Notes

Kadsuralignans H−K from *Kadsura coccinea* and Their Nitric Oxide Production Inhibitory Effects

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Received June 7, 2007

One new arylnaphthalene lignan named kadsuralignan H (**1**) and three new dibenzocyclooctadiene lignans, named kadsuralignans I (**2**), J (**3**), and K (**4**), respectively, were isolated from an EtOAc fraction of the 80% acetone extract of *Kadsura coccinea.* The structures were elucidated on the basis of spectroscopic evidence. Compound **1** is the second arylnaphthalene lignan isolated from the *Kadsura* genus. Compounds **1** and **3** showed inhibitory activity on nitric oxide (NO) production by the murine macrophage-like cell RAW264.7, which was activated by lipopolysaccharide (LPS) and recombinant mouse interferon-γ (INF-γ).

The Schisandraceae family of plants is known to be a rich source of lignans and triterpenoids with various biological activities.[1] The genus *Kadsura* (Schisandraceae) is closely related to *Schisandra*, and many of its species are extensively used as a substitute for *Schisandra* in Chinese Medicine in Taiwan, Japan, and mainland China.

Kadsura coccinea (Lem.) is widely distributed throughout southwest China. Plant extracts have been used in Chinese folk medicine for treatment of cancer and dermatosis and as an anodyne to relieve pain in China.[2] Previous investigations of *K. coccinea* have yielded some lignans and triterpenoids.[3–12]

In our previous study of anti-inflammatory compounds from *K. coccinea*, a series of lignans were isolated from the $CHCl_3$ extract.[13,14] To continue our search for anti-inflammatory compounds from *K. coccinea*, we studied an EtOAc fraction of the 80% acetone extract of this plant. Bioassay-directed fractionation led to the isolation of one new arylnaphthalene lignan named kadsuralignan H (**1**) and three new dibenzocyclooctadiene lignans, named kadsuralignans I (**2**), J (**3**), and K (**4**), respectively, together with the two known spirobenzofuranoid dibenzocyclooctadiene lignans kadsulignans E and F (**5**, **6**). Herein, we describe the structure elucidation and biological evaluation of these compounds.

Kadsuralignan H (**1**) was obtained as a colorless powder. Its molecular formula, $C_{22}H_{26}O_6$, was determined by HREIMS ($[M]^+$, *m/z* 386.1733) with 10 degrees of unsaturation. Analysis of the ^{13}C NMR and DEPT spectra showed that **1** contained three methoxy groups and two methylene, six methine, two aliphatic methyl, and nine quaternary carbons. The IR spectrum showed an absorption band at 3435 cm^{-1} for a hydroxy group.

The ^1H NMR spectrum of **1** accounted for the presence of three aromatic hydrogens at δ_H 6.13, 6.25, and 6.26 (H-2′, H-6′, and H-6, respectively). In the HMBC spectrum (Figure 1), H-2′ has cross-peaks with carbons at δ_C 142.0, 148.4, and 133.1 (C-1′, -3′, and -4′, respectively), and H-6′ has cross-peaks with carbons at δ_C 142.0, 133.1, and 143.1 (C-1′, -4′ and -5′, respectively). This suggested that these two aromatic hydrogens are on the same phenyl ring. In addition, the HMBC spectrum of **1** showed correlations from the protons of the methylenedioxy group at δ_H 5.89 and 5.90 (each 1H, s, OCH_2O) to C-3′ and C-4′ and from OCH_3-5′ at δ_H 3.84 to

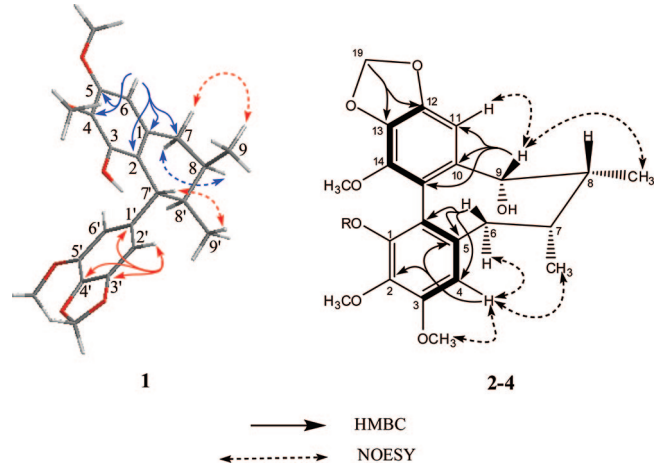
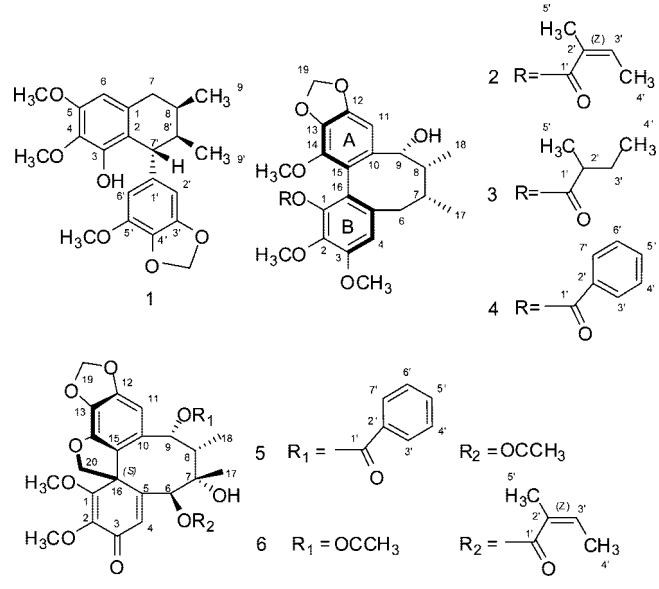


Figure 1. Key HMBC and NOESY correlations of compounds **1**–**4**.



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10.1021/np070269x CCC: $37.00
Published on Web 11/21/2007

Table 1. ^{1}H and ^{13}C NMR Data (600 MHz, $CDCl_3$) for **1**

position	δ_C	δ_H (mult; *J*, Hz)	position	δ_C	δ_H (mult; *J*, Hz)
1	133.0		4′	133.1	
2	116.0		5′	143.1	
3	147.6		6′	107.7	6.25, d (1.3)
4	133.5		7′	46.9	3.98, d (2.0)
5	150.6		8′	40.8	1.87, m
6	103.2	6.26, s	9′	13.5	0.93, d (7.0)
7	33.3	H_β 2.39, dd (17.2, 11.8)	4-OCH_3	61.0	3.83, s
		H_α 2.66, dd (17.2, 5.5)	5-OCH_3	55.6	3.86, s
8	25.9	1.98, m	5′-OCH_3	56.6	3.84, s
9	19.0	0.90, d (6.7)	OCH_2O	101.1	5.90, s
1′	142.0				5.89, s
2′	102.4	6.13, d (1.3)	OH		5.65, s
3′	148.4				

carbon C-5′. These HMBC correlations showed that the methylenedioxy and one methoxy group are on the same phenyl ring. The MS spectrum of **1** exhibited an intense peak at *m/z* 234 [$M^+ - C_8H_7O_3$], which indicated the elimination of a 1,2-methylenedioxy-3-methoxybenzene group.

In the HMBC spectrum, correlations of the aromatic hydrogen at δ_H 6.26 (1H, s) with the carbons at δ_C 133.0, 116.0, 133.5, 150.6, and 33.3 (C-1, C-2, C-4, C-5, and C-7, respectively) suggested this proton should be H-6. Furthermore, the correlations between the protons of two methoxy groups with C-4 and C-5 confirmed these two methoxy groups should be located at C-4 and C-5, respectively. In addition, a butano functional group was indicated by cross-peaks of H-7′ (δ_H 3.98, d, $J = 2.0$), H-8′ (δ_H 1.87, m), H-8 (δ_H 1.98, m), and H-7 ($\delta_{H\beta}$ 2.39, dd, $J = 17.2$, 11.8, $\delta_{H\alpha}$ 2.66, dd, $J = 17.2$, 5.5) in the ^{1}H−^{1}H COSY spectrum. From the data above, the skeleton of **1** was deduced as a substituted aryltetrahydronaphthalene lignan. The correlations of the hydroxy proton at δ_H 5.65 (O*H*-3) with carbons C-2 and C-4 in the HMBC spectrum suggested this hydroxy was located at C-3.

The ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC spectra supported the planar structure of **1** (Figure 1). The NOESY spectrum of **1** (Figure 1) showed correlations between H-7β and both H-9 and H9′, and between H-7′ and CH_3-9′. The coupling constant between H-7β and H-8 ($J = 11.8$) indicated that these protons are diaxial. In addition, a positive CD Cotton effect at 272 nm indicated that **1** is a (7′*R*,8′*R*,8*R*)-aryltetralin derivative.[15] Thus, the structure of **1** was assigned as shown in Figure 1.

Kadsuralignan I (**2**) was obtained as colorless needles. Its molecular formula, $C_{27}H_{32}O_8$, was determined by HREIMS ([M]$^+$, *m/z* 484.2104) with 12 degrees of unsaturation. The UV spectrum of **2** gave characteristic peaks (λ_{max} 220, 254 sh, and 290 sh nm) of dibenzocyclooctadiene lignans.[16] The ^{13}C NMR spectrum of **2** clearly indicated the presence of 12 aromatic carbons (δ_C 141.8, 138.8, 151.5, 113.0, 134.8, and 123.0 for C-1, C-2, C-3, C-4, C-5, and C-16, respectively; δ_C 139.9, 102.4, 148.7, 134.7, 140.8, and 118.4 for C-10, C-11, C-12, C-13, C-14, and C-15, respectively), indicative of two aromatic moieties. A butano functional group was indicated by cross-peaks of H-6 (δ_H 2.65, m), H-7 (δ_H 2.00, m), H-8 (δ_H 1.85, m), and H-9 (δ_H 4.75, s) in the ^{1}H−^{1}H COSY spectrum. Moreover, in the HMBC spectrum correlations were found between H-9 and C-11 and C-15, and between H-6 and C-4 and C-16. Combining these data, the skeleton of **2** was deduced as a substituted dibenzocyclooctadiene lignan, a class of compounds previously isolated from Schisandraceae plants.[16]

The functional groups evident from the ^{1}H and ^{13}C NMR data included three methoxy, one methylenedioxy, and four methyl groups. In the HMBC spectrum, the correlations between the methylenedioxy hydrogens (δ_H 5.91 and 5.92, each 1H, d, $J = 1.4$ Hz, OCH_2O-19) and C-12, and C-13, and between two methyl groups (CH_3-17, CH_3-18) and C-6 and C-9, respectively, as well as the correlations between three methoxy groups at δ_H 3.80, 3.84, and 3.90 (OCH_3-2, OCH_3-14, and OCH_3-3) and C-2, C-14, and C-3, respectively, confirmed the positions of these functional groups. After assigning the proton chemical shifts of three methoxy groups, their carbon chemical shifts were assigned at δ_C 61.1, 59.6, and 55.9 (for C-2, C-14, and C-3, respectively), in the HMQC spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **1** (Tables 2 and 3) with those of kadsuphilins A[17] indicated the same substitution pattern on the biphenyl unit A. The correlations between H-4 and C-2 and C-3 confirmed the two methoxy groups were located at C-2 and C-3, respectively. Moreover, characteristic signals of a 2-methyl-2-butenoate group were observed in the ^{1}H and ^{13}C NMR spectra. The MS spectrum of **2** exhibited a molecular ion at *m/z* 484 and an intense peak at *m/z* 384 [$M^+ - C_4H_7COOH$], indicating the 1,2-elimination of a 2-methyl-2-butenoic acid via McLafferty ester rearrangement, providing further evidence for the presence of a 2-methyl-2-butenoic acid ester in **2**.[16] A downfield shift at δ_H 4.75 for H-9 was due to the presence of a hydroxy group at C-9.[18] Thus, the 2-methyl-2-butenoic acid ester should be located on the aromatic ring. The NMR signals on the biphenyl unit B were found to be almost identical to those of propinquanin D.[18]

Table 2. ^{13}C NMR Data (600 MHz, $CDCl_3$) for **2**, **3**, and **4**

position	**2**	**3**	**4**	position	**2**	**3**	**4**
1	141.8	141.7	140.7	16	123.0	122.8	123.0
2	138.8	138.8	139.0	17	15.4	15.3	15.3
3	151.5	151.5	151.5	18	20.4	20.1	20.1
4	113.0	113.0	113.3	19	100.9	101.0	100.9
5	134.8	134.8	135.0	1′	167.3	176.4	165.9
6	38.9	38.8	38.9	2′	127.2	41.4	129.2
7	35.2	35.1	35.1	3′	138.7	26.7	130.2
8	42.9	43.0	42.9	4′	15.2	11.5	128.4
9	82.8	82.7	82.9	5′	20.1	16.7	133.5
10	139.9	140.0	139.9	6′			128.4
11	102.4	102.5	102.5	7′			130.2
12	148.7	148.8	148.7	2-O*C*H_3	61.1	61.1	61.3
13	134.7	135.0	134.8	3-O*C*H_3	55.9	55.9	56.0
14	140.8	140.8	142.1	14-O*C*H_3	59.6	59.7	59.8
15	118.4	118.3	118.1				

The configuration of the double bond of the 2-methyl-2-butenoic acid ester was deduced as *Z*, on the basis of the correlation between H-3′ and CH_3-5′ in the NOESY spectrum (Figure 1). Thus, the facts above confirm the planar structure of **2** shown in Figure 1.

The IR and NMR spectra of kadsuralignans J (**3**) and K (**4**) revealed that, like **2**, these compounds also have a C18 dibenzocyclooctadiene lignan skeleton with one hydroxy, one methylenedioxy, and three methoxy groups. The NMR spectra of **3** and **4** revealed a 2-methylbutanoic acid ester and a benzoic acid ester, respectively, instead of a 2-methyl-2-butenoic acid ester found in **2**. Moreover, the mass spectrum of **3** exhibited a molecular ion at *m/z* 486 and an intense peak at *m/z* 384 [$M^+ - C_4H_9COOH$], which are further evidence for the presence of a 2-methyl-2-butanoic acid ester in **3**. The mass spectrum of **4** exhibited a molecular ion at *m/z* 506 and a characteristic fragmentation ion at *m/z* 384 [M^+ − benzoic acid] corresponding to the benzoic acid ester in **4**. In addition, the ^{1}H NMR spectra of **3** and **4** showed a downfield shift

Table 3. ^{1}H NMR Data (600 MHz, $CDCl_3$) for **2**, **3**, and **4**

position	2	3	4
4	6.73, s	6.72, s	6.77, s
6	2.65, m	2.63, m	2.66, m
7	2.00, m	2.00, m	2.02, m
8	1.85, m	1.80, m	1.84, m
9	4.75, s	4.73, s	4.76, s
11	6.32, s	6.33, s	6.27, s
17	1.00, d (7.4)	1.00, d (7.4)	1.02, d (7.4)
18	1.17, d (7.4)	1.16, d (7.4)	1.18, d (7.4)
19	5.91, 5.92, each 1H, d (1.4)	5.94, 5.91, each 1H, d, (1.4)	5.74, 5.85, each 1H, d (1.6)
2′		2.41, m	
3′	6.00, m	1.40, 1.57, each 1H, m	8.05, 1H, m
4′	1.80, m	0.81, d (7.4)	7.43, 1H, m
5′	1.85, m	0.92, d (6.9)	7.57, 1H, m
6′			7.43, 1H, m
7′			8.05, 1H, m
1-OCH_3	3.84, s	3.87, s	3.86, s
2-OCH_3	3.80, s	3.79, s	3.80, s
3-OCH_3	3.90, s	3.89, s	3.92, s

at δ_H 4.73 and 4.76 for H-9, respectively, due to the presence of a hydroxy group at C-9. Thus, the ester group of **3** and **4** was located at C-1, respectively. The configuration of C-2′ of compound **3** is not yet defined.

In the NOESY spectrum, the correlations between H-11 and H-9, H-4 and H-6, H-4 and CH_3-17, H-9 and CH_3-18, and H-7 and CH_3-18 suggested that **2**, **3**, and **4** have a stable twist-boat chair conformation, similar to schisantherin P.[5]

To determine the configuration of these new compounds (**2**, **3**, and **4**), we examined their circular dichroism (CD) spectra. The CD spectra of **2**−**4** showed a positive Cotton effect around 215−225 nm and a negative Cotton effect around 225−245 nm, suggesting that these dibenzocyclooctadiene lignans (**2**−**4**) possessed an *S*-biphenyl configuration identical to gomisin B.[20,21]

Compounds **5** and **6**[22] are known compounds, whose structures were confirmed by comparison with literature reports.

We examined the inhibitory effects of these compounds on the production of NO induced by LPS/INF-γ. Compounds **1** and **3** showed inhibitory activity [**1**: IC_{50} = 7.6 μg/mL (19.6 μM); **3**: IC_{50} = 16.3 μg/mL (33.5 μM)] (Figure 2). Compound **2** showed very weak inhibitory activity [**2**: IC_{50} = 42.2 μg/mL (87.2 μM)]. Compounds **4**–**6** did not exhibit any inhibitory activity. Compound **1** showed stronger inhibition on NO production than quercetin, used as a positive control (IC_{50} = 25.0 μM). Quercetin is reported to have an inhibitory effect on the production of NO by LPS-stimulated macrophage cells RAW264.7 (IC_{50} = 26.8 μM).[23,24] Cytotoxic effects of these compounds were measured using the MTT assay. Compounds **1** (3–30 μg/mL) and **2/3** (10–100 μg/mL) did not show any significant cytotoxcity with LPS/INF-γ treatment for 24 h.

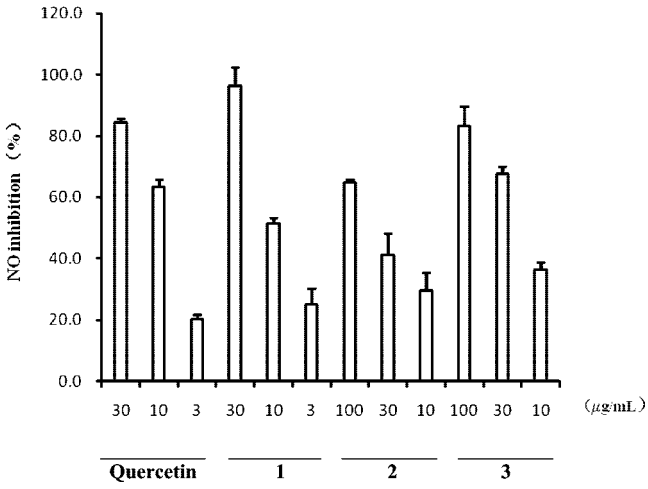

Figure 2. Inhibitory effects of quercetin and compounds **1**–**3** on NO production stimulated by LPS and INF-γ. RAW264.7 cells were treated with LPS/INF-γ alone or together with each compound at the concentrations indicated. After 16 h incubation, the supernatants were tested by Griess assay and the inhibitory rates were calculated. The experiment was performed three times, and the data are expressed as mean ± SD values. The inhibitory rate on NO production was calculated by the NO_2^- levels as follows: Inhibitory rate (%) = (1 − (LPS/INF/sample − untreated)/(LPS/INF − untreated)) × 100.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a JASCO DIP-360 polarimeter. The UV spectra were obtained in MeOH on a Shimadzu UV-160 spectrophotometer, the CD spectra were obtained in MeOH on a JASCO J-600 spectrophotometer, and the IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were recorded on a JEOL-ECA600 MHz spectrometer, with TMS as an internal standard. MS data were obtained on a JEOL GC mate spectrometer. Column chromatographies were carried out with a CHP-20P column (Mitsubishi Chemical Corp.). Thin-layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 instrument equipped with a JASCO UV-2075 detector. A COSMOSIL (Cholester Waters 10 × 250 mm) column was used for preparative purposes.

Plant Materials. The dried rhizomes of *K. coccinea* were collected in Guangxi Province, People's Republic of China, in April 2004 and were identified by Dr. Bao-Lin Guo, Peking Union Medical College, Beijing, China. Voucher specimens (NK04012) were deposited in the Department of Pharmacognosy, College of Pharmacy, Nihon University.

Extraction and Isolation. The dried rhizomes of *K. coccinea* (1.75 kg) were extracted three times with 80% acetone. Evaporation of the solvent under reduced pressure gave the extract (82.5 g). The extract was dissolved and suspended in water (2.0 L) and partitioned with $CHCl_3$ (3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L). The amounts extracted were 44.7, 4.0, and 14.2 g, respectively, and the residual aqueous extract yielded 20.6 g of material. The $CHCl_3$ and EtOAc extracts exhibited the strongest inhibitory activities (IC_{50}: 24.5 and 26.5 μg/mL, respectively). The *n*-BuOH and the aqueous extracts did not show any inhibitory activities. The EtOAc fraction was subjected to CHP-20P column chromatography (40 × 1000 mm, eluted with H_2O and MeOH in increasing polarity, H_2O−MeOH, 100:0 → 60:40). The column chromatographic fractions (100 mL each) were combined according to TLC monitoring into seven portions (toluene−EtOAc−HOAc, 70:33:3). Portion seven was further purified by HPLC (COSMOSIL, 10 × 250 mm, H_2O−CH_3CN, 30:70, 3 mL/min, UV detector λ = 210 nm) to give **1** (7 mg). Portion four was further purified by HPLC (COSMOSIL, 10 i.d. × 250, H_2O−CH_3CN, 30:70, 3 mL/min, UV detector λ = 210 nm) to give **2** (11 mg), **3** (12 mg), and **4** (9 mg). Portion two was further purified by HPLC (COSMOSIL, 10 i.d. ×

250 mm, H_2O-CH_3CN, 30:70, 3 mL/min, UV detector $\lambda = 210$ nm) to give **5** (7 mg) and **6** (3 mg).

Kadsuralignan H (1): colorless powder; $[\alpha]^{25}_D$ +49.1 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3210 (OH), 1712, 1633, 1508, 1454, 1426, 1120; UV (MeOH) (log ε) λ_{max} 219 (4.29), 270 (3.28) nm; CD (*c* 0.1, MeOH) [θ] (nm) +1100 (272), +4450 (219); EIMS *m*/*z* (rel int %) 386 $[M]^+$ (100), 234 (75), 219 (40), 180 (43), 73 (29); HREIMS *m*/*z* 386.1733 ($[M]^+$, calcd 386.1729 for $C_{22}H_{26}O_6$); ^1H and ^{13}C NMR data, see Table 1.

Kadsuralignan I (2): colorless, amorphous powder; $[\alpha]^{25}_D$ −23 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3200 (OH), 2933, 2876, 1716, 1616, 1055; UV (MeOH) (log ε) λ_{max} 220 (4.77) nm; CD (*c* 0.1, MeOH) [θ] (nm) −11120 (239), +19950 (217); ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* (rel int %) 484 $[M]^+$ (60), 466 (35), 384 (100), 369 (20), 353 (30), 208 (30), 83 (92); HREIMS *m*/*z* 484.2104 ($[M]^+$, calcd 484.2097 for $C_{27}H_{32}O_8$).

Kadsuralignan J (3): colorless, amorphous powder; $[\alpha]^{25}_D$ −59 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3200 (OH), 2965, 2876, 1734, 1683, 1619, 1101; UV (MeOH) (log ε) λ_{max} 220 (4.70) nm; CD (*c* 0.1, MeOH) [θ] (nm) −17300 (239), +28750 (219); ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* (rel int %) 486 $[M]^+$ (34), 402 (44), 384 (100), 369 (31), 346 (35), 208 (37), 105 (20); HREIMS *m*/*z* 486.2260 ($[M]^+$, calcd 486.2254 for $C_{27}H_{34}O_8$).

Kadsuralignan K (4): colorless, amorphous powder; $[\alpha]^{25}_D$ −37 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3200 (OH), 2922, 1717, 1633, 1558, 1506, 1455, 1382, 1110; UV (MeOH) (log ε) λ_{max} 219 (4.63) nm; CD (*c* 0.1, MeOH) [θ] (nm) −17300 (239), +35110 (219); ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* (rel int %) 506 $[M]^+$ (36), 488 (52), 384 (25), 208 (25), 105 (100); HREIMS *m*/*z* 506.1941 ($[M]^+$, calcd 506.1941 for $C_{29}H_{30}O_8$).

Inhibitory Activity on NO Production from Activated Macrophages-like Cell Line, RAW 264.7[23,24] The cells were seeded at 1.2×10^6 cells/mL onto a 96-well flat bottom plate and then incubated at 37 °C for 2 h. Next, the test sample was added to the culture simultaneously with both *Escherichia coli* LPS (100 ng/mL) and recombinant mouse IFN-γ (0.33 ng/mL). Then cells were incubated at 37 °C for approximately 16 h and subsequently chilled on ice. The culture supernatant (100 μL) was placed in duplicate in the wells of 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was placed in alternate wells on the same plate. To quantify nitrite, 50 mL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthylethylenediamide dihydrochloride) was added to each well. After 10 min the reaction products were colorimetrically quantified at 550 nm using a model 3550 microplate reader, and the background absorbance (630 nm) was subtracted. Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.

Acknowledgment. This investigation was supported by "Academic Frontier" Project for private universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) 2002−2007 of Japan. The authors are grateful to Dr. J. Zhu at Johns Hopkins University School of Medicine for manuscript reading.

Supporting Information Available: 1D and 2D NMR spectra of **1**, **2**, **3**, and **4** are available free charge via the Internet at http://pubs.acs.org.

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NP070269X