and the carbonyl at δ_C 196.2. In addition, the methine at δ_H 6.48 (H-4) correlated to the quaternary carbon at δ_C 63.3 (C-16), as well as C-2 (δ_C 131.9) and C-6 (δ_C 82.3). These data agreed with the presence of a carbonyl moiety at C-1 and an oxymethylene at C-20 forming spirobenzo-dihydrofuran ring (Li and Xue, 1990; Chen et al., 1992). The CD curve exhibited a negative Cotton effect at 228 and 319 nm, and a positive Cotton effect at 249 and 369 nm, indicating the same configuration as kadsulignan H (Liu et al. 1992). The NOESY correlations of **5** were similar to those of **2** (Fig. 3), indicating the same orientation at C-6, C-7, C-8 and C-9. It was concluded that **5** was a C_{19}-homolignan and unambiguously identified as kadsuphilol M.

The HRESIMS, ^{1}H NMR, ^{13}C NMR spectroscopic data, together with HMBC and NOESY correlations of **6** established that the structure of **6** was identical with kadangustin E (Gao et al., 2008). The NOESY and CD data of **7** were similar to those of **5** allowing the assignment of structure **7** to that of heteroclintin J (Xu et al, 2007). X-ray crystallographic analysis eventually established (Fig. 4) its relative stereochemistry, especially at C-6, C-7, C-8, C-9 and C-16.



1 R_1 = Ac, R_2 = H
6 R_1 = Bz, R_2 = Ac
8 R_1 = H, R_2 = Ac



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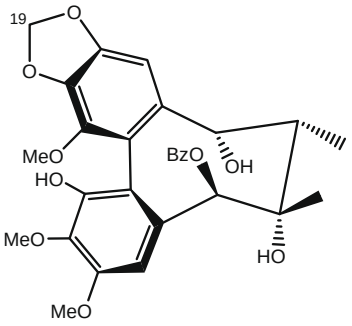

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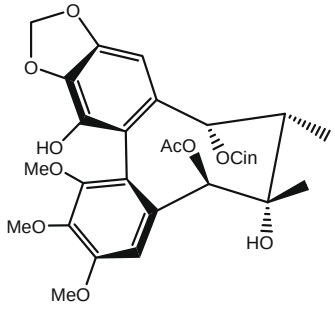

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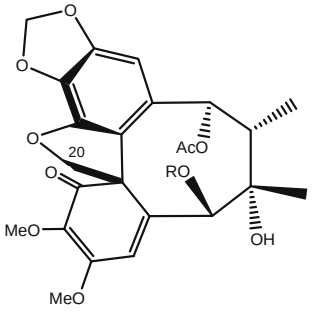
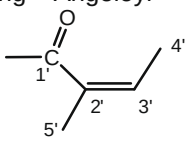


5 R = Ang

Ang = Angeloyl



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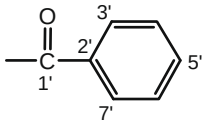
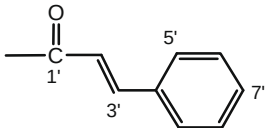


Cin = cinnamoyl



During the course of fractionation schizanrin G (Wu et al., 2003), kadsuphilin G (Shen et al., 2007), kadsulignan C (Liu et al., 1991) were also isolated and identified through comparing their spectroscopic data with the reported values.

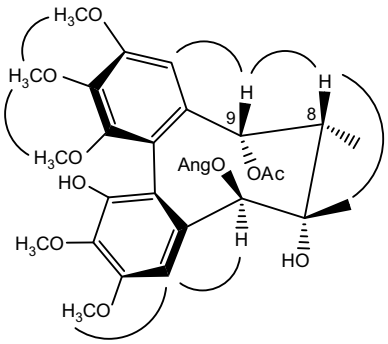


Fig. 3. Key NOESY correlations of **2**.

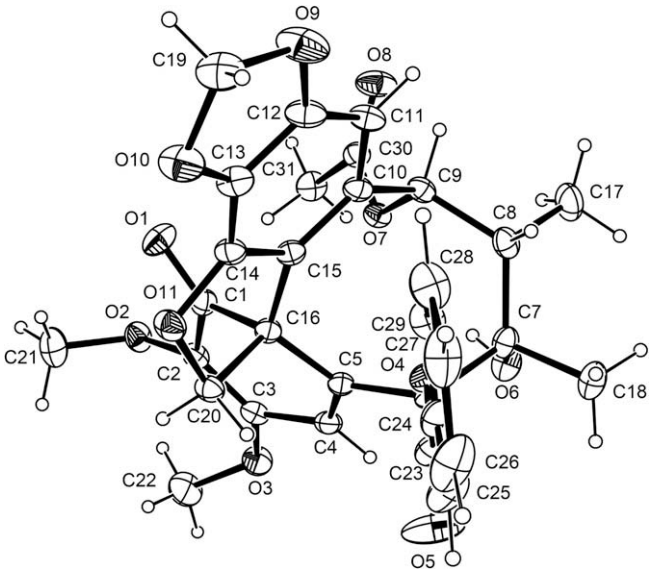


Fig. 4. ORTEP diagram showing the solid state conformation of **7**.

2.2. Antioxidative experiments

Lignans **1–8** were tested and evaluated for their antioxidative activity by application of the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical test. Among them, kadsuphilin K (**8**) showed moderate DPPH free radical-scavenging activity at 6.25 μM (Table 3).

2.3. Conclusions

The aerial parts of *K. philippinensis* were investigated chemically. Four new lignans with dibenzocyclooctadiene skeleton, kadsuphilols I–L, and one new C_{19}-homolignan, kadsuphilol M, were isolated and the structures identified. The structure of heteroclitin J (**7**) was further determined by single crystal X-ray crystallographic analysis. In vitro radical-scavenging activities of these compounds using DPPH were also tested, with kadsuphilin K (**8**) exhibiting significant DPPH free radical-scavenging activity.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO DIP-1000 polarimeter, whereas IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. CD spectra were acquired using a JASCO J-720 spectrophotometer, whereas ^{1}H, ^{13}C NMR, COSY, HMQC, HMBC and NOESY spectra were recorded on a Bruker FT-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively) or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using TMS as internal standard. Chemical shifts are given in δ (ppm) and coupling constants in Hz. Low-resolution EIMS and FABMS spectra were recorded on a VG Quattro 5022 mass spectrometer, and high-resolution ESIMS spectra were measured on a JEOL HX 110 mass spectrometer. Silica gel 60 (Merck) was used for column chromatography (CC), and pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) was used for either purification or separation. LiChrospher® Si 60 (5 μm, 250-10, Merck) and LiChrospher® 100 RP-18e (5 μm, 250-10, Merck) were used for NP-HPLC and RP-HPLC (Hitachi), respectively.

Table 3
Free radical-scavenging activities of compounds **1–8** at various concentrations.[a,b]

Compound	100 μM	50 μM	25 μM	12.5 μM	6.25 μM
1	1.6	1.9	3.6	1.7	0.3
2	4.7	1.5	1.2	1.3	1.9
3	1.6	1.8	4.0	1.9	1.9
4	10.3	10.4	9.9	10.3	10.3
5	17.2	18.5	16.2	16.3	16.5
6	14.9	12.7	12.3	12.4	14.8
7	11.0	11.3	13.1	14.4	14.6
8	35.6	32.8	29.6	31.5	25.4
Vitamin C[c]	43.9	26.4	23.1	21.1	20.1
Vitamin E[c]	24.4	14.0	11.2	8.6	8.1

[a] Radical-scavenging activities were measured by the DPPH method.
[b] Data are shown as % inhibition.
[c] Positive control substances.

3.2. Plant material

Aerial parts of *K. philippinensis* were collected at Green Island, Taiwan in November, 2002. A voucher sample (specimen code: TP 93-2) was deposited in the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

3.3. Extraction and isolation

Dry leaves and twigs (8.5 kg) were extracted three times with acetone with the combined extract evaporated in vacuum and then partitioned between EtOAc and H_2O (1:1). The resulting EtOAc extract (250 g) was subjected to column chromatography on silica gel CC using *n*-hexane/EtOAc (gradient 100:1 to 0:1) for elution to furnish 24 fractions. Fraction 20 (4.9 g) was fractionated on a column of Sephadex LH-20 using MeOH for elution to produce five fractions (L_1–L_5). Fraction L_2 (2.8 g) was separated on a silica gel flash column using a gradient of *n*-hexane/EtOAc (20:1 to 0:1) to afford 8 subfractions (L_2-1 to L_2-8). Subfraction L_2-4 (200 mg) was re-applied to a silica gel column using *n*-hexane/EtOAc (15:1 to 0:1) as eluant to give three fractions (L_2-4-a to L_2-4-c). Fraction L_2-4-b was repeatedly subjected to normal phase (NP)-HPLC using *n*-hexane/CH_2Cl_2/MeOH (70:30:1) then (80:20:1) to yield **5** (17 mg). Subfraction L_2-4-c (43 mg) was fractionated by NP-HPLC using a *n*-hexane/CH_2Cl_2/MeOH (75:25:1.5) followed by RP-HPLC using MeOH/H_2O (7:3) to yield schizanrin G (7 mg) and kadsuphilin G (4 mg). Subfractions L_2-5 and L_2-6 were combined (2 g) and fractionated on a silica gel column using *n*-hexane/acetone/EtOAc (20:1:1 to 1:1:1) to give three fractions (L_2-56-a to L_2-56-c). Fraction L_2-56-b (920 mg) was subjected to silica gel CC using *n*-hexane/acetone (20:1 to 0:1) to yield 12 fractions (L_2-56-b-1 to L_2-56-b-12). Fraction L_2-56-b-4 (50 mg) was separated further using NP-PTLC with *n*-hexane/CH_2Cl_2/MeOH (64:40:1) as eluant to give **6** (7 mg) and

additional **5** (14 mg). On the other hand, fraction 21 (23 g) was separated on a column of Sephadex LH-20 using MeOH for elution to produce five fractions (S_1–S_5). Fraction S_2 (13.2 g) was then subjected to silica gel flash CC using a gradient of *n*-hexane/EtOAc/acetone (15:1:1 to 1:1:1) to produce four fractions (S_2-1 to S_2-4). Fraction S_2-2 (158 mg) was subjected to NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (62:38:1) to yield **7** (7 mg). Fraction S_2-3 (516 mg) was separated on NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (30:70:1) to afford kadsulignan C (12 mg) and three lignan mixtures A-C. Mixture A (104 mg) was separated on NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (70:30:1) followed by RP-HPLC using MeOH/H_2O (7:3) to give **5** (6 mg) and **8** (3 mg). Mixture B (69 mg) was separated on RP-HPLC using MeOH/H_2O (65:35) to afford **2** (26 mg). Mixture C (34 mg) was similarly separated like B to produce **1** (7 mg). In addition, Fraction S_4 (1.5 g) applied to a silica gel column using a gradient of *n*-hexane/EtOAc (25:1 to 0:1) to produce seven subfractions (S_4-1 and S_4-7). Subfraction S_4-5 (94 mg) was subjected to NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (60:40:1) then RP-HPLC using MeOH/H_2O (65:35) to yield **3** (2 mg).

3.3.1. Kadsuphilol I (*1*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 22.6 (CH_2Cl_2; c = 0.6); UV (MeOH) λ_{max} (log ε): 222 (4.62), 254 (4.21), 287 (3.92) nm; CD (MeOH): $[\theta]_{224}$ (+23 3465), $[\theta]_{249}$ −20 0059; IR (CH_2Cl_2) υ_{max} 3503 (OH), 1734, 1598, 1491, 1236, 1105, 734 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic analysis, see Tables 1 and 2, respectively; HRESIMS *m/z*: 499.1942 $[M + Na]^+$ calcd. 499.1944 for $C_{25}H_{32}O_9Na$.

3.3.2. Kadsuphilol J (*2*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 10.2 (CH_2Cl_2; c = 2.0); UV (MeOH) λ_{max} (log ε): 224 (4.65), 255 (4.45), 286 (4.16) nm; CD (MeOH): $[\theta]_{222}$ +24 5117, $[\theta]_{245}$ −8 5303; IR (CH_2Cl_2) υ_{max} 3568, 3431 (OH), 1746, 1715, 1645, 1586, 1497, 1456, 850, 736 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic analysis, see Tables 1 and 2, respectively; HRESIMS *m/z*: 597.2313 $[M + Na]^+$ calcd. 597.2312 for $C_{32}H_{36}O_{10}Na$.

3.3.3. Kadsuphilol K (*3*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 14.0 (CH_2Cl_2; c = 0.2); UV (MeOH) λ_{max} (log ε): 223 (4.37), 257 (3.30), 288 (2.54) nm; CD (MeOH): $[\theta]_{224}$ +13 0467, $[\theta]_{245}$ −11 9778; IR (CH_2Cl_2) υ_{max} 3444 (OH), 1715, 1614, 1463, 1371, 1261, 1108, 738, 713 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic analysis, see Tables 1 and 2, respectively; HRESIMS *m/z*: 561.1738 $[M + Na]^+$ calcd. 561.1737 for $C_{29}H_{30}O_{10}Na$.

3.3.4. Kadsuphilol L (*4*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 3.2 (CH_2Cl_2; c = 0.25); UV (MeOH) λ_{max} (log ε): 219 (4.67), 281 (4.16) nm; CD (MeOH): $[\theta]_{223}$ +14 1969, $[\theta]_{254}$ −13 8057, $[\theta]_{281}$ 1 5992; IR (CH_2Cl_2) υ_{max}: 3564 (OH), 1715, 1634, 1596, 1505, 1487, 1455, 1234, 936, 736 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic analysis, see Tables 1 and 2, respectively; HRESIMS *m/z*: 629.1996 $[M + Na]^+$ calcd. 629.1999 for $C_{33}H_{34}O_{11}Na$.

3.3.5. Kadsuphilol M (*5*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 4.6 (CH_2Cl_2; c = 0.6,); UV (MeOH) λ_{max} (log ε): 222 (4.68), 253 (4.10), 283 (3.53) nm; CD (MeOH): $[\theta]_{224}$ +24 2388, $[\theta]_{238}$ −46 7346; IR (CH_2Cl_2) υ_{max}: 3444 (OH), 1715, 1599, 1584, 1495, 1455, 1334, 1106, 1026, 1006, 735, 714 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic analysis, see Tables 1 and 2; HRESIMS *m/z*: 603.2209 $[M + Na]^+$ calcd. 603.2206 for $C_{32}H_{36}O_{10}Na$.

3.3.6. Kadangustin E (*6*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 0.3 (CH_2Cl_2; c = 1.2); UV (MeOH) λ_{max} (log ε) 219 (4.46) nm; CD (MeOH) $[\theta]_{228}$ −4 75763, $[\theta]_{249}$ +2 05665, $[\theta]_{319}$ −12 696, $[\theta]_{368}$ −7 0168; IR (CH_2Cl_2) υ_{max} 3567 (OH), 1716, 1645, 1455, 1135, 934, 736 cm^{-1}; HRESIMS *m/z*: 579.1842 $[M + Na]^+$ calcd. 579.1842 for $C_{29}H_{32}O_{11}Na$.

3.3.7. Heteroclitin J (*7*)

Amorphous yellowish powder; $[\alpha]_D^{25}$ – 2.0 (CH_2Cl_2; c = 0.6); UV (MeOH) λ_{max} (log ε): 221 (4.54) nm; CD (MeOH) $[\theta]_{220}$ −20 1028, $[\theta]_{244}$ +1 9844, $[\theta]_{320}$ −22 7976, $[\theta]_{369}$ −11.4894; IR (CH_2Cl_2) υ_{max}: 3565 (OH), 1747, 1721, 1660, 1651, 1645, 1582, 1258, 736, 713 cm^{-1}; HRESIMS *m/z*: 601.1689 $[M + Na]^+$ calcd. 601.1686 for $C_{31}H_{30}O_{11}Na$.

3.4. Crystallographic data and X-ray structure analysis of *7*

Crystal data: $C_{31}H_{30}O_{11}$, M = 578.55, monoclinic system, space group $P21$, a = 11.985(2), b = 9.104(2), c = 12.760(3) Å, V = 1388.5(5) $Å^3$, Z = 2, d = 1.384 g/cm^3. A crystal of dimensions 0.60 × 0.60 × 0.80 mm was used for measurements on a RIGAKU AFC7S diffractometer with a graphite monochromator (−2θ scans, 2θmax = 52.0), Mo Kα radiation. The total number of independent reflections measured was 2922, of which 2335 were observed ($|F|^2 \geqslant 2\sigma|F|^2$). The crystal structure was solved by the direct method SHELX-86 (Sheldrick, G.M. University of Gottingen, Gottingen, Germany, 1985) and expanded using difference Fourier techniques, refined by the program SHEXTL 97 (Sheldrick, G.M. University of Gottingen, Gottingen, Germany, 1997) and full-matrix least-squares calculations. Final indices: Rf = 0.041, Rw = 0.107 ($w = 1/[\sigma^2(Fo^2) + (0.078P)^2 + 0.840P]$ where $P = (Fo^2 + 2Fc^2)/3$). Copies of the deposited crystal data can be obtained, free of charge, on application to CCDC (658220), 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.5. Antioxidative assay

DPPH radical-scavenging activity was measured according to a published protocol (Gerhauser et al, 2003). Dilutions of compounds **1**–**8** (in 100% DMSO) were treated with a solution of 100 μM DPPH in ethanol at 37 °C. The mixtures were shaken vigorously and stood for 30 min. The absorbances at 517 nm were measured using a UV–vis spectrophotometer. Vitamins C and E were used as positive control.

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