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NOTE

Lignans from the Stem of *Styrax japonica*

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A new lignan (Styrlignan A), together with four known lignans, were isolated from the steam of *Styrax japonica* (Styracaceae). Their structures were determined by means of HRESIMS, extensive 1D and 2D NMR spectroscopic studies and chemical evidence. The anti HIV-1 activity of Styrlignan A was also evaluated and it shows an anti HIV-1 activity with a TI (therapeutic index) above 38.5.

Key Words: Lignans, *Styrax japonica*, Anti HIV-1 activity.

Styrax japonica Sieb. et Zucc. (Styracaceae) is a deciduous tree grown in the mainland of China, Korea and Japan[1]. The resin from this species has been used in traditional medicine to treat inflammatory diseases and was also used by Romans, Egyptians, Phoenicians and Ionians as both incense and a medicine[2,3]. The previous investigation on the chemical constituents from the seeds or leaves of the styrax species revealed them to be a rich source of lignan[4-6], terpenoids[7,8] and sapogenins[8,9], from the fresh fruits were showed to have an antisweet activity and the egonol from the seeds has attracted the attention of synthetic organic chemists due to its activity against human cancer cells[10,11]. The phytochemical constituents of the stem bark of *S. japonica* had been studied by Byung-Sun Min *et al*., a serial of new furofuran, dibenzyl-γ-butyrolactone lignans, norlignan and terpenes were also isolated from this plants[5,6,12].

In order to investigate the components of the aerial parts and search for potential leads for drug development, phytochemical investigation on *Styrax japonica Sieb*. et Zucc. (Styracaceae) was carried out. This study led to the isolation of a new lignan, Styrlignan A (**1**), together with four known lignans, dihydroguaiaretic acid (**2**)[12], 4-[4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl]-2-methoxy-phenol (**3**)[13,14], 14 prinsepiol (**4**)[15], dehydrodiconiferyl alcohol (**5**)[16]. Their structures were established by means of HRESIMS and extensive NMR spectra. The anti HIV-1 activity of Styrlignan A (Fig. 1) was also evaluated.

Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instruments with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh) or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China). On second separate used Agilent 1100 HPLC equipped with ZORBAX-C_{18} (9.4 mm × 250 nm, 5.0 μm) column and DAD detector.

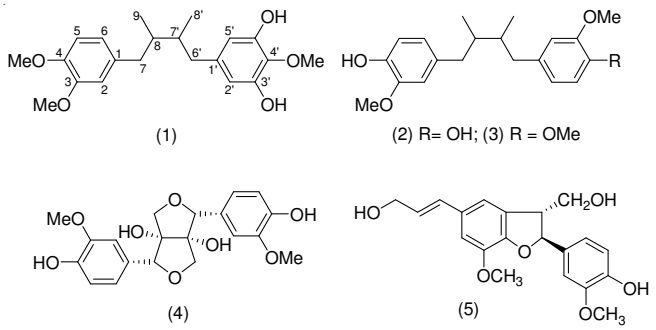

Fig. 1. Structure of lignans in *Styrax japonica*

The stem of *S. japonica* was collected in Dali County, Yunnan Province, P.R. China, in October 2007 and was identified by Prof. N. Yuan. A voucher specimen (No. YNNi 07-8-07) was deposited in our laboratory.

Extraction and isolation: The dried sample (2.2 kg) was extracted with MeOH at room temperature (4 × 5 L) to obtain 0.56 kg of the solid extract. The MeOH extract was suspended in H_2O and extracted successively with hexane (3 × 3 L) and EtOAc (3 × 3 L) to give the hexane-(28.5 g) and EtOAc-soluble fractions (21.6 g), respectively. The EtOAc-soluble fraction (21.6 g) was chromatographed on a silica gel column eluted

with a stepwise gradient of $CHCl_3$ and MeOH to yield five fractions (A-E: 2.18, 1.65, 5.1, 3.16 and 1.54 g, respectively). Fraction B was further purified by HPLC with mobile phase (MeOH-H_2O 65:35) to yield **1** (16.4 mg), **2** (31.2 mg), **3** (12.8 mg), **4** (6.4 mg), **5** (15.4 mg).

Anti HIV-1 Assay: The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50})[17].

Styrlignan A (**1**) was obtained as a white, amorphous solid. Its molecular formula was determined as $C_{21}H_{28}O_5$ by the HRESIMS at m/z 383.1831 $[M + Na]^+$ (calcd. 383.1834 %). The functional group signals appearing in the ^1H and ^{13}C NMR spectra (Table-1) included two aromatic rings, three methoxy groups, two phenolic hydroxy group, two methyl groups, two methylene groups and seven methine groups. The strong IR absorption bands indicated the presence of aromatic rings (1594, 1516 and 1465 cm^{-1}). HMBC correlations found from H-2 and H-6 to C-7 and from H-2' and H-6' to C-7' implied that the two substituted aromatic moieties were not linked directly (Fig. 2). Moreover, the HMBC correlations of H3-9 with C-7, C-8 and C-8', of H3-9' with C-7', C-8' and C-8, of H2-7 with C-1, C-2, C-6, C-8 and C-9 and of H2-7' with C-1', C-2', C-6', C-8' and C-9', together with the spin systems of H-7/H-8/H-8'/H-7', H-8/H-9 and H-8'/H-9' in the ^1H-^1H COSY spectrum, suggested that **1** is a substituted 1,4-biphenyl-2,3-dimethylbutane-type lignan (Fig. 2)[18]. The correlations from the protons of three methoxy groups to C-3, C-4 and C-4', were used to locate these groups at C-3, C-4, C-4', respectively. The correlations from the protons of two phenolic hydroxy groups to C-3' and C-5' suggested that the two phenolic hydroxy groups were locate at C-3' and C-4'. Since the C-C bonds can rotate randomly, the relative configuration of **1** could not be determined on the basis of ROESY spectra. Thus, the structure of **1** was established as shown and gives the name of styrlignan A.

TABLE-1
^1H AND ^{13}C NMR DATA OF STYRLIGNAN A IN $CDCl_3$

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	134.1 s		4'	139.2 s	
2	112.8 d	6.65 (1H, s)	5'	147.8 s	
3	148.2 s		6'	106.0 d	6.38 (1H, s)
4	146.8 s		7'	39.7 t	2.76 (1H, dd, 13.2, 5.0)
5	111.8 d	6.72 (1H, d, 8.0)			2.35 (1H, dd, 13.2, 8.8)
6	121.5 d	6.64 (1H, d, 8.0)	8'	39.4 d	1.85 (1H, m)
7	39.2 t	2.72 (1H, dd, 13.2,5.2)	9'	16.2 q	0.84 (3H, d, 9.5)
		2.31 (1H, dd, 13.4, 8.8)	OMe-3	55.6 q	3.85 (3H, s)
8	39.8 d	1.82 (1H, m)	OMe-4	55.4 q	3.88 (3H, s)
9	16.8 q	0.87 (3H, d, 9.5)	OMe-4'	60.5 q	3.84 (3H, s)
1'	138.5 s		OH-3'		5.84 (1H, Brs)
2'	106.0 d	6.38 (1H, s)	OH-5'		5.84 (1H, Brs)
3'	147.8 s				

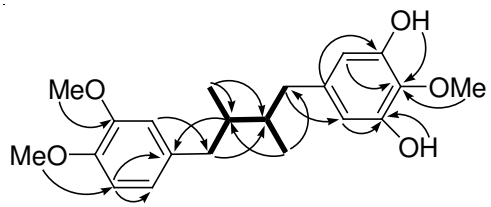

Fig. 2. Selected HMBC (→) and ^1H-^1H COSY (–) of **1**

The potencies of Styrlignan A in preventing the cytopathic efects of HIV-1 in MT4 cells, as well as compound-induced cytotoxicity in MT4 cells in parallel with the antiviral activity were evaluated[17]. The results from the cell-based assays demonstrated potent anti HIV-1 activity with EC_{50} (median effect concentration) value of 6.28 μg/mL and a TI (therapeutic index) of greater than 38.5. Styrlignan A shows weak anti HIV activity.

Styrlignan A (1): White, amorphous solid; UV ($CHCl_3$) λ_{max} (log ε): 276 (3.47), 242 (3.78), 230 (2.16), 206 (5.21), 193 (3.29) nm; IR (KBr, ν_{max}, cm^{-1}) 3426, 2955, 2931, 2870, 1594, 1516, 1465, 1375, 1330, 1260, 1235, 1192, 1105, 1012, 975, 926, 832, 762; ^1H and ^{13}C NMR data, Table-1; HRESIMS (positive ion mode) m/z 383.1831 $[M + Na]^+$ (calcd. (%) 383.1834 for $C_{21}H_{28}O_5$).

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REFERENCES

1. D.K. Lee, H.S. Kang and Y.D. Park, *Forest. Ecol. Manag*., **201**, 23 (2004).
2. K. Yoshikawa, H. Hirai, M. Tanaka and S. Arihara, *Chem. Pharm. Bull*., **48**, 1093 ((2000).
3. H.I. Moon, J. Lee and J.H. Chung, *Phytomedicine*, **13**, 707 (2006).
4. Y.Y. Akgul and H. Anil, *Phytochemistry*, **63**, 939 (2003).
5. M.R. Kim, H.T. Moon, D.G. Lee and E.R. Woo, *Arch. Pharm. Res*., **30**, 425 (2007).
6. B.S. Min, M.K. Na, S.R. Oh, K.S. Ahn, G.S. Jeong, G. Li, S.K. Lee, H. Joung and H.K. Lee, *J. Nat. Prod*., **67**, 1980 (2004).
7. M.C. Das and S.B. Mahato, *Phytochemistry*, **22**, 1071 (1983).
8. J.P. Vincken, L. Heng, A. Groot and H. Gruppen, *Phytochemistry*, **68**, 275 (2007).
9. Y.K. Kwon and S.U. Kim, *J. Korean. Soc. Appl. Biol. Chem*., **45**, 28 (2002).
10. K.J. Yun, B.S. Min, J.Y. Kim and K.T. Lee, *Biol. Pharm. Bull*., **30**, 139 (2007).
11. B.S. Min, S.R. Oh, K.S. Ahn, J.H. Kim, J. Lee, D.Y. Kim, E.H. Kim and H.K. Lee, *Planta. Med*., **70**, 1210 (2004).
12. T.M. Hung, M.K. Lee, B.S. Min, J.C. Kim, J.S. Choi and H.K. Lee, *J. Korean Soc. Appl. Biol. Chem*., **52**, 560 (2009).
13. L.N. Li, X. Hong and X. Li, *Planta. Med*., **57**, 169 (1991).
14. J.N. Grabre, I. Yoichiro, Y. Ma and R.C. Huang, *J. Chromatogr. A*, **719**, 353 (1996).
15. S.B. Kilidhar, M.R. Parthasarathy and P. Sharma, *Phytochemistry*, **21**, 796 (1982).
16. H. Kasahara, Y. Jiao, D.L. Bedgar, S.J. Kim, A.M. Patten, Z.Q. Xia, L.B. Davin and N.G. Lewis, *Phytochemistry*, **67**, 1765 (2006).
17. J.H. Wang, S.C. Tam, H. Huang, D.Y. Yang, Y.Y. Wang and Y.T. Zheng, *Biochem. Biophys. Res. Commun*., **317**, 965 (2004).
18. V.J.C. Martinez and C.R. Torres, *Phytochemistry*, **44**, 1179 (1997).