A Novel Anti-HBeAg Homolignan, Taiwanschirin D from *Kadsura matsudai*

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A novel C_{19} homolignan, taiwanschirin D (1), possessing a 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton, was isolated from the stem of *Kadsura matsudai* Hayata. Its structure was determined from physical and spectral data including 2D NMR spectra. The Anti-HBeAg test revealed that taiwanschirin D (1) had moderate activity at a concentration of 94.3 μM (50 μg/ml).

Key Words *Kadsura matsudai* Hayata; Anti-HBeAg; C_{19} homolignans; taiwanschirin; 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]} pentano (2,3-dihydrobenzo[*b*] furan)-3(2-oxoacetate)

Several C_{18} dibenzocyclooctadiene type lignans have been isolated from plants of Schizandraceae.[1—7] These lignans showed some pharmacological effects such as antioxidant, antihepatitis, antihepatotoxic and antilipid peroxidative effects.[1—5] We have reported the isolation of two types of novel cytotoxic C_{19} homolignans, 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano(2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton [taiwanschirins A, B, C] and 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]-furan) skeleton [schiarisanrins A—D] from *Schizandra arisanensis*.[8,9] Recently, we obtained a new anti-HBeAg C_{18} dibenzocyclooctadiene lignan, kadsumarin A from another Taiwanese Schizandraceae plant, *Kadsura matsudai* Hayata.[10] Further investigation of the *K. matsudai* led to the isolation and characterization of a novel analog of taiwanschirins, namely taiwanschirin D (**1**), along with a known cytotoxic taiwanschirin C (**2**) and two C_{18} dibenzocyclooctadiene lignans, kadsurarin (**3**) and kadsulignan N (**4**). We report herein the structural elucidation of compound **1** determined by employing two dimensional (2D) NMR techniques including ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMQC), ^{1}H–^{13}C heteronuclear multiple bonds coherence (HMBC), and (NOESY) spectra.

Taiwanschirin D (**1**) was obtained as colorless, cubic crystals. The IR spectrum indicated the presence of ester carbonyl (1745, 1735 cm^{-1}), conjugated ester carbonyl (1718 cm^{-1}), vinyl (1655 cm^{-1}) and aromatic (1620 cm^{-1}) groups. The ^{13}C-NMR and DEPT of **1** indicated the signals of an aromatic group at δ_C 132.9 (s), 102.3 (d), 130.4 (s), 150.8 (s), 145.5 (s) and 118.1 (s) (C-4′, -5′, -6′, -7′, -8′ and -9′, respectively). HMBC correlations from oxygenated methylene protons (H-2′) to C-8′, -9′ and from a methylenedioxy proton (H-10′) to C-6′ and -7′, indicated the presence of an oxygenated benzene moiety. The correlations between H-2′ and a quaternary carbon (C-3′) and C-9′ indicated the connectivity of a dihydrofuran linkage an oxygenated benzene, which suggested a 2,3-dihydrobenzo[*b*]furan with a methylenedioxy moiety. A butyl chain (C_2–C_3–C_4–C_5) was predicted due to the prominent cross peaks of H-3, 2, 4, and of H-4, 3, 5, as well as two methyl groups correlated with H-3 and 4 in the ^{1}H–^{1}H COSY spectrum. Other evidence for structural elucidation comes from HMBC studies, the spectrum showing the couplings between H-2′ and C-1, between H-2 and C-1, and between H-4′ and C-5 suggested that the dihydrobenzo[*b*]-furan was connected to a pentano moiety at 3′–4′ positions. This evidence together with the signals of an oxoacetate and a 2-methoxy-2-oxoethylidene groups occurring in the NMR spectra suggested that **1** was the analogue of taiwanschirin C (**2**)[9] which possesses a substituted dihydrobenzo[*b*]furan, a substituted cyclooctene, a substituted oxoacetate, and a substituted methoxy oxoethylidene moiety.[2] However, proton signals (δ_H 2.22, 2.36, 1.52, 1.30×2, 0.87) and carbon signals [δ_C 33.7 (t), 24.5 (t), 22.4 (t), 31.3 (t), 13.9 (q), 173.9 (s)] for a caproxyl group (*n*-hexanoic acid, $C_6H_{12}O_2$) instead of a benzoyl ester group were observed in the NMR spectra.

Moreover, the mass spectrum of **1** exhibited a molecular ion at *m*/*z* 530 and an intense peak at *m*/*z* 327 [$M^+ - CH_3OCOCO - C_6H_{12}O_2$] corresponding to the fragmentation of an oxoacetate, and reflected the 1,2-elimination of an *n*-hexanoic acid *via* McLafferty ester rearrangement. In detailed inspection of the HMBC spectrum, cross signals between methylene-2‴′, H-5 and ester carbonyl carbon at δ_C 173.9 (C-1‴′) consequently suggested that the n-hexanoic acid ester is located at C-5. On the basis of the above corroboration, the entire structure of taiwainschrin D (**1**) was deduced to have a 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]} pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton with a caproxyl group at C-5.

These lignans and homolignans **1**—**4** were assayed for cytotoxicity against human epidermoid carcinoma of nasophar-

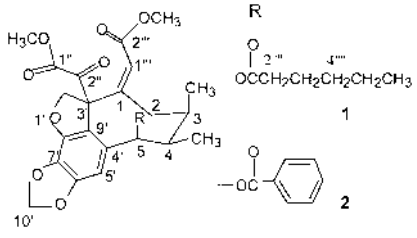

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Table 1. ^{1}H- (300 MHz) and ^{13}C- (75 MHz) NMR Data ($CDCl_3$) for Compound **1**[a)]

	Carbon	Proton	C–H connectivities[b)]
1	154.9 s		H-2, 2′
2	41.6 t	2.43 (m)	H-1‴, H-2
3	35.9 d	2.03 (m)	H-5, 3-CH_3
4	43.6 d	1.92 (m)	H-2, 3, 5
5	80.7 d	5.82 (s)	H-5′, 4-CH_3
CH_3-3	19.9 q	1.03 (d, 8.0)	H-5, 3
CH_3-4	13.8 q	1.01 (d, 8.0)	H-3, 5
2′	85.4 t	4.68, 5.92 (ABq, 10.2)	
3′	64.9s		H-1‴
4′	132.9 s		H-5
5′	102.3 d	6.32 (s)	H-5
6′	130.4 s		H-5′
7′	150.8 s		H-10′
8′	145.5 s		H-2′
9′	118.1 s		H-5′, 5
10′	102.3 t	5.98 (s)	—
1″	166.0 s		1″-OCH_3
2″	184.5 s		H-2′a, b
OCH_3-1″	51.4 q	3.57 (s)	—
1‴	124.2 d	6.09 (s)	H-2
2‴	161.6 s		2‴-OCH_3
OCH_3-2‴	52.5 q	3.68 (s)	—
1⁗	173.9 s		c)
2⁗	33.7 t	2.22, 2.36 (m)	c)
3⁗	24.5 t	1.56 (m)	c)
4⁗	22.4 t	1.30 (m)	c)
5⁗	31.3 t	1.30 (m)	c)
6⁗	13.9 q	0.87 (t, 5.5)	c)

a) Multiplicity was determined from DEPT spectra. *b*) HMBC corresponded to two or three bonds. *c*) The assignments were explained in the text.

ynx (KB), colon carcinoma (COLO-205), hepatoma (Hepa-3B), and cervix (Hela) tumor cells. Compound **2** exhibited the cytotoxic effect against hepatoma (ED_{50}=2.3 g/ml) as previously reported,[7)] while ED_{50} values for compounds **1**, **3**, and **4** exceeded 10 μg/ml. Moreover, biological evaluation of compounds **1**—**4** in anti-HBsAg and anti-HBeAg for the human type B hepatitis was also established. Only compound **1** showed the marginal inhibition in anti-HBeAg at a concentration of 94.3 μM (50 μg/ml). To our knowledge, this is the first report that the C_{19} homolignan with 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano (2,3-dihydrobenzo[*b*]-furan)-3(2-oxoacetate) skeleton as compound **1** has anti-HBeAg activity.

Experimental

General Experimental Procedures NMR spectra were measured at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Heteronuclear long range correlation (HMBC) spectra were performed using coupling constants of 8 Hz. Samples for IR spectral measurements were prepared as KBr discs. Electron impact (EI)-MS were performed in the electron impact mode (20 eV). HPLC was employed using the semipreparative $5C_{18}$ column.

Plant Material The stems of *Kadsura matsudai* HAYATA were collected in July 1997 in Taichung County, Taiwan. A voucher specimen was deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

Extraction and Isolation The dried stems of *K. matsudai* HAYATA (6.2 kg) were extracted exhaustively with ethanol. The crude ethanol syrup was extracted five times with hexane. The ethanol layer was partitioned with EtOAc–H_2O (1 : 1) three times to give EtOAc and H_2O layers. After the EtOAc layer was evaporated *in vacuo*, its extract (82 g) was chromatographed on a silica gel column with *n*-hexane–EtOAc (8 : 1, 6 : 1, 4 : 1, 2 : 1, 1 : 1, EtOAc) to give 12 fractions, fr. 1—12. Bioactive fraction 5 was further separated by column chromatography on Silica gel eluting with $CHCl_3$: MeOH (15 : 1, 12 : 1, 10 : 1, 8 : 1, 6 : 1, 4 : 1) to yield 12 fractions, fr. 5-1—5-12. Compounds **1** (3.3 mg) and **4** (4.2 mg) were furnished from fr. 5—4 by HPLC ($5C_{18}$, 250×10 mm, MeOH–H_2O=70 : 30). Compound **3** (186 mg) was obtained from fraction 5-5 after recrystallization.

Taiwanschirin D (**1**): Light yellow powder; mp 47—50 °C, IR (KBr), 1740, 1735,1715, 1655, 1615 cm^{-1}; $[\alpha]_D$ +315° (CH_2Cl_2, *c*=0.3); EI-MS *m/z* (rel. intensity) 530 (M^+, 8), 443 (15), 327 (100), 295 (70), 267 (68), 253 (25); HR-EI-MS *m/z* 530.2153 $[M]^+$ (Calcd for $C_{28}H_{34}O_{10}$, 530.2152); ^{1}H- and ^{13}C- NMR, see Table 1.

Cytotoxicity Assay The *in vitro* cytotoxicity assay against KB (nasal pharyngeal carcinoma), Hepa-3B (hepatoma), Hela (cervix carcinoma), and COLO-205 (colon carcinoma) tumor cells by the methylene blue dying method was conducted as described in a previous paper.[10)]

Anti-HBsAg and Anti-HBeAg Test The assay for *in vitro* antiviral activity against hepatitis B virus (HBV) followed to our previously described procedure.[11)]

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