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Taiwankadsurins A, B, and C, Three New C19 Homolignans from *Kadsura philippinensis*

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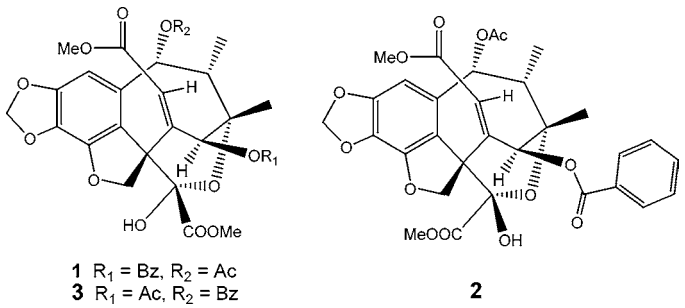
ABSTRACT



1 R_1 = Bz, R_2 = Ac
3 R_1 = Ac, R_2 = Bz

2

Three novel C19 homolignans, designated taiwankadsurins A (1), B (2), and C (3), were isolated from the aerial parts of Taiwanese medicinal plant *Kadsura philippinensis*. The structures of 1–3, which have a 3,4-{1′-[(*Z*)-2″-methoxy-2″-oxo-ethylidene]}-pentano(2,3-dihydro-benzo[*b*]-furano)-3-(2‴-methoxycarbonyl-2‴-hydroxy-2‴,3′-epoxide) skeleton, were determined by spectroscopic analyses, especially 2D NMR techniques (HMBC and NOESY). Compound 2 exhibited mild cytotoxicity against human KB and Hela tumor cells.

The genus *Kadsura* (Schisandraceae) is closely related to *Schisandra*, and many of its species are extensively used as a Chinese medicine in Taiwan, Japan, and mainland China, mostly as a substitute for *Schisandra*.[1] Phytochemical investigations of *Kadsura* revealed that it is a rich source of lignans possessing structures similar to those isolated from *Schisandra*.[2−5] The genus *Kadsura* is a promising source of bioactive lignans. Some lignans and triterpene lactones from *Kadsura* species proved their effectiveness as antitumor, antiviral, and hepatoprotective agents.[6−9] The bioactivities and structures activity relationship (SAR) of the lignans isolated from species *Kadsura* had prompted us to work toward discovery and development of potential antitumor and antiviral drugs from plants belonging to these genera.[10] The successful synthesis of the dibenzocyclooctadienes based on the natural lignans provided a new route to manufacture a practical and potential anti-HIV agent.[11]

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Kadsura philippinensis Elmer, indigenous to southern Taiwan, has been used as a folk medicine for the treatment of rheumatism and headache.[12] Previously, we have reported two new triterpenoids from the aerial part of *K. philippinensis*.[13] In the continuous search for bioactive metabolites from this plant, three novel C19 homolignans, taiwankadsurins A (**1**), B (**2**), and C (**3**), have been isolated and characterized. This paper decribes the isolation, structural elucidation, and biological activity, as well as biogenetic pathway, of the new **1**−**3**.

The leaves and stems of *K. philippinensis* were extracted with a mixture of CH_2Cl_2 and acetone, and the extract was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble fraction was subjected to a silica gel column (*n*-hexane/EtOAc, 1:0 to 0:1), from which a fraction (fr 21) was eluted with LH-20 (MeOH) to give fr 21-3 (4 g). This residue was chromatographed on a flash column (silica gel, *n*-hex/EA, 15:1−0:1) and further separated by normal phase HPLC (*n*-hexane/CH_2Cl_2/MeOH, 35:65:1) to furnish taiwankadsurins A (**1**, 38 mg), B (**2**, 4 mg), and C (**3**, 7 mg).

Taiwankadsurin A (**1**) was obtained as an amorphous powder ($[\alpha]_D$ +20°, CH_2Cl_2) and possesses the molecular formula $C_{31}H_{30}O_{13}$, as derived from its HREIMS at *m*/*z* 610.1690 ($[M]^+$, calcd 610.1681) indicating 17 degrees of unsaturation.[14] The UV bands (245, 276 nm) and IR absorption at 1731, 1650 cm^{-1} indicated a benzyl, a benzoyl ester, and an α,β-unsaturated ester functionalities. The ^1H NMR spectrum (Table 1) of **1** showed the presence of a

Table 1. ^1H NMR Data ($CDCl_3$, 300 MHz) of **1**−**3**[a]

position	1	2	3
4	6.08 d (2.7)	6.17 d (2.7)	6.04 brs
6	6.49 d (2.7)	6.91 d (2.7)	6.21 brs
8	2.36 m	2.38 m	2.37 m
9	6.69 d (3.0)	6.80 d (3.0)	6.74 brs
11	6.60 s	6.60 s	6.61 s
17	1.34 s	1.37 s	1.32 s
18	1.05 d (6.9)	1.04 d (6.6)	1.13 d (6.9)
19	5.99 s, 6.02 s	5.95 s, 5.96 s	5.98 s
20	4.54 d (10.5)	4.63 d (9.9)	4.50 d (10.2)
20	5.00 d (10.5)	4.98 d (9.9)	5.00 d (10.2)
OMe-2	3.96 s	3.61 s	3.93 s
OMe-3	3.57 s	3.56 s	3.63 s
OAc	2.21 s	2.21 s	2.33 s
3′, 7′	8.31 d (7.5)	8.32 d (7.2)	8.12 d (7.2)
4′, 6′	7.53 t (7.5)	7.52 t (7.2)	7.49 t (7.2)
5′	7.65 t (7.5)	7.64 t (7.2)	7.61 t (7.2)

[a] Chemical shifts in ppm, *J* values in Hz are in parentheses. Assignments were made using HMQC and HMBC techniques.

benzoyl group (δ 8.31d, 7.65t, and 7.53t, J = 7.5 Hz), two methoxyl singlets (δ 3.96, 3.57), an acetyl singlet (δ 2.21), a methyl singlet (δ 1.34), and a methyl doublet (δ 1.05, J = 6.9 Hz). Two oxymethylene AB quartets appeared at δ 5.00 and 4.54 (J = 10.5 Hz) in addition to two usual dioxymethylene singlets at δ 5.99 and 6.02. In the downfield region, a singlet at δ 6.60 represented an isolated H-11 of the phenyl ring. Moreover, two oxymethines were observed at δ 6.69 (H-9) and 6.49 (H-6). The latter signal was correlated with a doublet at δ 6.08 (H-4) through allylic coupling (J = 2.7 Hz) in the COSY spectrum of **1**. The ^{13}C NMR spectrum (Table 2) and DEPT revealed that **1** possessed corresponding

Table 2. ^{13}C NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**−**3**[a]

position	1	2	3
1	97.6 s	97.8 s	97.5 s
2	171.1 s	170.2 s	171.0 s
3	165.5 s	165.7 s	165.4 s
4	117.3 d	118.5 d	117.6 d
5	150.4 s[b]	149.6 s[c]	150.3 s[d]
6	73.3 d	73.4 d	73.1 d
7	79.3 s	78.6 s	79.0 s
8	45.3 d	45.4 d	45.3 d
9	70.5 d	70.5 d	71.1 d
10	127.8 s	127.8 s	127.8 s
11	99.1 d	99.4 d	98.9 d
12	150.3 s[b]	149.9 s[c]	150.4 s[d]
13	129.0 s	128.8 s	129.5 s
14	144.6 s	142.5 s	144.2 s
15	118.2 s	120.7 s	118.1 s
16	57.1 s	58.8 s	56.8 s
17	28.3 q	28.6 q	28.4 q
18	8.8 q	8.5 q	9.1 q
19	101.9 t	101.7 t	101.9 t
20	80.5 t	78.6 t	80.5 t
OMe-2	53.7 q	54.1 q	53.6 q
OMe-3	51.8 q	51.8 q	51.8 q
OAc	169.1 s	169.1 s	169.9 s
	21.3 q	21.3 q	20.8 q
1′	165.5 s	165.6 s	164.7 s
2′	128.4 s	130.0 s	130.1 s
3′, 7′	128.9 d	128.8 d	128.6 d
4′, 6′	130.6 d	130.6 d	128.6 d
5′	134.1 d	133.9 d	133.3 d

[a] Assignments were made using HMQC and HMBC techniques. [b−d] Data interchangeable.

signals including four carbonyl esters (δ 171.1, 169.1, 165.5, 165.5), two aromatic rings (including monosubsitituted and pentasubstituted benzene moieties, total 12 carbons), two olefinic carbons (C-4, δ 117.3 and C-5, δ 150.4), one dioxymethylene carbon (δ 101.9), one oxymethylene carbon (δ 80.5), two oxymethine carbons (δ 73.3, 70.5), two

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(14) $[\alpha]^{26}_D$ +20° (*c* 0.5, CH_2Cl_2); UV λ_{max} (MeOH) 245, 276 nm; CD (MeOH, *c* 0.37) nm (ϵ) 227 (−1.30), 254 (+1.27), 324 (+0.40); IR (neat) ν_{max} 3443, 2954, 1731, 1561, 1505, 1487, 1452, 1435, 1372, 1266, 1094, 1026, 935, 736, 713 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; FABMS *m*/*z* 633 $[M + Na]^+$, 611 $[M + H]^+$, 610 $[M]^+$; EIMS *m*/*z* 610 ($[M]^+$, 0.2), 550 ($[M - AcOH]^+$, 0.2), 506 (0.2), 488 (0.2), 446 (0.1), 384 ($[506 - benzoic\ acid]^+$, 1.1), 341, 324 ($[506 - benzoic\ acid - AcOH]^+$, 4), 285, 281, 269, 253, 239, 215, 105, 77; HREIMS *m*/*z* 610.1690 (calcd for $C_{31}H_{30}O_{13}$, 610.1681).

oxygenated tertially carbons (δ 97.6, 79.3), two methoxyl (δ 53.7, 51.8), one acetyl (δ 21.3), and two methyl (δ 28.8, 8.8) groups. The structure and relative stereochemistry of compound **1** were elucidated from its COSY, HMQC, and HMBC studies. The COSY spectrum of **1** showed the correlations of Me-18/H-8/H-9 and H-20a/H-20b in addition to H-4/H-6. The HMBC experiment (Figure 1) of **1** revealed

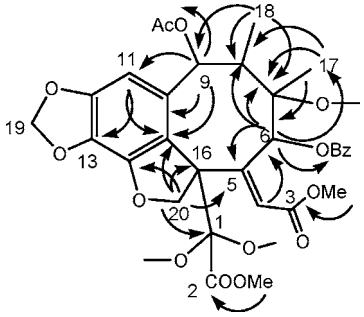

Figure 1. HMBC and partial structure of compound **1**.

the correlations of H-11/C-13,C-15 and H-20/C-14,C-15,C-16, indicating that **1** contains a dihydrobenzofuran system. The correlations of H-9/C-10,C-11,C-15; H-18/C-7,C-8,C-9; H-17/C-6,C-7,C-8; H-6/C-4,C-5,C-7,C-8 and H-20/C-5 disclosed the partial structuture of the ethylidene-octane ring, in which the H-9 (δ 6.69) was correlated with the acetyl carbonyl and H-6 (δ 6.49) was connected with the benzoyl carbonyl.

On the other hand, one methoxyl (δ 3.96) was correlated with the carbonyl C-2 (δ 171.1). The other methoxyl (δ 3.57) was correlated with the carbonyl C-3 (δ165.5). The connection of C-1 (δ 97.6) and C-16 (δ 57.1) was inferred from HMBC correlation of H-20 with C-1. Because compound **1** occupied 17 degrees of unsaturation, one more ether ring was required between C-1 and C-7. The relative stereochemistry of taiwankadsurin A (**1**) was determined by the NOESY experiment (Figure 2). Thus, cross-peaks of H-4/H-9, H-9/

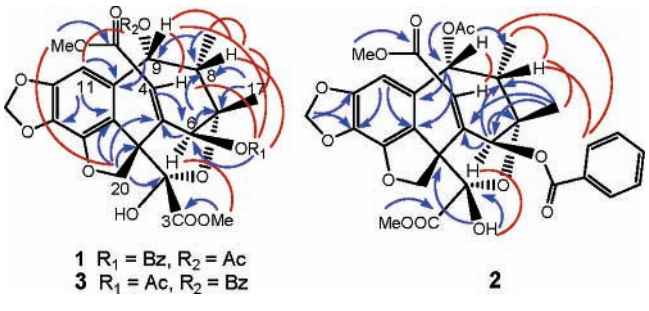

Figure 2. Selective HMBC (hook) and NOESY (curve) of **1**−**3**.

H-8 (δ 2.36), H-8/H-17, H-4/H-3′, H-8/H-3′ and H-9/H-3′ suggested that the H-9, H-8, H-17 and 6-*O*-benzoyl group should be positioned on the β-face of the molecule. The correlation between H-6 and the C-3 methoxyl indicated that H-6 was α−orientation. The computer-modeled structure of **1** was generated by CS Chem 3D version 9.0 using MM2 force field calculation for energy minimization as shown in Figure 3. The result was consistent with the stereochemistry

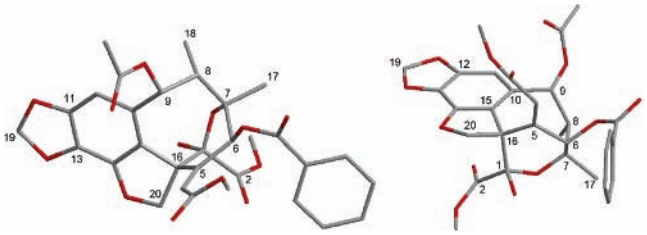

Figure 3. Computer-generated perspective models of **1** and **2** using MM2 force field calculation. Protons were omitted for clarity.

of **1** as established by NOESY experiments. Moreover, the circular dichroism (CD) spectrum of **1** exhibited a negative Cotton effect at 227 nm and positive Cotton effect at 254 and 324 nm. On the basis of above findings, the structure of taiwankadsurin A was unambiguously established as **1**, which possesses a novel skeleton of 3,4-{1′-[(*Z*)-2″-methoxy-2″-oxo-ethylidene]}-pentano-(2,3-dihydro-benzo[*b*]furano)-3-(2‴-methoxycarbonyl-2‴-hydroxy-2‴,3′-epoxide). According to IUPAC sequence rule, the relative stereochemistry of the chiral carbons was assigned as 6*S**,7*S**,8*S**,9*R**.

Taiwankadsurin B (**2**), $[\alpha]_D$ +62° (CH_2Cl_2), had the same molecular formula ($C_{31}H_{30}O_{13}$) as **1**, as derived from HRESIMS at *m*/*z* 633.1581 (calcd 633.1584).[15] The UV and IR absorptions of **2** were similar to those of **1**, suggesting a close analogue. The ^{1}H NMR spectral data (Table 1) of **2** resembled those of **1** except that the signal of H-6 shifted downfield to δ 6.91, while the C-2 methoxyl protons shifted upfield to δ 3.61. Comparison of the ^{13}C NMR spectral data (Table 2) of **2** with those of **1** revealed that the only difference between them were C-14 (δ 142.5, 144.6), C-15 (δ 120.7, 118.2), C-16 (δ 58.8, 57.1) and C-20 (δ 78.6, 80.5) for **2** and **1**, respectively. The location of benzoyl, acetyl and methoxyl groups were same as **1** as observed from HMBC correlation of **2** (Figure 2). Furthermore the fact that compound **2** can be spontaneously obtained from **1** confirmed that all of the substituents in **1** and **2** are regiochemically at the same positions. The stereochemistry of **2** was further established from NOESY, in which most of the cross-peaks were identical to those of **1**. However, the correlation

(15) $[\alpha]^{26}_D$ +62° (*c* 0.4, CH_2Cl_2); UV λ_{max} (MeOH) 248, 274 nm; CD (MeOH, *c* 0.35) nm (ϵ) 230 (−0.80), 242 (−0.64), 250 (+1.10), 280 (+0.75), 298 (−0.28), 365 (+0.07); IR (neat) ν_{max} 3446, 2988, 2950, 1731, 1650, 1486, 1390, 1261, 1096, 1058, 1027, 934, 713 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; FABMS *m*/*z* 633 $[M + Na]^+$, 610 $[M]^+$, 609 $[M - H]^+$; EIMS *m*/*z* 610 ($[M]^+$, 1), 550 ($[M - AcOH]^+$, 1), 506 (1), 488 (0.2), 446 (3.4), 384 ($[506 - benzoic\ acid]^+$, 1.1), 341, 324 ($[506 - benzoic\ acid - AcOH]^+$, 4), 285, 281, 269, 253, 239, 215, 105, 77; HRESIMS *m*/*z* 633.1581 (calcd for $C_{31}H_{30}O_{13}Na$, 633.1584).

between H-6 and the carbomethoxyl of C-1 was missing in **2**. Instead, correlation between H-6 and the hydroxyl of C-1 was observed and illustrated in Figure 2. A computer-generated perspective model for **2** was also established as shown in Figure 3. The CD spectrum of **2** exhibited negative Cotton effect at 230, 242 and 298 nm and positive Cotton effect at 250, 280 and 365 nm. The above findings suggested that compound **2** was an epimer of **1** and designated as taiwankadsurin B.

Taiwankadsurin C (**3**), $[\alpha]_D$ −30° (CH_2Cl_2), is an isomer of **1** and **2** due to HRESIMS at *m*/*z* 633.1581 (calcd 633.1584).[16] The UV and IR bands of **3** resemble those of **1** and **2**, indicating a similar structure. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of **3** were very similar to those of **1** except for the signals of H-6 (δ 6.21) and H-3′ (δ 8.12), which appeared at higher field region as compared with those of **1**. Detailed analysis of the ^{13}C NMR spectral data of **3** indicated that the signals of benzoyl and acetyl carbonyls had a difference in resonances of about 0.8 ppm (δ 169.9 and 164.7 for **3**; δ 169.1 and 165.5 for **1**). Thus, the location of the benzoyl and the acetyl groups should be changed in **3**. This finding was supported by an HMBC experiment of **3** (Figure 4), in which the correlations between H-6 and the benzoyl carbonyl and between H-9 and the acetyl carbonyl were observed.

The relative configuration of **3** was determined by comparison of the coupling constants of **3** and **1** and further confirmed by NOESY experiment (Figure 2). The correlations between H-11 and the benzoyl H-3′,7′, between H-4 and the acetyl protons, and between H-4 and H-9 assigned the H-9 at β-configuration and H-6 as α- disposition same as **1**. The circular dichroism spectrum of **3** exhibited a strong negative Cotton effect at 312 and 372 nm. Therefore, compound **3** was designated as taiwankadsurin C.

A plausible biogenetic pathway of **1**−**3** was postulated as shown in Scheme 1 based on dimerization of phenylpropanoid units[17,18] and recently published structures such as schiarisanrins A−D and taiwanschirins A−C.[19,20] These homolignans might be derived from dibenzocyclooctadiene lignans. This pathway involves several steps of oxidative coupling reactions, C−C bond formation, breakage of a C═C bond, and finally hemiacetal formation.

Compounds **1**−**3** were tested for cytotoxicity against human KB and Hela tumor cells using the MTT method as reported previously.[21] As shown in Table 3, taiwankadsurins A (**1**) and B (**2**) exhibited mild cytotoxicity, whereas compound **3** was inactive in vitro against these tumor cell lines.

Scheme 1. Plausible Biogenetic Pathway of **1** and **2**

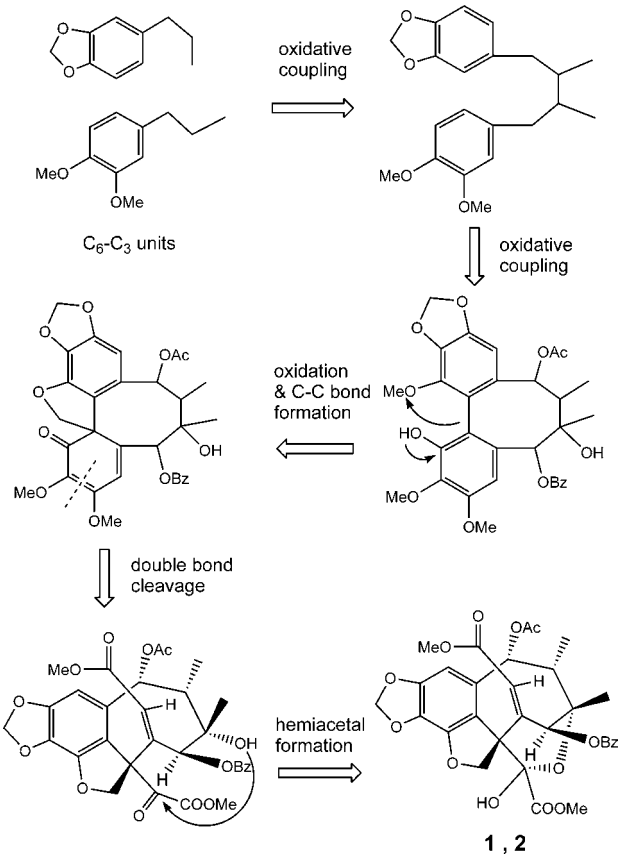

Table 3. Cytotoxicity (IC_{50}, μg/mL)[a] of Isolated Homolignans **1**−**3**

compound	KB[b]	Hela[c]
taiwankadsurin A (**1**)	18.6	>20
taiwankadsurin B (**2**)	13.0	12.7
taiwankadsurin C (**3**)	>20	>20

[a] The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the method published previously.[21] [b] Human oral epidermoid carcinoma, [c] Human cervical epitheloid carcinoma.

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Supporting Information Available: ^1H and ^{13}C NMR spectral data for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) $[\alpha]^{26}_D$ −30° (*c* 0.7, CH_2Cl_2); UV λ_{max} (MeOH) 246, 275, 282 nm; CD (MeOH, *c* 0.37) nm (ϵ) 226 (−0.95), 278 (+0.79), 312 (−1.34), 372 (+0.68); IR (neat) ν_{max} 3446, 2988, 2950, 1728, 1650, 1488, 1392, 1273, 1218, 1092, 942, 713 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; FABMS *m*/*z* 633 [M + Na]$^+$, 611 [M + H]$^+$; EIMS *m*/*z* 610 ([M]$^+$, 0.4), 550 ([M − AcOH]$^+$, 0.2), 506 (0.2), 488 (1), 446 (1), 384 ([506 − benzoic acid]$^+$, 1.1), 341, 324 ([506 − benzoic acid − AcOH]$^+$, 4), 285, 281, 269, 253, 239, 215, 105, 77; HRESIMS *m*/*z* 633.1581 (calcd for $C_{31}H_{30}O_{13}Na$, 633.1584).

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