A New Anti-HBeAg Lignan, Kadsumarin A, from *Kadsura matsudai* and *Schizandra arisanensis*

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A new C₁₈ dibenzocyclooctadiene lignan, kadsumarin A (1) was isolated from *Kadsura matsudai* HAYATA and *Schizandra arisanensis* HAYATA. The anti-HBeAg test revealed that kadsumarin A had activity at a concentration of 40 µg/ml (=90.1 µM). Its structural elucidation by spectral analysis was discussed in this note.

Key words *Kadsura matsudai*; *Schizandra arisanensis*; anti-HBeAg; kadsumarin A; lignan; Schizandraceae

During the course of studies on the antitumor agents,^{1,2)} we have recently reported the isolation of novel C₁₉ homolignans with 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton, schiarisanrins from *Schizandra arisanensis*.³⁾ The isolated C₁₉ homolignans have a substituted cyclohexadienone moiety with an oxygenated methylene group, instead of the substituted benzene moiety found before in C₁₈ dibenzocyclooctadiene lignans from *Schizandra* spp. which showed some pharmacological effects such as antioxidant, antihepatitis, antihepatotoxic and antilipid peroxidative effects.^{4–8)} After further investigation of plants of the Schizandraceae family in Taiwan, we reported herein the isolation and characterization of a new C₁₈ dibenzocyclooctadiene lignan, kadsumarin A (1) from *Kadsura matsudai* HAYATA and *Schizandra arisanensis* HAYATA.

Kadsumarin A, mp 175–178 °C, [α]_D +13° (CHCl₃, *c* = 0.1), had a molecular weight of 444 corresponding to the molecular formula C₂₄H₂₈O₈. The nature of the structure deduced from ¹³C- and ¹H-NMR spectra, together with the IR spectrum with bands at 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm⁻¹, suggest that 1 might possess dibenzocyclooctadiene lignan with a hydroxyl and an ester groups. The ¹³C-NMR spectrum of 1 clearly indicates the presence of 12 carbon atoms (δ_C 146.5, 148.9, 136.1, 102.7, 119.0, 135.6 for C-1, -2, -3, -4, -5 and -16, respectively; δ_C 133.7, 107.1, 133.3, 150.3, 141.2, 117.0 for C-10, -11, -12, -13, -14 and -15, respectively), revealing a biphenyl moiety. A butano moiety was predicted due to the prominent cross peaks of H-6 (δ_H 5.50, s), H-7 (δ_H 2.07, m), H-8 (δ_H 2.07, m), and H_b-9 (δ_H 2.61, m) in the ¹H-¹H homonuclear correlation spectroscopy (¹H-¹H COSY) spectrum. Moreover, ¹H-¹³C long-range correlation (HMBC) spectrum showed the couplings between H_b-9 and C-15 and C-11, and between H-6 and C-4 of respective aromatic rings, implying that 1 pos-

sesses a substituted butano moiety (C₆-C₇-C₈-C₉) at C-5 and C-10, and two substituted aromatic moieties by C-15 and -16 linkage. From the above evidence, the skeleton of 1 was deduced to be a substituted dibenzocyclooctadiene lignan isolated previously from Schizandraceae plants.^{8,9)}

The other signals of functional groups including three methoxy groups, an oxygenated methylene group, and two secondary methyl groups appearing in the ¹³C- and ¹H-NMR spectra, were assigned as C-19, 20, 21, and C-22, and C-17, 18, respectively, based on the HMBC and ¹³C-¹H COSY studies. The presence of an acetic acid ester was shown by carbon signals at δ_C 170.0 (C-23) and δ_C 21.7 (C-24) in the ¹³C NMR spectrum. Also, a methyl signal at δ_H 1.52 (s) was found in the ¹H-NMR spectrum. The mass spectrum of 1 exhibited a molecular ion at *m/z* 444 and a characteristic peak

Table 1. ¹H (300 MHz) and ¹³C (75 MHz) NMR Data (CDCl₃) for Compound 1^{a)}

	Carbon	Proton	C-H Connectivities ^{b)}
1	146.54 s	—	c)
2	148.92 s	—	H _b -22, 4
3	136.05 s	—	H _b -22, 4
4	102.70 d	6.46 (s)	H-6, 4
5	118.98 s	—	H-6, 4
6	82.59 d	5.50 (s)	H-4
7	41.54 d	2.07 (m)	H-6
8	34.98 d	2.07 (m)	H _b -9, H _b -6, 17, 18
9	38.59 t	2.61 (m)	H-11, 17
10	133.70 s	—	H _b -9, 11
11	107.14 d	6.37 (s)	H _b -9
12	133.27 s	—	H-11, 19
13	150.27 s	—	H-11, 20
14	141.22 s	—	H-21
15	116.99 s	—	H-11, H _b -9
16	135.57 s	—	H-6, 4
17	14.79 q	0.88 (d, 6.9)	H _b -9
18	19.69 q	1.06 (d, 6.9)	H-6
19	59.78 q	3.82 (s)	c)
20	60.82 q	3.88 (s)	c)
21	55.76 q	3.87 (s)	c)
22	101.21 t	5.92, 5.95 (d, 1.2)	c)
23	170.02 s	—	H-6
24	21.65 q	1.52 (s)	c)

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

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at m/z 384 ($M^+ - H_3CCOO$), which also correspond to the presence of an acetoxyl group. The ion at m/z 384 reflected the 1,2-elimination of acetic acid *via* McLafferty rearrangement involving the acetoxyl group. Detailed examination of the HMBC spectrum of **1** showed that the methyl proton (H-24) was correlated with carbonyl carbon (C-23), and the correlation between H-6 and C-23 revealed an acetic acid ester at C-6. On the basis of NOESY spectrum, three methoxy groups correlated with each other, and the methoxy group at C-12 correlated with H-11, providing further evidence that the three methoxy groups are adjacent at the same aromatic ring. In addition, the correlation between H-6 and H-4, CH_3 -18 and between CH_3 -17 and H-19, H-11 determine the exact orientation of functional groups at C-6, 7, 8, respectively, as shown. Consequently, the remaining quaternary carbon at C-1 (δ_C 146.5) would have a phenolic hydroxyl group for fitting the molecular weight of **1**, so its structure was established unambiguously.

To study the stereochemistry of compound **1**, the circular dichroism (CD) was also examined. The CD showing a negative Cotton effect at 260 nm suggested that compound **1** possessed an *S*-biphenyl configuration as gomisin B.^{9,10)}

Furthermore, kadsumarin A was assayed for *in vitro* antiviral activity against hepatitis B virus (HBV) according to our previously described procedure.¹¹⁾ At a concentration of 40 $\mu\text{g/ml}$ ($=90.1 \mu\text{M}$), the effect of **1** was maximal at 6 to 9 days with a 40% inhibition of HBeAg production without evidence of cytotoxicity. To our knowledge, this is the first report that C_{18} lignans with a dibenzooctadiene such as compound **1** has anti-HBeAg activity.

Experimental

The instruments applied for obtaining spectral data and chromatography were the same as in the previous paper.³⁾

Plant Material The stems of *K. matsudai* and *S. arisanensis* were collected in July 1995 in Taipei 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* were extracted exhaustively with ethanol. The crude ethanol syrup was extracted five times with hexane. The *n*-hexane extract (58 g) was chromatographed on a silica gel column with *n*-hexane-EtOAc (10:1, 8:1, 6:1, 4:1, 2:1, 1:1) to give 12 fractions, fr. 1–12. Fraction 6 was further separated by column chromatography on silica gel eluting with CH_2Cl_2 -Acetone (10:1, 6:1, 4:1, 1:1) to yield 12 fractions, fr. 6-1–6-12. Compound **1** (4.2 mg) was obtained from fr. 6-7 by HPLC ($5C_{18}$, 250×10 mm, MeOH- H_2O = 1.8:1). The crude $CHCl_3$ extract (67 g) of *S. arisanensis* was yielded as reported.⁵⁾ After chromatography on silica gel with hexane-EtOAc by gradually increasing EtOAc as the eluent, 10 fractions were furnished. Fraction 4 was further rechromatographed over silica gel with hexane-EtOAc (10:1, 7:1, 5:1, 3:1, 1:1) as the eluent to acquire nine fractions, 4-1–4-9. Fraction 4-3 was further separated repeatedly by HPLC ($5C_{18}$, 250×10 mm) with MeOH- H_2O (2.7:1) as the eluent to afford **1** (1.7 mg).

Kadsumarin A (**1**): Light pink powder; mp 175–178 °C; IR (KBr) 3400 (OH), 1715 (ester), 1610, 1590 (aromatic) cm^{-1} ; $[\alpha]_D^{25} +13^\circ$ ($CHCl_3$, $c=0.1$); EI-MS m/z (rel. intensity) 444 (M^+ , 20), 418 (7), 384 (100), 369 (30), 353 (27), 327 (26), 295 (25), 267 (25); 1H - and ^{13}C -NMR, see Table 1.

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