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# TWO NEW FUROFURAN LIGNANS FROM *KANDELIA OBOVATA*

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**Abstract** – Two new furofuran lignans, named kandelisesquilignan A and kandelisesquilignan B, were isolated from the aerial part of *Kandelia obovata*, together with seven known compounds *β*-sitosterol, betulinic acid, daucosterol, friedelin, lupeol, apigenin and acacetin. Their structures were characterized on the basis of spectral data. The two new compounds showed significant antioxidant activity by the DPPH method ($IC_{50}$ = 31.9 and 27.8 μg/mL).

*Kandelia obovata*, a kind of mangrove plant grows in the tropical and subtropical regions of East Asia, belongs to Rhizophoraceae. The plants of Kandelia in China and Japan were included within *K. candel* (Linnaeus) Druce before 2003. But *K. candel* is now recognized as an allopatric species ranging from India to Borneo.[1,2] In traditional medicine, the bark of *K. obovata* is used for constringency, hemostasia, and anti-bacteria, and the root of *K. obovata* is used for treating rheumatoid arthritis.[3] Ethanol and water extracts from this plant had been found to possess antifungal activity.[4] Previous phytochemical research of *K. obovata* had yielded lipids,[5] tannins,[6] flavonoids,[6] and polysaccharides.[7]

The extracts of some mangrove plants have significant antioxidant activity.[8] The antioxidant activity of extracts from *K. obovata* had been tested. It was supposed that phenolic compounds including condensed tannins may be responsible for the antioxidant activity.[9] In this research, we report the structural elucidation and antioxidant activity of two new furofuran lignans, together with seven known compounds from the aerial part of *K. obovata*.

The 80% aqueous ethanol extract prepared from the aerial part of *K. obovata* was suspended in $H_2O$, and further extracted with petroleum ether, ethyl acetate, and *n*-BuOH successively. The ethyl acetate extract was subjected repeatedly to column chromatography on silica gel and sephadex LH-20 to afford two new

furofuran lignans, kandelisesquilignan A and B (**1**-**2**), together with seven known compounds (**3**-**9**). The structures of compounds **1**-**2** were as shown in Figure 1. The seven known compounds, compared with literature spectroscopic data, were identified as β-sitosterol (**3**),[10] betulinic acid (**4**),[11] daucosterol (**5**),[12] friedelin (**6**),[13] lupeol (**7**),[14] apigenin (**8**)[15] and acacetin (**9**).[15]

Compound **1**, named kandelisesquilignan A, colorless optically active oil ($[\alpha]_D^{20}$ -6.8, MeOH; c=1), gave a molecular ion peak at *m/z* 584.2253 in the HREIMS, indicating a molecular formula of $C_{31}H_{36}O_{11}$ (calc. 584.2258). The $^1$H-NMR spectrum of **1** showed signals of four aromatic methoxy groups at $\delta_H$ 3.91 (12H, brs, 3,3',3",5"- OMe), along with signals due to eight aromatic protons in low field, including 2 ABX spin systems ($\delta_H$ 6.95, 1H, d, $J$ = 2.0 Hz, H-2, $\delta_H$ 6.84, 1H, d, $J$ = 8.5 Hz, H-5, $\delta_H$ 6.82, 1H, dd, $J$ = 2.0, 8.5 Hz, H-6; $\delta_H$ 6.89, 1H, d, $J$ = 2.0 Hz, H-2', $\delta_H$ 6.88, 1H, d, $J$ = 8.0 Hz, H-5', $\delta_H$ 6.85, 1H, dd, $J$ = 2.0, 8.0 Hz, H-6'). This indicated that **1** possibly consisted of two coniferyl and one syringyl phenylpropanoid residues. The $^{13}$C-NMR spectrum of **1** exhibited signals of eighteen aromatic carbons, nine aliphatic carbons, and four methoxy carbons. Comparison of $^1$H-, $^{13}$C-NMR data of **1** with those of ficusesquilignan A indicated that **1** possessed the same skeletal structure, and they differed in the presence of methoxy.[16] The $^1$H- and $^{13}$C-NMR spectrum showed signals at $\delta_H$ 4.75 (2H, d, $J$ = 5.0 Hz), 3.08 (1H, m), 3.10 (1H, m), 3.87 (2H, m), 4.27 (2H, m) and $\delta_C$ 85.9, 85.8, 54.2, 54.6, 71.6, 72.3 which are typical of the furofuran protons and carbons in lignans with 7,7'-diequatorial diaryl substitution with different aryl groups.[17,18] It also showed the signals at $\delta_C$ 74.3, 89.2 typical of the carbons of the aryl glycerol. The coupling constant for $J_{7",8"}$ (8.5 Hz) indicated a *threo* configuration for the aryl glycerol-8"-yloxy moiety.[18-20] In HMBC spectrum, crosspeaks between protons ($\delta_H$ 3.91, 12H, brs) and carbons resonated at $\delta$ 149.7, 146.5, 147.3, 147.3 indicated four methoxy groups attached to C-3,3',3",5" in **1** respectively. A crosspeak between H-8" and C-4 indicated an aryl glycerol portion with C-8" attached to C-4 by an ether linkage. Some HMBC correlations were shown in Figure 1. All protons and carbons were assigned with the help of interpretation of HSQC and HMBC spectra.

Compound **2**, named kandelisesquilignan B, colorless optically active oil ($[\alpha]_D^{20}$ -5.3, MeOH; c=1), gave a molecular ion peak at *m/z* 614.2357 in the HREIMS, indicating a molecular formula of $C_{32}H_{38}O_{12}$ (calc. 614.2363). Comparison of spectral data of compound **1** with those of **2** indicated that they shared the same type of structure, i.e. a furofuran with two aromatic substituents, one of which was attached to an aryl glycerol. Using information from the integration of the methoxy region of the $^1$H-NMR and HMBC spectrum of **2**, it showed that the extra methoxy attached to the C-5'. All protons and carbons were assigned with the help of interpretation of HSQC and HMBC spectra. Some HMBC correlations were shown in Figure 1.

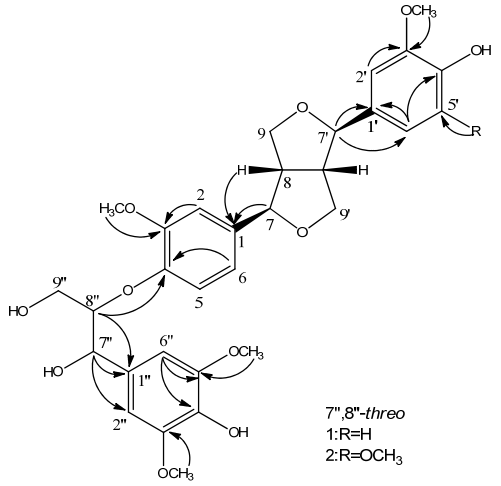


**Figure 1.** Selected HMBC correlations of compound **1** and **2**.

Furofuran lignans have antioxidant activity, antitumor activity, phosphodiesterase inhibition activity, and the effect on the central nervous system. They are widely biosynthesized in plants. The extracts and compounds were tested for their antioxidant activity based on the DPPH radical scavenging effect by the spectrophotometric method.[21]

Results of the DPPH assay on four crude extracts and four isolated compounds were shown in Table 1. Ascorbic acid was used as positive control. Kandelisesquilignan A and kandelisesquilignan B had significant antioxidant activity with $IC_{50}$ 31.9 and 27.8 μg/mL respectively. But *β*-sitosterol, betulinic acid, daucosterol, friedelin and lupeol showed no antioxidant activity.

**Table 1.** $IC_{50}$ values of the ethanolic extract, partitioned layers and some compounds isolated from the aerial part of *K. obovata* against DPPH free radical.

| Extract/Compound | $IC_{50}$(μg/mL) ± S.D. |
|---|---|
| crude ethanol extract | 68.7 ± 0.45 |
| petroleum ether extract | 75.2 ± 0.38 |
| ethyl acetate extract | 23.5 ± 0.57 |
| *n*-butanol extract | 357.1 ± 0.76 |
| kandelisesquilignan A | 31.9 ± 0.61 |
| kandelisesquilignan B | 27.8 ± 0.82 |
| apigenin | 39.2 ± 0.93 |
| acacetin | 107.4 ± 0.79 |
| ascorbic acid | 21.7 ± 0.65 |

## EXPERIMENTAL

**General**. Optical rotations were measured on a Jasco 1020 polarimeter. HREIMS spectra were recorded on a Finnigan MAT TSQ 700 mass spectrometer. UV spectra were obtained in a Beckman DU-640 UV spectrophotometer. IR spectra were obtained in KBr disc on a Nicolet 670 FT-IR spectrophotometer. The NMR spectra were obtained on a Bruker AV-500 spectrometer with TMS as internal standard (500 MHz for $^{1}$H NMR, 125 MHz for $^{13}$C NMR). Column chromatography was performed on silica gel (200-300 mesh), or on Sephadex LH-20.

**Plant material**. The aerial part of *K. obovata* was collected in August 2010 from the mangrove area of Ximen island, Yueqing, Zhejiang Province, China. The material was identified by Prof. Shaobo Chen, Zhejiang Mariculture Research Institute. A voucher specimen (No. WMC1005) is deposited at the herbarium of Wenzhou Medical College.

**Extraction and Isolation**. The dry powdered aerial part of *K. obovata* (4.0 kg) was extracted with EtOH-water (80:20, v/v, 10 L) at room temperature (3 days each time) three times. After evaporation of the solvent under reduced pressure, the residue (700 g) was suspended in water, and further extracted with petroleum ether, EtOAc, and *n*-BuOH successively. After removal of solvent, the EtOAc extract (43 g) was fractionated into 12 fractions (F1~F12) by silica gel column chromatography using a gradient of mixtures of $CHCl_3$-MeOH (99:1 to 60:40). F2 (3.2 g) was subjected to a silica gel column chromatography eluting with a gradient of petroleum ether- EtOAc (9:1 to 1:1) to give 10 fractions (F2-1~F2-10). Crude compounds **3**, **4**, **6** and **7** were eluted respectively and recrystallized to give **3** (12 mg), **4** (8.7 mg), **6** (7.9 mg) and **7** (11 mg). Subfraction F2-5 (79 mg) was purified by repeated Sephadex LH-20 column chromatography eluting with MeOH –water (3:1) to give compound **1** (28.1 mg). Subfraction F2-6 (56 mg) was purified by repeated Sephadex LH-20 column chromatography eluting with MeOH –water (3:1) to give compound **2** (17.6 mg). F5 (2.3 g) was separated by silica gel column chromatography (petroleum ether- EtOAc (4:1 to 1:1)) to give 5 fractions (F5-1~F5-5). Crude compounds **5**, **8** and **9** were eluted respectively and purificated to give **5** (25 mg), **8** (13 mg) and **9** (16 mg).

**Kandelisesquilignan A (1)**. colorless oil, $[\alpha]_D^{20}$ -6.8 (MeOH; c=1), HREIMS *m/z*: 584.2253 $[M]^+$, UV (MeOH) $\lambda_{max}$ nm: 233.4, 275.6, IR (KBr) $\nu_{max}$ 3445, 2927, 1587, 1521, 1468, 1421, 1275, 1237, 1129, 1035, 827 cm$^{-1}$, $^{1}$H-NMR (500 MHz, $CDCl_3$): $\delta$ 6.95 (1H, d, $J$ = 2.0 Hz, H-2), 6.84 (1H, d, $J$ = 8.5 Hz, H-5), 6.82 (1H, dd, $J$ = 2.0, 8.5 Hz, H-6), 4.75 (1H, d, $J$ = 5.0 Hz, H-7), 3.10 (1H, m, H-8), 3.87 (3H, m, H-9, H-9', H-8''), 6.89 (1H, d, $J$ = 2.0 Hz, H-2'), 6.88 (1H, d, $J$ = 8.0 Hz, H-5'), 6.85 (1H, dd, $J$ = 2.0, 8.0 Hz, H-6'), 4.75 (1H, d, $J$ = 5.0 Hz, H-7'), 3.08 (1H, m, H-8'), 4.27 (2H, m, H-9, H-9'), 6.60 (1H, brs, H-2''), 6.60 (1H, brs, H-6''), 5.01 (1H, d, $J$ = 8.5 Hz, H-7''), 3.31 (1H, m, H-9''), 3.56 (1H, m, H-9''), 3.91 (12H, brs, 3-OMe, 3'-OMe, 3''-OMe, 5''-OMe); $^{13}$C-NMR (125 MHz, $CDCl_3$): $\delta$ 134.7 (C-1), 109.8 (C-2), 149.7 (C-3), 147.1 (C-4), 114.3 (C-5), 119.0 (C-6), 85.9 (C-7), 54.2 (C-8), 71.6 (C-9), 132.8 (C-1'), 108.3 (C-2'),

146.5 (C-3'), 145.4 (C-4'), 114.3 (C-5'), 119.0 (C-6'), 85.8 (C-7'), 54.6 (C-8'), 72.3 (C-9'), 132.2 (C-1''), 102.9 (C-2''), 147.3 (C-3''), 134.2 (C-4''), 147.3 (C-5''), 102.9 (C-6''), 74.3 (C-7''), 89.2 (C-8''), 60.1 (C-9''), 56.3 (3-OMe), 56.0 (3'-OMe, 3''-OMe, 5''-OMe).

**Kandelisesquilignan B (2)**. colorless oil, $[\alpha]_D^{20}$ -5.3 (MeOH; c=1), HREIMS *m/z*: 614.2357 $[M]^+$, UV (MeOH) $\lambda_{max}$ nm: 235.1, 276.4, IR (KBr) $\nu_{max}$ 3441, 2932, 1588, 1525, 1467, 1422, 1275, 1235, 1123, 1031, 823 $cm^{-1}$, $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ 6.96 (1H, d, $J$ = 2.0 Hz, H-2), 6.85 (1H, d, $J$ = 8.5 Hz, H-5), 6.82 (1H, dd, $J$ = 2.0, 8.5 Hz, H-6), 4.74 (1H, d, $J$ = 5.0 Hz, H-7), 3.09 (1H, m, H-8), 3.92 (2H, m, H-9, H-9'), 6.62 (1H, brs, H-2'), 6.62 (1H, brs, H-6'), 4.74 (1H, d, $J$ = 5.0 Hz, H-7'), 3.09 (1H, m, H-8'), 4.30 (2H, m, H-9, H-9'), 6.58 (1H, brs, H-2''), 6.58 (1H, brs, H-6''), 5.02 (1H, d, $J$ = 8.0 Hz, H-7''), 3.90 (1H, m, H-8''), 3.32 (1H, m, H-9''), 3.56 (1H, m, H-9''), 3.90 (15H, brs, 3-OMe, 3'-OMe, 5'-OMe, 3''-OMe, 5''-OMe); $^{13}$C-NMR (125 MHz, $CDCl_3$): $\delta$ 134.6 (C-1), 109.9 (C-2), 149.7 (C-3), 147.3 (C-4), 114.3 (C-5), 120.4 (C-6), 86.0 (C-7), 54.5 (C-8), 71.8 (C-9), 132.0 (C-1'), 102.9 (C-2'), 147.3 (C-3'), 134.6 (C-4'), 147.3 (C-5'), 102.9 (C-6'), 85.9 (C-7'), 54.5 (C-8'), 72.1 (C-9'), 131.4 (C-1''), 102.9 (C-2''), 147.2 (C-3''), 134.6 (C-4''), 147.2 (C-5''), 102.9 (C-6''), 74.1 (C-7''), 89.2 (C-8''), 60.7 (C-9''), 56.5 (3-OMe), 56.2 (3'-OMe, 5'-OMe), 56.0 (3''-OMe, 5''-OMe).

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