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

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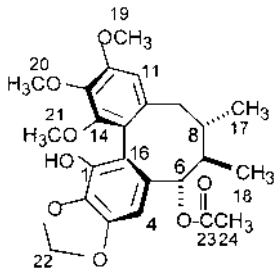
**A new $C_{18}$ dibenzocyclooctadiene lignan, kadsumarin A (1) was isolated from *Kadsura matsudai* HAYATA and *Schizandra arisanensis* HAYATA. The anti-HBeAg test revealed that kadsumarin A had activity at a concentration of 40 $\mu$g/ml (=90.1 $\mu$M). Its structural elucidation by spectral analysis 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 $C_{19}$ homolignans with 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton, schiarisanrins from *Schizandra arisanensis*.[3] The isolated $C_{19}$ homolignans have a substituted cyclohexadienone moiety with an oxygenated methylene group, instead of the substituted benzene moiety found before in $C_{18}$ 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 $C_{18}$ dibenzocyclooctadiene lignan, kadsumarin A (**1**) from *Kadsura matsudai* HAYATA and *Schizandra arisanensis* HAYATA.

Kadsumarin A, mp 175—178 °C, $[\alpha]_D$ +13° ($CHCl_3$, $c$= 0.1), had a molecular weight of 444 corresponding to the molecular formula $C_{24}H_{28}O_8$. The nature of the structure deduced from $^{13}$C- and $^1$H- 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 $^{13}$C- NMR spectrum of **1** clearly indicates the presence of 12 carbon atoms ($\delta_C$ 146.5, 148.9, 136.1, 102.7, 119.0, 135.6 for C-1, -2, -3, -4, -5 and -16, respectively; $\delta_C$ 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 ($\delta_H$ 5.50, s), H-7 ($\delta_H$ 2.07, m), H-8 ($\delta_H$ 2.07, m), and $H_b$-9 ($\delta_H$ 2.61, m) in the $^1$H–$^1$H homonuclear correlation spectroscopy ($^1$H–$^1$H COSY) spectrum. Moreover, $^1$H–$^{13}$C long-range correlation (HMBC) spectrum showed the couplings between $H_b$-9 and C-15 and C-11, and between H-6 and C-16 and C-4 of respective aromatic rings, implying that **1** possesses a substituted butano moiety ($C_6$–$C_7$–$C_8$–$C_9$) 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 $^{13}$C- and $^1$H-NMR spectra, were assigned as C-19, 20, 21, and C-22, and C-17, 18, respectively, based on the HMBC and $^{13}$C–$^1$H COSY studies. The presence of an acetic acid ester was shown by carbon signals at $\delta_C$ 170.0 (C-23) and $\delta_C$ 21.7 (C-24) in the $^{13}$C NMR spectrum. Also, a methyl signal at $\delta_H$ 1.52 (s) was found in the $^1$H-NMR spectrum. The mass spectrum of **1** exhibited a molecular ion at *m/z* 444 and a characteristic peak

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 | 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 ($\delta_C$ 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 $\mu$g/ml (=90.1 $\mu$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) $cm^{-1}$; $[\alpha]_D$ +13° ($CHCl_3$, *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); $^1$H- and $^{13}$C- NMR, see Table 1.

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