Hepatoprotective Diastereomeric Lignans from *Saururus chinensis* Herbs

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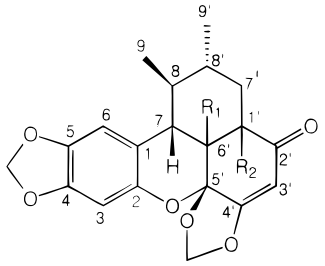
Received October 8, 1999

Two new diastereomeric sauchinones (**2**, **3**), along with the known sauchinone (**1**), a phenylpropanoid (sarisan), and two known lignans (galbacin and saucernetin), were isolated from the *n*-hexane fraction of *Saururus chinensis* using reversed-phase HPLC. The stereostructures of the lignans, sauchinone A (**2**) and 1′-*epi*-sauchinone (**3**), were established by analysis of spectroscopic data. Incubation of cultured rat hepatocytes intentionally injured by CCl_4 with each of the compounds **1**, **2**, and **3** significantly reduced the levels of glutamic pyruvic transaminase released by the damaged hepatocytes.

Saururus chinensis (Lour.) Baill. (Saururaceae) has been used in Korean folk medicine for the treatment of edema, jaundice, and gonorrhea.[1] Lignans, neolignans, aristolactams, and flavonoids have been isolated from *Saururus* species.[2−7] In our previous work on the aqueous fraction of *S. chinensis* herbs, we reported two flavonol glucuronides that have hepatoprotective activities.[8] Further work on the *n*-hexane fraction of this plant resulted in the isolation of three diastereomeric lignans identified as sauchinone (**1**), sauchinone A (**2**), and 1′-*epi*-sauchinone (**3**) by NMR spectroscopic methods. Sauchinone A (**2**) is a new sauchinone. This is the first report of 1′-*epi*-sauchinone (**3**) from plants, which has been previously reported via synthesis by reduction of carpanone.[9]



Sauchinone (**1**) R_1 = —H R_2= ····H

Sauchinone A (**2**) R_1 = ····H R_2= ····H

1′-Epi-sauchinone (**3**) R_1 = —H R_2= —H

In our present work on the *n*-hexane fraction of *S. chinensis*, we isolated three diastereomeric lignans using reversed-phase HPLC. Among the isolated diastereomers, **1** was identified as sauchinone by comparison to previously reported spectroscopic data.[10] Sauchinone is a unique lignan with a structure closely related to that of carpanone. Wang et al.[10] isolated sauchinone from *S. chinensis* and evaluated its stereostructure using NMR spectroscopy and X-ray crystallography.

Compound **2** was obtained as an amorphous powder. The molecular formula $C_{20}H_{20}O_6$ was established by HREIMS. The spectral data (IR, UV, MS, and NMR) closely resembled those of sauchinone (Table 1). The NMR spectra showed the presence of two aromatic protons (δ 6.53 and 6.73) and two methylenedioxy groups, one (^{1}H δ 5.93, 5.91; ^{13}C δ 101.24) attached to an aromatic ring and the other (^{1}H δ 5.74, 5.52; ^{13}C δ 98.19) attached to aliphatic carbons. ^{13}C NMR signals at δ 143.00, 145.83, and 146.86 indicated that, in addition to aromatic carbon−oxygen bonds for a methylenedioxy group, there is an additional oxygen attached to the aromatic ring. The ^{13}C NMR signal at δ 197.81 was attributed to the carbonyl group of an enone, while the ^{13}C NMR signal at δ 163.81 indicated the presence of a methylenedioxy group attached to the γ-carbon of an enone. ^{1}H NMR signals at δ 1.14 (d, 5.7 Hz) and 1.05 (d, 6.1 Hz) showed two methyl groups, each coupled to a vicinal proton. Comparison of ^{1}H and ^{13}C NMR spectral data between **2** and sauchinone suggested that the two compounds are stereoisomers. Their ^{13}C NMR spectra are largely in agreement except for the carbon shifts of 1, 7, 1′, 2′, 6′, and 7′ (Table 1). From COSY, NOE, and HMBC data, we concluded that **2** is a diastereomer of sauchinone (**1**) (Table 2 and Figure 1). The methine resonance at δ 2.20 (H-6′) displayed NOEs with the methine proton of δ 2.68 (H-1′), and the signal at δ 2.09 (H-7) displayed NOEs with the methine protons at δ 6.73 (H-6) and δ 1.14 (9-CH_3). Hence, the configurations of 7-H, 6′-H, and 1′-H are a *trans*−*cis* form by comparison with the *cis*−*trans* form in sauchinone (**1**). Thus, **2** is a new sauchinone diastereomer, which we have designated sauchinone A.

Compound **3** was also obtained as an amorphous powder. The molecular formula $C_{20}H_{20}O_6$ was also established by HREIMS, *m*/*z* 356.1266 $[M]^+$ (calcd for $C_{20}H_{20}O_6$, *m*/*z* 356.1260). The spectral data (IR, UV, MS, and NMR) closely resembles the data of sauchinone (Table 1). Comparison of ^{13}C NMR spectra between **3** and sauchinone suggested that these two compounds are also stereoisomers. Their ^{13}C NMR spectra are largely in agreement except for carbon shifts of 7′, 6′, 1′, and 7 (Table 1). From the NOESY spectrum, we found that the methine resonance at δ 2.20 (H-6′) displayed positive NOEs with the methine protons of δ 2.60 (H-1′) and 3.18 (H-7) and, hence, the configurations of 7-H, 6′-H, and 1′-H are a *cis*−*cis* form by comparison with the *cis*−*trans* form in sauchinone (Table 2).[1] H and ^{13}C NMR spectral assignments of **3** were based on its COSY and HMBC data. Although compound **3** has been previously synthesized by reduction of carpanone,[9] this is the first report of its occurrence in nature.

The hepatoprotective activities of **1**, **2**, and **3** were assessed by measuring their effects on the release of glutamic pyruvic transaminase (GPT) from primary cul-

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10.1021/np990499e CCC: $19.00
Published on Web 05/19/2000

Table 1. ^{1}H and ^{13}C NMR Data of **1**, **2**, and **3**

	1		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	115.73		126.00		118.77	
2	145.70		145.83		144.95	
3	99.50	6.38 (1H, s)	100.94	6.53 (1H, s)	100.32	6.34 (1H, s)
4	143.26		143.00		143.26	
5	146.72		146.86		146.49	
6	105.54	6.82 (1H, s)	104.83	6.73 (1H, d, 0.6)	107.33	6.76 (1H, s)
7	35.06	3.03 (1H, d, 5.4)	45.54	2.09 (1H, d, 9.7)	36.13	3.18 (1H, dd, 10.7, 1.5)
8	34.82	2.44 (1H, m)	36.56	1.55 (1H, m)	35.22	2.22 (1H, dd, 14.4, 6.3)
9	21.29	1.22 (3H, d, 7.3)	18.32	1.14 (3H, d, 5.7)	24.54	1.20 (3H, d, 7.6)
1′	37.55	2.52 (1H, td, 11.9, 3.5)	45.10	2.68 (1H, m)	39.35	2.60 (1H, td, 12.7, 2.9)
2′	194.69		197.81		194.53	
3′	101.34	5.57 (1H, s)	101.38	5.61 (1H, s)	99.50	5.57 (1H, s)
4′	168.66		163.81		168.26	
5′	100.42		105.00		100.73	
6′	37.60	2.48 (1H, d, 5.4)	42.34	2.20 (dd, 12.5, 2.7)	42.16	2.20 (1H, t, 8.52)
7′eq	25.27	1.74 (1H, m)	32.04	2.30 (1H, td, 13.7, 2.7)	30.78	1.98 (1H, m)
7′ax		1.92 (1H, m)		1.09 (1H, m)		1.09 (1H, m)
8′	33.45	1.88 (1H, m)	32.72	1.45 (1H, m)	32.72	1.50 (1H, m)
9′	20.92	0.71 (3H, d, 7.4)	19.85	1.05 (3H, d, 6.1)	23.58	0.68 (3H, d, 7.1)
Ar. OCH_2O	101.24	5.90 (1H, s) 5.87 (1H, s)	101.24	5.93 (1H, s) 5.91 (1H, s)	101.24	5.91 (1H, s) 5.88 (1H, s)
Al. OCH_2O	98.19	5.65 (1H, s) 5.60 (1H, s)	98.19	5.74 (1H, s) 5.52 (1H, s)	98.19	5.61 (1H, s) 5.60 (1H, s)

Table 2. NOE Interactions Observed for **1**, **2**, and **3**

protons	**1**	**2**	**3**
7-H	6-H, 9′-CH_3, 6′-H	6-H, 9-CH_3,	6-H, 8-H, 9-CH_3, 6′-H
1′-H	8-H, 9-CH_3	6′-H	9-CH_3, 8′-H, 6′-H, 7′-Heq
6′-H	7-H, 9-CH_3	1′-H	7-H, 9-CH_3, 1′-H

Table 3. Hepatoprotective Activities of **1**, **2**, and **3**

compound	GPT assay[a] (%)
control[b]	100 ± 0.1
control[c]	0 ± 4.5
silybin[d]	76.6 ± 2.2***
1	70.1 ± 4.6**
2	78.2 ± 2.7***
3	75.9 ± 3.7***

[a] The percentage of activity is calculated as 100 × (GPT activity of control[c] − GPT activity of sample)/(GPT activity of control[c] − GPT activity of control[b]). [b] Control value for hepatocytes cultures not challenged with CCl_4. [c] Control value for untreated hepatocytes challenged with CCl_4. [d] Silybin was used as a positive control. Each sample was tested at 100 μM concentration. Significantly different from control[c] value: **$p < 0.01$ *** $p < 0.001$ ($n = 3$).

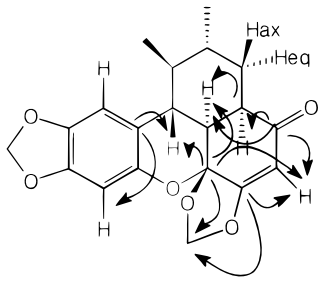

Figure 1. HMBC correlations of compound **2**.

tures of rat hepatocytes injured with CCl_4.[8,11−14] Compounds **1**, **2**, and **3** markedly blocked the release of GPT from CCl_4-damaged hepatocytes (Table 3). These effects were comparable to silybin, which was used as a positive control. The present study has shown that compounds **1**, **2**, and **3** have a significant hepatoprotective effect on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes. As such, **1**, **2**, and **3** might hold significant therapeutic value in the prevention or treatment of liver disease.

Experimental Section

General Experimental Procedures. Merck Si gel 60 (230−400 mesh) was used for flash column chromatography. UV spectra were obtained on a Shimadzu UV-2101 spectrophotometer, and IR spectra on a Perkin-Elmer 1710 spectrophotometer. The ^{1}H and ^{13}C measurements were carried out in a Bruker AMX 400 spectrometer operating at 400 and 100 MHz, respectively. Data processing was carried out on an Aspect ×32 computer with UXNMR software with Bruker microprograms. Standard pulse sequences were used for ^{1}H−^{1}H COSY (PO = 45 or 90°), NOESY (mixing time varying between 0.5 and 1.2 s), and HMBC [1/2J = 70 ms for $J_{C,H}$ = 7 Hz]. The experiments were carried out at 300 K unless stated otherwise. An internal lock was applied, and the reference was set to the solvent peak ($CDCl_3$, 7.27 for ^{1}H and 77.23 for ^{13}C). HRMS were measured on a JEOL JMS AX 505 WA spectrometer and optical rotations on a JASCO DIP-1000 polarimeter.

Plant Material. *S. chinensis* was cultivated in the Medicinal Plant Garden, College of Pharmacy, Seoul National University. The specimen was identified by Dr. Dae S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. The air-dried, aerial part of *S. chinensis* (9 kg) was cut into pieces and extracted three times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent in vacuo yielded a methanolic extract (900 g). This MeOH extract was then suspended in distilled water and partitioned successively with *n*-hexane, $CHCl_3$, and *n*-BuOH. The *n*-hexane fraction (50 g) was fractionated by extensive column chromatography over Si gel using a *n*-hexane−EtOAc gradient and yielded five fractions. After Si gel column chromatography of fraction 1, three compounds were isolated and subsequently identified as the known compounds, sarisan, galbacin, and saucernetin. Fraction 2 was a complex mixture of lignans; Si gel column chromatography of fraction 2 with a solvent gradient of ethyl acetate in *n*-hexane yielded a mixture of three isomeric compounds, which were resolved by reversed-phase HPLC to give **1**, **2**, and **3**. An HPLC system (Hitachi L-6200, Japan), equipped with a UV−visible detector and

Microsorb C_{18} semipreparative column (Rainin Inst. Co.), was used for isolation. The conditions for HPLC were a mixture of AcCN−MeOH−H_2O (60:12:25) as mobile phase and UV detection at 254 nm.

Sauchinone (1): colorless powder (500 mg); $C_{20}H_{20}O_6$; mp 224−226 °C; $[\alpha]^{26}_D$ −140° (*c* 1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 300 (2.55), 253 (2.74) nm; IR (neat) ν_{max} 2916, 1676, 1664, 1418, 1433, 1321, 1240, 1184, 1155, 979, 926, 892, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 356 $[M]^+$ (100), 270 (13), 257 (18), 205 (26), 175 (57), 151 (39), 138 (23).

Sauchinone A (2): colorless powder (20 mg); $C_{20}H_{20}O_6$; mp 240−242 °C; $[\alpha]^{26}_D$ −80° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 299 (2.12), 253 (2.54) nm; IR (neat) ν_{max} 2918, 1668, 1478, 1346, 1240, 1163, 936, 888, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 356 $[M]^+$ (100), 298 (11), 270 (18), 257 (19), 205 (11), 175 (34), 151 (34), 138 (16); HREIMS *m*/*z* 356.1262 $[M]^+$ (calcd for $C_{20}H_{20}O_6$, *m*/*z* 356.1260).

1′-*epi*-Sauchinone (3): colorless powder (20 mg); $C_{20}H_{20}O_6$; mp 235−237 °C; $[\alpha]^{26}_D$ −100° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 300 (2.32), 255 (2.51) nm; IR (neat) ν_{max} 2916, 1651, 1477, 1187, 1039, 915 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 356 $[M]^+$ (89), 270 (9), 257 (8), 205 (42), 175 (100), 147 (17), 138 (9); HREIMS *m*/*z* 356.1266 $[M]^+$ (calcd for $C_{20}H_{20}O_6$, *m*/*z* 356.1260).

Biological Evaluation. Isolated rat hepatocytes were prepared from male Wistar rats by the collagenase perfusion technique of Berry and Friend[15] with minor modifications.[16,17] One day after isolated rat hepatocytes were plated, the cultured cells were exposed to a medium containing 5 mM CCl_4/EtOH (final concentration, 0.07%) for 1.5 h to induce hepatotoxicity.[11,12] The activity of GPT released into the culture medium was determined by the method of Reitman−Frankel.[16]

Statistical Analysis. All data are expressed as the mean ± SD. The evaluation of statistical significance was determined by the one-way ANOVA test.

Acknowledgment. This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the Research Center for New Drug Development at Seoul National University. We thank Dr. G. J. Markelonis of the University of Maryland School of Medicine for his editorial assistance.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP990499E