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

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A novel C₁₉ 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; C₁₉ homolignans; taiwanschirin; 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]} pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate)

Several C₁₈ 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 C₁₉ 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-butan-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton [schiarianrins A—D] from *Schizandra arisanensis*.^{8,9)} Recently, we obtained a new anti-HBeAg C₁₈ 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 C₁₈ 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 ¹H–¹H correlation spectroscopy (COSY), ¹H–¹³C heteronuclear multiple quantum coherence (HMQC), ¹H–¹³C 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 ¹³C-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 (C₂–C₃–C₄–C₅) 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 ¹H–¹H 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 caproxy group (*n*-hexanoic acid, C₆H₁₂O₂) 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⁺ – CH₃OCOCO – C₆H₁₂O₂] 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 taiwanschirin 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 caproxy 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. ¹H- (300 MHz) and ¹³C- (75 MHz) NMR Data (CDCl₃) 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'	64.9 s		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 (ED₅₀=2.3 g/ml) as previously reported,⁷⁾ while ED₅₀ 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 C₁₉ 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 ¹H and 75 MHz for ¹³C. 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 5C₁₈ 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–H₂O (1:1) three times to give EtOAc and H₂O 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 CHCl₃: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 (5C₁₈, 250×10 mm, MeOH–H₂O=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; [α]_D²⁵ +315° (CH₂Cl₂, 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 C₂₈H₃₄O₁₀, 530.2152); ¹H- and ¹³C- 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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