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Kadsuphilactones A and B, Two New Triterpene Dilactones from *Kadsura philippinensis*

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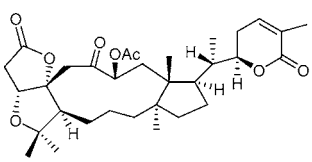
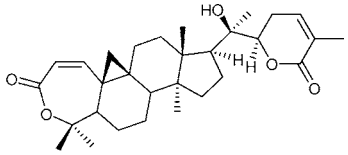
ABSTRACT



kadsuphilactone A (**1**)



kadsuphilactone B (**2**)

Two novel triterpene dilactones, kadsuphilactones A (1) and B (2), were isolated from the Taiwanese medicinal plant *Kadsura philippinensis*. The structures of 1 and 2 were elucidated on the basis of extensive spectroscopic methods, including two-dimensional NMR techniques, and confirmed by X-ray crystallographic analysis. Kadsuphilactone B (2) exhibited in vitro anti-HBV activity with IC_{50} values of 6 μg/mL by HBsAg enzyme immunoassay.

Hepatitis B virus (HBV) infection commonly results in chronic and acute hepatitis and is associated with a high risk of developing liver cancer in humans.[1] Recently, it was demonstrated that the hepatocellular carcinoma cell line, HepA2, containing the HBV genome, which continually secretes hepatitis B surface antigen (HBsAg) into the culture medium, can serve as a quick assay system for screening agents for anti-HBV activity.[2,3] Plants of the genus *Kadsura* (Schizandraceae) have been used in traditional Chinese medicine to treat a variety of diseases.[4−10] Phytochemically, isolates from *Kadsura* species also showed antihepatitis activities and inhibitory activities against cholesterol biosynthesis. A literature survey about a related shrub is well-known to produce novel triterpenes[11−15] that showed significant antiviral activity such as anti-HIV activity.

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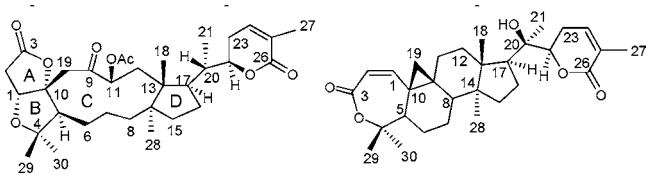
Kadsura philippinensis Elmer, indigenous to southern Taiwan, has not been studied chemically.[16] This plant has been used as a folk medicine for the treatment of rheumatism and headache. To explore the origin of its bioacivity, an investigation of the chemical constituents of this plant was carried out that led to the isolation of two novel triterpene dilactones, kadsuphilactones A (**1**) and B (**2**), from the EtOAc extract. This paper decribes the isolation and structural elucidation of the unprecedented triterpene **1** and a new triterpene **2**.



The leaves and stems of *K. philippinensis* were extracted with a mixture of CH_2Cl_2 and acetone (1:1), and the extract was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble fraction was subjected to a Si gel column (*n*-hexane/EtOAc, 1:0 to 0:1), from which a fraction (fraction 21) was separated by Sephadex LH-20 (MeOH) followed by normal-phase HPLC (*n*-hexane/CH_2Cl_2/MeOH, 35:65:1), and recrystallization from MeOH−EtOH to furnish kadsuphilactones A (**1**, 0.00092%) and B (**2**, 0.0012%). Kadsulactone (**3**, 0.002%)[17] was directly obtained from fraction 9, while schisanlactone B (**4**, 0.0001%)[18] was isolated from extensive Sephadex LH-20 (MeOH), Si gel column (*n*-hexane/acetone, 7:1 to 1:1), and preparative thin-layer chromatography (*n*-hexane/CH_2Cl_2/MeOH, 3:2:0.5) of fraction 19.

Kadsuphilactone A (**1**) was obtained as colorless prisms (mp 193 °C) and possessed the molecular formula $C_{32}H_{46}O_8$, as derived from its HRESIMS (*m*/*z* 581.3086 $[M + Na]^+$, calcd 581.3090), indicating 10 degrees of unsaturation.[19] The IR absorption bands at 1781 and 1724 cm^{-1} indicated a γ-lactone functionality and a carbonyl functionality. This was supported by CD absorption bands at 260 and 310 nm. The ^1H NMR spectrum (Table 1) of **1** showed the presence of

Table 1. ^1H and ^{13}C NMR Data of **1**[a]

no.	δ_H (mult, *J*, Hz)	δ_C[b]	HMBC ($^1H-^{13}C$)
1	4.15 (d, 5.7)	81.3	H-2
2	2.83 (dd, 6.3, 18.9)	35.1	H-1
2	2.61 (d, 18.9)		
3		174.9	H-2
4		84.1	H-5, Me-29, Me-30
5	1.94 (m)	60.4	Me-29, Me-30
6	1.50 (overlap)	33.3	
7	1.51 (overlap)	27.4	
8	2.37 (m), 2.04 (m)	23.5	Me-28
9		198.4	
10		94.9	H-1, H-2, H-5
11	5.54 (brs)	75.5	
12	1.90 (m), 2.50 (m)	37.8	H-11
13		50.7	
14		47.9	
15	1.42 (m), 1.74 (m)	25.7	Me-28
16	2.44 (m), 2.50 (m)	32.4	
17	1.66 (m)	46.8	H-16, Me-21, Me-18
18	0.86 (s)	13.7	
19	2.40 (d, 10.5)	43.3	
19	3.19 (d, 10.5)		
20	2.00 (m)	39.9	Me-21, H-22
21	1.03 (d, 6.6)	14.1	H-22
22	4.44 (d, 12.9)	80.3	Me-21
23	2.08 (m)	26.1	
24	6.59 (d, 5.4)	139.6	H-22, Me-27
25		128.2	Me-27
26		166.6	Me-27
27	1.88 (s)	17.0	
28	0.82 (s)	20.4	
29	1.26 (s)	27.7	Me-30
30	1.09 (s)	21.0	Me-29
OAc	2.10 (s)	20.5, 170.9	H-11

[a] Data were recorded in $CDCl_3$ on a Bruker AM-300 MHz apparatus. [b] Data assigned by HMQC and HMBC.

an acetyl methyl singlet, five methyl singlets, and a methyl doublet. The ^{13}C NMR spectrum (Table 1) and DEPT revealed that **1** contained four carbonyl groups of one ketone and three esters, five quarternary carbons, including one olefinic carbon and one oxygenated carbon, seven methines, including one olefinic carbon, and three oxygenated carbons, nine methylenes, and seven methyl groups. Among them, the oxygenated carbons appeared at δ 81.3 (C-1), 84.1 (C-4), 94.9 (C-10), 75.5 (C-11) and 80.3 (C-22). Compound **1**, possessing partial structures of ring A and ring B, was observed from its HMBC studies, which indicated correlations of H-2 (δ 2.83, dd, J = 6.3, 18.8 Hz; δ 2.61,d, J = 18.8 Hz) with C-1, C-3 (δ 174.9), and C-10 (δ94.9), correlations of H-5 (δ 1.93 m) with C-10 and C-4.[21] The

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(19) Mp 193 C; $[\alpha]^{25}_D$ +211 ° (*c* 0.1, CH_2Cl_2); UV λ_{max} (CH_3CN) nm (log ϵ) 207 (4.36); CD (CH_3CN, *c* 0.17) nm ($\Delta\epsilon$) 260 (+3.27), 310 (+4.07); IR (neat) ν_{max} 2974, 2940, 1781, 1724, 1466, 1375, 1227, 1123, 1064, 735 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* 581 $[M + Na]^+$, 559 $[M + H]^+$; EIMS *m*/*z* 454, 426, 315, 151, 111, 95, 83, 55; HREIMS *m*/*z* 581.3086 (calcd for $C_{32}H_{46}O_8$, 581.3090). Crystal data: $C_{32}H_{46}O_8 \cdot CH_3OH$, M = 590.73, trigonal system, space group $P3_1$, $a = b = 10.4666(14)$, $c = 25.264(6)$ Å, $V = 2396.8(6)$ Å^3, $Z = 3$, $d = 1.228$ g/cm^3. A crystal of dimensions 0.20 × 0.60 × 0.80 mm was used for measurements on a RIGAKU AFC7S diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max}$= 52.0°), Mo Kα radiation. The total number of independent reflections measured was 3473, of which 2284 were observed ($|F|^2 \geq 2\sigma|F|^2$). 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: $R_f = 0.043$, $R_w = 0.113$ ($w = 1/[\sigma^2(F_o^2) + (0.0726P)^2 + 0.3796P]$ where $P = (F_o^2 + 2F_c^2)/3$).
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latter carbon was also found to correlate with H-29 (δ_H 1.26) and H-30 (δ_H 1.09). The six-membered α-methyl, α,β-unsaturated-δ-lactone was elucidated from mass fragment at *m/z* 111 ($[C_6H_7O_2]^+$),[22] as well as HMBC correlations of H-22 (δ 4.44, d, J = 12.9 Hz) with C-20 (δ 39.9) and C-24 (δ 139.6) and correlations of the methyl singlet (H-27, δ 1.88) with C-24, C-25, and C-26 (δ 166.6). This was further supported by the COSY spectrum of **1**, in which correlations between H-24, H-23, H-22, H-20, and H-21 were observed. Two isolated COSY correlations were also observed among H-11 (δ 5.54)/H-12 (δ 2.50, 1.90) and H-19a/H-19b (δ 2.40 d, 3.19 d, J = 10.5 Hz). Since compound **1** contains an acetyl group (δ 2.10) and a carbonyl group (δ_C 198.4), two more ring (rings C and D) were required after deduction of the degrees of unsaturation. The relative stereochemistry of kadsuphilactone A (**1**) was disclosed from the NOESY correlations, which indicated cross-peaks between H-1 and Me-29 and between H-11 and Me-28 (δ 0.82s). The location of the acetyl was revealed from HMBC correlation (H-11/*C*OCH_3). These findings suggested that the acetyl group should be positioned on the β-face of the molecule. Mutual NOESY correlations were observed between Me-28/H-5 and Me-28/H-17 but not between Me-18 and Me-28, indicating that Me-18 was β-oriented whereas H-5, H-17, and Me-28 possessed α-orientation. However, the HRMS and even the extensive two-dimensional NMR analyses, including the HMQC, HMBC, and NOESY could not determine the structure of the rings C and D due to overlapping of ^{1}H NMR signals in ring C. Fortunately, single-crystal X-ray diffraction analysis (Figure 1) allowed the structure of **1** to be solved.

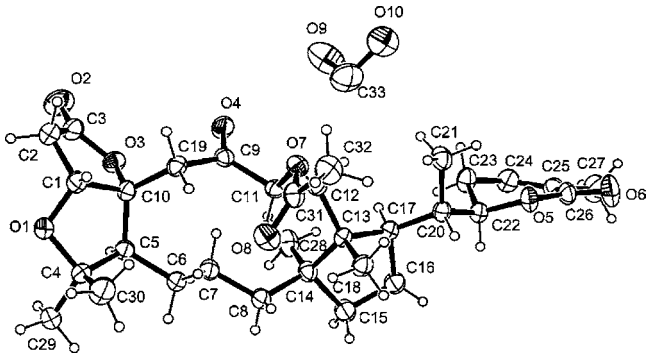

Figure 1. Perspective drawing of the X-ray structure of **1**.

Ring D is five-membered, while ring C reveals an unusual eleven-membered system with a carbonyl group at C-9. Therefore, the structure of **1** was unambiguously established. The ^{1}H and ^{13}C NMR assignment of rings C and D was further determined by detailed analysis of COSY, HMQC, and HMBC as shown in Table 1. The CD spectrum of **1** exhibited positive Cotton effects at 260 nm, indicating that **1** should have a C-22*R* configuration.[23] According to IUPAC sequence rules, the absolute configuration of the chiral carbons was assigned as 1*R*,5*S*,10*R*,11*S*,13*R*,14*R*,17*R*,20*S*,22*R*.

Kadsuphilactone B (**2**), crystallized as colorless prisms, had the molecular formula $C_{30}H_{42}O_5$, as derived from its HRESIMS (*m/z* 483.3106 $[M + H]^+$, calcd 483.3110).[24]

Table 2. ^{1}H and ^{13}C NMR Data of **2**[a]

no.	δ_H (mult, J, Hz)	δ_C[b]	HMBC (^{1}H−^{13}C)
1	6.11 (d, 12.6)	150.7	H-5, H-9
2	5.91 (d, 12.6)	120.2	
3		167.4	H-1, H-2
4		84.5	H-5, Me-29, Me-30
5	2.38 (dd, 4.2, 12.6)	46.4	H-1, H-19
6	0.79 (m), 1.87 (m)	24.1	
7	1.22 (m), 1.45 (m)	24.6	
8	1.80 (m)	44.9	H-19, Me-28
9		28.4	H-1, H-12, H-19
10		33.5	H-2, H-19
11	2.10 (m), 1.61 (m)	29.0	
12	1.75 (m)	33.0	Me-18
13		48.9	H-12, Me-18, Me-28
14		46.1	H-12, H-17, Me-18, Me-28
15	1.38 (m)	34.6	Me-28
16	1.58 (m), 2.05 (m)	21.5	
17	1.94 (m)	50.5	Me-18, H-20, Me-21
18	1.16 (s)	19.1	
19	1.04 (d, 5.1),	32.9	H-1
19	1.20 (d, 5.1)		
20		75.4	H-17, Me-21, Me-22
21	1.27 (s)	20.8	H-22
22	4.25 (dd, 4.5, 12.0)	83.1	H-17, Me-21
23	2.30 (m)	25.6	
24	6.58 (d, 5.1)	139.0	H-22, Me-27
25		128.3	Me-27
26		165.4	Me-27
27	1.90 (s)	16.8	
28	0.89 (s)	19.1	
29	1.33 (s)	22.1	Me-30
30	1.35 (s)	29.2	Me-29

[a] Data were recorded in $CDCl_3$ on a Bruker AM-300 MHz apparatus. [b] Data assigned by HMQC and HMBC.

The presence of the seven-membered α,β-unsaturated lactone and a six-membered α,β-unsaturated lactone was revealed from the IR absorption bands (1730, 1713, 1690,

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(24) Mp 167 °C, $[\alpha]^{25}_D$ +128 ° (*c* 0.2, CH_2Cl_2); UV λ_{max} (CH_3CN) nm (log ϵ) 206 (4.14), 248 (4.16); CD (CH_3CN, *c* 0.14) nm ($\Delta\epsilon$) 237 (−8.54), 272 (+12.6); IR (neat) ν_{max} 3497, 2950, 1730, 1713, 1690, 1667, 1650, 909, 858, 739 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* 505 $[M + Na]^+$, 483 $[M + H]^+$; EIMS *m/z* 482 $[M]^+$, 464 $[M - H_2O]^+$, 439, 420, 353, 328, 285, 191, 173, 155, 107, 83, 71, 55; HRESIMS *m/z* 483.3106 (calcd for $C_{30}H_{43}O_5$, 483.3110). Crystal data: $C_{30}H_{43}O_5 \cdot 0.5H_2O$, M = 491.64, monoclinic system, space group $P2_1$, a = 15.305(2), b = 11.735(3), c = 15.339(3) Å, β = 106.94(1)°, V = 2636(1) Å^3, Z = 4, d = 1.239 g/cm^3. A crystal of dimensions 0.20 × 0.50 × 0.50 mm was used in diffraction experiment (same as compound **1**). The total number of independent reflections measured was 5648, of which 3413 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_f = 0.044, R_w = 0.117 ($w = 1/[\sigma^2(F_o^2) + (0.0829P)^2 + 0.3416P]$ where $P = (F_o^2 + 2F_c^2)/3$).

1667, and 1650 cm^{-1}) and NMR spectral data (Table 2). The DEPT spectra showed six methyl singlets (δ 16.8, 19.1, 19.2, 20.8, 22.1, and 29.2). The mutually coupled doublets at δ 1.04 and 1.20 (J = 5.1 Hz) indicated a cyclopropane.

The location of a tertiary hydroxyl group was revealed by HMBC, in which H-22 (δ 4.25, dd, J = 4.5, 12.0 Hz), H-17 (δ 1.94 m), and Me-21 (δ 1.27 s) were correlated with C-20 (δ 75.4). Compound **2** showed a strong positive Cotton effect at 272 nm similar to that of kadsudilactone, [23] indicating an *R* configuration of C-22. The complete structure and stereochemistry of **2** were established from NOESY and X-ray crystallographic analysis, and the solid-state conformation is illustrated in Figure 2.

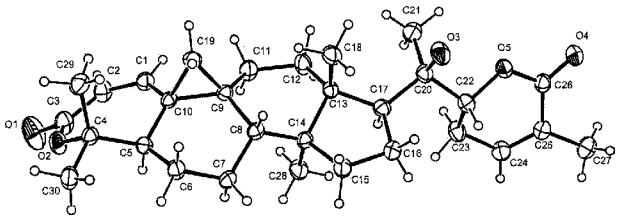
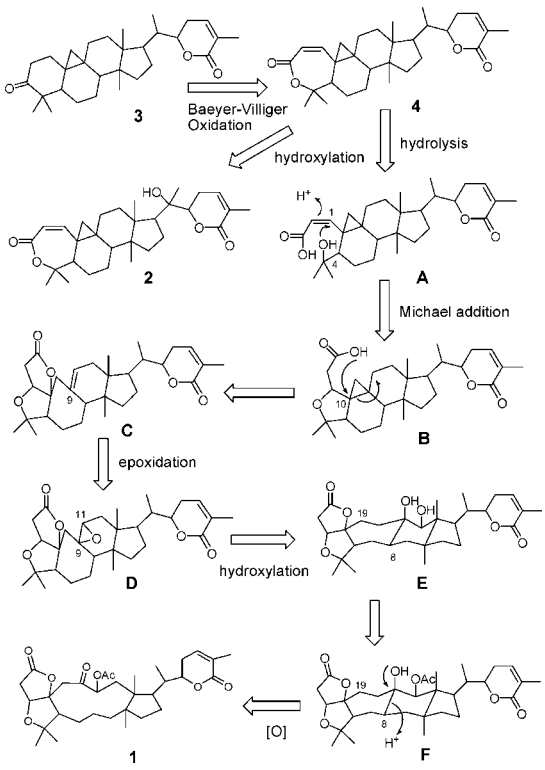


Figure 2. Perspective drawing of the X-ray structure of **2**.

A plausible biogenetic pathway of **1** and relationships for these triterpenoids were proposed as shown in Scheme 1 on the basis of recently published structures such as lancilactone C,[12] micrandilactone A,[11] and lancifodilactone A.[21] Kadsuphilactone A (**1**) might be produced from kadsulactone (**3**) and schisanlactone B (**4**) through intermediates **A**−**F**. This pathway involves Baeyer−Villiger oxidation, hydrolysis, Michael addition, epoxidation, hydroxylation, and finally oxidation with ring expansion.

Compounds **1** and **2** were tested for cytotoxicity against hepA2 cells using the MTT method as reported previously.[25] These compounds exhibited cytotoxicity against hepA2 cells with TC_{50} > 200 μg/mL. The production of HBsAg in the culture medium was measured by enzyme immunoassay (EIA; kit from Bio-Rad). The IC_{50} is the concentration of tested compounds required for a 50% reduction in HbsAg secretion in hepA2 cells. Kadsuphilactone B (**2**) exhibited anti-HBV activity with IC_{50} value at 6 μg/mL in vitro.

Scheme 1. Plausible Biogenetic Relationships for Compounds **1**−**4**.



Acknowledgment. This work was supported by a grant from the National Science Council of the Republic of China (Grant NSC-93-2320-B-110-003) awarded to Y. C. Shen.

Supporting Information Available: ^1H and ^{13}C NMR spectra and X-ray data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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