The Anti-HBsAg (Human Type B Hepatitis, Surface Antigen) and Anti-HBeAg (Human Type B Hepatitis, e Antigen) C_{18} Dibenzocyclooctadiene Lignans from *Kadsura matsudai* and *Schizandra arisanensis*

Ming-Der Wu,[a,b] Ray-Ling Huang,[b] Li-Ming Yang Kuo,[b] Chia-Cheng Hung,[b] Chi-Wi Ong,*[,a] and Yao-Haur Kuo*[,b]

[a] *Department of Chemistry, National Sun Yat-Sen University; Kaohsiung, 804, Taiwan, R.O.C.: and* [b] *National Research Institute of Chinese Medicine; No. 155–1, Li-Nong St., Sec. 2, Shih-Pai, Taipei, 112, Taiwan, R.O.C.*

Received April 28, 2003; accepted August 4, 2003

The C_{18} dibenzocyclooctadiene lignans including three novel schizanrin F (1), G (2), H (3), along with the known kadsurarin (4), were isolated from *Kadsura matsudai*. A new C_{19} homolignan named schiarisanrin E (5), together with the known C_{18} lignans, gomisin B (6), G (7) and (+)-gomisin K_3 (8) were obtained from *Schizandra arisanensis*. Gomisin B, G and (+)-gomisin K_3 showed moderate to strong activity for antihepatitis in anti-HBsAg (human type B hepatitis, surface antigen) and/or anti-HBeAg (human type B hepatitis, e antigen) tests. The structural elucidations of new compounds 1—3 and 5 were based on two-dimensional (2D) NMR techniques including COSY, HMQC, HMBC, NOESY and CD spectra. Preliminary structure–activity relationship studies for these isolated lignans are also discussed.

Key words *Kadsura matsudai*; *Schizandra arisanensis*; schizanrin; schiarisanrin E; anti-HBsAg (human type B hepatitis, surface antigen); anti-HBeAg (human type B hepatitis, e antigen)

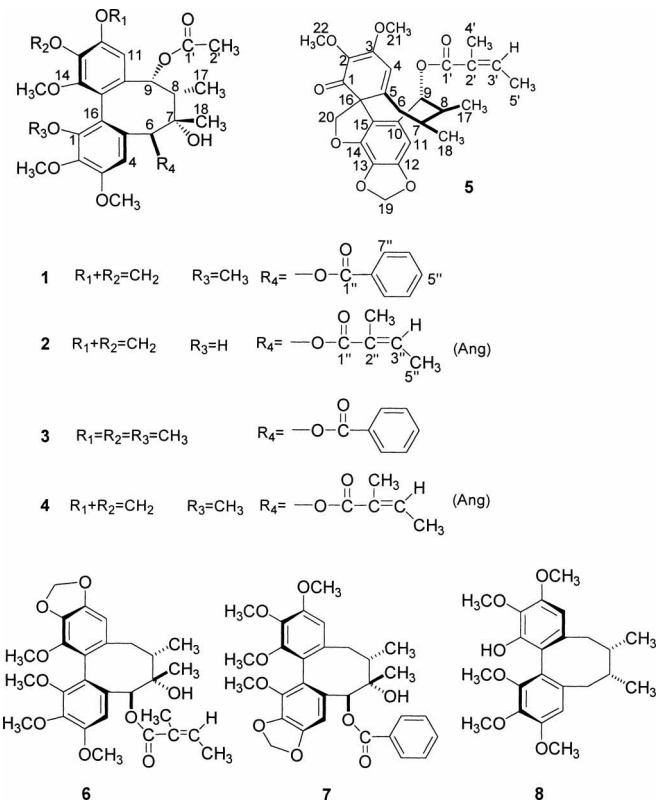
Several C_{18} dibenzocyclooctadiene lignans have been isolated from plants of Schizandraceae and exhibited some pharmacological effects such as antioxidant, antihepatitis, antihepatotoxic, and antilipid peroxidative activities.[1—5] In the course of our searching for the development of antitumor agents from Schizandraceous plants (*Schizandra arisanensis* and *Kadsura matsudai*) in Taiwan, we recently reported the isolation of two types of novel cytotoxic C_{19} homolignans.[6,7] We also reported that several novel C_{18} dibenzocyclooctadiene lignans (schizanrins B, C, D and E) were isolated from *K. matsudai*, which showed the anti-HBeAg (human type B hepatitis, e antigen) and anti-HBsAg (human type B hepatitis, surface antigen) effects.[8] Preliminary results concluded that C_{18} dibenzocyclooctadiene lignans possessed the anti-HBeAg and anti-HBsAg effects, instead of the cytotoxic effects caused by C_{19} homolignans. These findings stimulated our further search for the bioactive constituents against human type B hepatitis from *K. matsudai* and *S. arisanensis*. We report herein that bioassay led to the isolation and characterization of three novel C_{18} dibenzocyclooctadiene lignans, schizanrin F (**1**), G (**2**), H (**3**), along with the known kadsurarin (**4**) from *K. matsudai*. In addition, new C_{19} homolignan schiarisanrin E (**5**), together with the known C_{18} lignans, gomisin B (**6**), G (**7**) and (+)-gomisin K_3 (**8**), were isolated from *S. arisanensis*. The conformations of these new lignans were elucidated by two-dimensional NMR techniques including ^1H–^1H correlation spectroscopy (COSY), ^1H–^{13}C heteronuclear multiple quantum coherence (HMQC), ^1H–^{13}C heteronuclear multiple bonds coherence (HMBC) and nuclear Overhauser effect spectroscopy (NOESY).

The isolated C_{18} lignans (**1**—**4**, **6**—**8**) and C_{19} homolignan (**5**) were evaluated for inhibitory activity against human type B hepatitis with e antigen (HBeAg) and surface antigen (HBsAg).

Results and Discussion

The EtOH extract of the dried stems of *K. matsudai* and *S. arisanensis* were extracted successively with *n*-hexane, EtOAc and BuOH, respectively. Repeated column chromatography of the EtOAc extract of *K. matsudai* yielded schizanrin F (**1**), schizanrin G (**2**), H (**3**) and kadsurarin (**4**). The EtOAc extract of *S. arisanensis* yielded schiarisanrin E (**5**), gomisin B (**6**), G (**7**) and (+)-gomisin K_3 (**8**).

Schizanrin F (**1**) has molecular weight of 594, corresponding to the molecular formula $C_{32}H_{34}O_{11}$, and the IR spectrum with bands at 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm^{-1}. It possessed a biphenyl moiety due to two aro-



* To whom correspondence should be addressed. e-mail: kuoyh@cma23.nricm.edu.tw

matic protons (δ_H 6.82, 6.49 for H-4 and H-11) and 12 carbon atoms (δ_C 151.3, 141.1, 151.9, 110.2, 129.6, 121.8 for C-1, 2, 3, 4, 5, and 16, respectively; δ_C 132.9, 101.6, 148.7, 135.4, 140.5, 120.1 for C-10, 11, 12, 13, 14, and 15, respectively). Moreover, one methylenedioxy moiety (δ_H 5.76, 5.61, each 1H, d, J=2.0 Hz) and four methoxyl groups (δ_H 3.60, 3.85, 3.93, 3.30, each 3H, s) were occurred, and predictably located them at the biphenyl rings. In the cyclooctadiene ring, one secondary methyl group (δ_H 1.29, 3H d, J=7.0 Hz) and one singlet methyl group (δ_H 1.37, 3H, s), together with oxygenated carbon (δ 73.9), were assigned to CH_3-8 and CH_3-7, respectively. Moreover, the signals of a benzoate and an acetate groups were found in the NMR spectrum. Detailed inspection of the HMBC spectrum, the couplings between H-6 and C-1″ (δ_C 164.6), and between H-9 and C-1′ (δ_C 168.8) were found and further suggested that two ester groups substituted at C-6 and C-9, respectively. Thus, the structure of **1** was similar to the known kasurarin (**4**) except for an angelate at C-6 in **4** replaced by a benzoate. Moreover, the mass spectrum of **1** showed two intense peaks at *m/z* 534 [M^+-CH_3COOH] and *m/z* 412 [$M^+-CH_3COOH-C_7H_5COOH$], which also reflected the 1,2-elimination of acetic acid and benzoic acid *via* McLafferty ester rearrangement. The NOESY spectrum of **1** showed correlations between H-4 and H-6α, and between H-11 and H-9β, indicating that the benzoyl and acetyl group were located at the 6β and 9α position, respectively. Moreover, like the other cyclooctadiene lignans,[9,10] **1** has a twist-boat-chair conformation due to the correlated peaks between Me-7 and Me-8, H-9β and Me-8, and Me-7 and H-6α (Fig. 1).

Structure of schizanrin G (**2**) has the molecular formula $C_{29}H_{34}O_{11}$ as revealed by its electron impact (EI)-MS (*m/z* 558). Its IR spectrum exhibiting the absorptive bands at 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm^{-1}, as well as NMR spectrum showing two aromatic protons (δ_H 6.87, 6.36 for H-4 and H-11), indicated that **2** might possess a dibenzocyclooctadiene lignan. The ^{1}H-NMR spectrum of **2** displayed the signals as a secondary methyl group (δ_H 1.26, 3H, d, J=7.0 Hz, CH_3-8), a tertiary methyl group (δ_H 1.35, 3H, s, CH_3-7), one methylenedioxy moiety (δ_H 5.90, 5.97, each 1H, d, J=1.2 Hz), and three methoxyl groups (δ_H 3.89, 3.93, 3.56, 3H each s, 2-OCH_3, 3-OCH_3, 14-OCH_3, respectively, based on the HMBC spectrum), which was similar to those of **1** except for the absence of a benzoate and a methoxyl group instead of an angelate (2-methyl-2-butenoic acid) group in **2**. In the mass spectrum of **2**, the intense fragment ion at *m/z* 398 [$M^+-CH_3COOH-C_4H_7COOH$] was consistent with the presence of an angeloyloxy group. This evidence also reflected the 1,2-elimination of an acetic acid and angelic acid *via* McLafferty rearrangement involving the acetoxyl and angeloyloxy groups. Further confirmation of the location of angelic acid was based on the HMBC spectrum. Thus, correlated signals between the carbonyl carbon at C-1′ (δ_C 168.6), H-6 (δ_H 5.55) and olefinic H-3″ (δ_H 5.95) clearly indicated the angelic acid ester at C-6, and therefore completely assigned the structure of schizanrin G (**2**).

In the MS spectrum, schizanrin H (**3**) gave *m/z* 610 [M^+], corresponding to $C_{33}H_{38}O_{11}$. Its ^{1}H and ^{13}C spectra were similar to those of **1**, and indicated the characteristic signals for a substituted dibenzocyclooctadiene lignan with a benzoate ester, two methyl groups and an acetate ester at C-6, 7, 8 and 9, respectively. Six methoxyl groups on two aromatic rings in **3** rather than a methylenedioxy moiety and four methoxyl groups in **1** were observed in the NMR spectra. However, these methoxyl groups (δ_H 3.65, 3.88, 3.97, 3.97, 3.43, 3.13, 3H each s) were assigned for 1-OCH_3, 2-OCH_3, 3-OCH_3, 12-OCH_3, 13-OCH_3, 14-OCH_3 on the basis of the HMBC spectrum exhibiting the cross peaks to their respective aromatic carbon. The mass spectrum of **3** exhibited a molecular ion at *m/z* 610 compared with the weight of **1** at *m/z* 594, which was also consistent with the replacement of a methylenedioxy by two methoxyl groups. Like **1**, the stereochemistry of **3** possessed 6β-benzoic acid and 9α-acetic acid ester which were established by the NOESY spectrum. Thus, the structure of schizanrin H (**3**) was determined unambiguously.

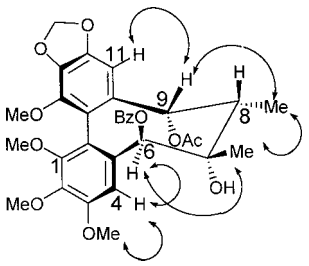


Fig. 1. Partial NOE Correlations of **1**

Table 1. ^{1}H-NMR Data[a] ($CDCl_3$) for Compounds **1**, **2** and **3**

Carbon	1	2	3
4	6.82 (s)	6.87 (s)	6.87 (s)
6	5.86 (s)	5.55 (s)	6.06 (s)
8	2.29 (m)	2.17 (m)	2.36 (m)
9	5.75 (s)	5.65 (s)	5.91 (s)
11	6.49 (s)	6.36 (s)	6.67 (s)
17	1.29 (d, 7.0)	1.26 (d, 7.0)	1.26 (d, 7.0)
18	1.37 (s)	1.35 (s)	1.35 (s)
OCH_2O	5.76 (d, 2.0)	5.97 (d, 1.2)	
	5.61 (d, 2.0)	5.90 (d, 1.2)	
1-OCH_3	3.60 (s)		3.65 (s)
2-OCH_3	3.85 (s)	3.89 (s)	3.88 (s)
3-OCH_3	3.93 (s)	3.93 (s)	3.97 (s)
12-OCH_3			3.97 (s)
13-OCH_3			3.43 (s)
14-OCH_3	3.30 (s)	3.56 (s)	3.13 (s)
2′	1.58 (s)	1.54 (s)	1.63 (s)
3″	7.44 (d, 7.0)	5.95 (br q, 6.3)	7.34 (d, 7.2)
4″	7.30 (t, 8.0)	1.42 (br s)	7.26 (t, 7.3)
5″	7.52 (t, 8.0)	1.82 (br d, 6.3)	7.47 (t, 7.2)
6″	7.30 (t, 8.0)		7.26 (t, 7.3)
7″	7.44 (d, 7.0)		7.34 (d, 7.2)

a) All assignments are based on 1D and 2D NMR experiments, including COSY 90, HETCOR, and HMBC spectra.

The configuration of biphenyl groups in all isolated dibenzocyclooctadiene lignans was determined basing on their characteristic circular dichroism (CD) spectra. The CD spectra of **1**—**3** showed a positive Cotton effect around 215—25 nm and a negative Cotton effect around 230—255 nm, suggesting that these dibenzocyclooctadiene lignans (**1**—**3**) possessed an *S*-biphenyl configuration as gomisin B.[11,12]

Schiarisanrin E (**5**) showed a molecular ion at *m/z* 482, which is consistent with the molecular formula of $C_{27}H_{30}O_8$. The IR and NMR spectra revealed that **5** possessed a C_{18} lig-

nan skeleton with an additional oxygenated methylene group, which is similar to those of previously reported C_{19} homolignans.[6] The presence of signals at δ_H 5.76 (br q, 1H, J=6.5 Hz), 1.71 (br s, 3H) and 1.73 (br d, 3H, J=6.5 Hz), and δ_C 168.2 (C-1′), 127.8 (C-2′), 135.5 (C-3′), 20.4 (C-4′) and 15.6 (C-5′) suggested an angeloyloxy group in **5**.[13] A proposed intense ion at m/z 382 [$M^+ - C_4H_7COOH$] and a molecular ion at m/z 482 were observed in the EI-MS, revealing that **5** is corresponding to the presence of an angeloyloxy group. Moreover, a HMBC experiment of **5** displayed cross signals between the carbonyl carbon at δ_C 168.2 (C-1′) and protons at δ_H 5.76 (H-3′), 1.71 (H-4′) and δ_H 5.70 (H-9), indicating that the angeloyloxy group is located at C-9, thus undoubtedly verifying the structure of **5** as shown.

The isolated lignans or homolignans (**1**—**8**) were evaluated for anti-HBsAg and anti-HBeAg (Table 4). The bioassay data exhibited that **1**—**5** were inactive (inhibition percentage: <25%) at concentrations of 50 and 100 μg/ml. On the con-

Table 2. ^{13}C-NMR Data[a] ($CDCl_3$) for Compounds **1**, **2** and **3**

Position	**1**	**2**	**3**	^{1}H–^{13}C long range correlations[b]
1	151.3 s	149.7 s	151.1 s	OCH_3-1
2	141.1 s	141.4 s	141.2 s	OCH_3-2, H-4
3	151.9 s	152.4 s	151.8 s	OCH_3-3, H-4
4	110.2 s	111.7 d	110.5 d	H-6
5	129.6 s	132.1 s	129.2 s	H-6
6	85.1 d	84.9 d	84.7 d	CH_3-18, H-4
7	73.9 s	73.6 s	74.2 s	CH_3-18, CH_3-17, H-8, H-6, H-9
8	43.1 s	43.1 d	42.8 d	CH_3-17, CH_3-18, H-6, H-9
9	83.3 d	83.6 d	83.6 d	CH_3-17, H-11
10	132.9 s	133.1 s	133.7 s	H-9
11	101.6 d	101.6 d	106.4 d	H-9
12	148.7 s	148.5 s	152.9 s	H-19, H-11
13	135.4 s	135.0 s	141.3 s	H-19, H-11
14	140.5 s	137.0 s	151.3 s	
15	120.1 s	117.2 s	121.4 s	H-9, H-11
16	121.8 s	119.5 s	122.3 s	H-4, H-6
17	17.0 q	16.8 q	17.0 q	H-8, H-9
18	28.7 q	28.7 q	28.7 q	H-6
OCH_2O	100.8 t	101.3 t		
1-OCH_3	60.4 q		60.0 q	
3-OCH_3	55.9 q	56.1 q	56.1 q	
2-OCH_3	60.4 q	60.8 q	60.4 q	
12-OCH_3			56.0 q	
13-OCH_3			59.8 q	
14-OCH_3	58.6 q	61.0 q	60.4 q	
1″	164.6 s	165.9 s	164.7 s	H-6, H-3″, H-7″
2″	129.3 s	126.6 s	129.0 s	H-3″, H-7″
3″	129.4 d	139.8 s	129.3 d	H-7″, H-5″
4″	127.8 d	19.9 q	128.1 d	H-3″, H-6″
5″	132.6 d	15.6 q	129.2 d	H-3″, H-7″
6″	127.8 d		128.1 d	H-4″
7″	129.4 d		129.3 d	H-3″, H-5″
1′	168.8 s	168.6 s	168.8 s	CH_3-2′, H-9
2′	20.4 q	20.4 q	20.4 q	

a) Multiplicity was determined from DEPT. *b*) ^{1}H–^{13}C long-range correlation (HMBC) corresponded to two or three bonds connectivities.

Table 3. ^{1}H- and ^{13}C-NMR Data[a] ($CDCl_3$) for Compound **5**

Position	Proton	Carbon	^{1}H–^{13}C long-range correlations[b]
1		194.9 s	H-20
2		156.1 s	H-22
3		132.4 s	H-4, 21
4	6.09 (d, 1.9)	120.6 d	H-6
5		146.8 s	H-6, 20
6a	2.54 (m)	40.2 t	H-18
6b	2.19 (dd, 12.5, 3.5)		H-18
7	1.81 (m)	31.6 d	H-6, 8, 9, 17, 18
8	1.98 (m)	42.6 d	H-9, 6, 17, 18
9	5.70 (d, 7.0)	78.2 d	H-11, 18
10		129.0 s	H-9
11	6.39 (s)	101.1 d	H-9
12		130.1 s	H-11, 19
13		150.3 s	H-11, 19
14		144.1 s	H-20
15		122.1 s	H-9, 11, 20
16		64.5 s	H-6, 4
17	1.02 (d, 7.5)	9.5 q	H-8, 18
18	0.90 (d, 6.5)	21.6 q	H-6
19	6.03, 5.97 (ABq, 15.3)	101.3 t	H-12
20	4.27, 4.54 (ABq, 9.0)	78.0 t	
21	3.69 (s)	59.1	
22	4.02 (s)	58.3	
1′		168.2 s	H-9, 4′
2′		127.8 s	H-3′, 4′, 5′
3′	5.76 (br q, 6.5)	135.5 d	H-2′
4′	1.71 (br s)	20.4 q	H-3′, H-5′
5′	1.73 (br d, 6.5)	15.5 q	H-3′, H-4′

a) All assignments are based on 1D and 2D NMR experiments, including COSY 90, HETCOR, and HMBC spectra. *b*) ^{1}H–^{13}C long-range correlation (HMBC) corresponded to two or three bond connectivities.

Table 4. Anti-HBsAg and Anti-HBeAg Effects of Compounds **6**, **7** and **8**

Entry	Conc. (μg/ml)	HBsAg[a] (Inhibition %)	HBeAg[a] (Inhibition %)	AST (Inhibition %)
Gomisin B (**6**)	100	74.1	34.1	−3.2
	50	28.9	28.2	−7.7
	25	3.3	15.7	−6.9
	10	−1.3	−3.9	−0.3
Gomisin G (**7**)	100	76.3	22.1	19.0
	50	42.4	20.0	13.3
	25	17.9	6.3	16.5
	10	−3.3	6.1	3.2
(+)-Gomisin K_3 (**8**)	100	76.3	Toxic[b]	63.6
	50	42.4	20.0	19.5
	25	17.9	16.1	12.6
	10	−3.3	5.2	15.9
DMSO	2.5 μl/ml	0	0	<25

a) Active inhibition: 25—35% (moderate inhibition), 35—45% (medium inhibition), >45% (strong inhibition); inactive inhibition: <25% inhibition. *b*) Toxic & AST were explained in the text (see anti-HBsAg and anti-HBeAg test).

trast, **6**, **7** and **8** displayed strong inhibition at concentrations of 100, 100 and 50 μg/ml, respectively, in the anti-HBsAg test. Compounds **6** and **7** also exhibited moderate inhibition in anti-HBeAg assay at concentrations of 50 and 100 μg/ml, respectively. These results revealed that the substituted moiety at C-9 in the C_{18} dibenzocyclooctadiene lignans such as **1**—**4** would decrease the inhibitory effects, whereas C-6 substituent seems not significant for the bioactivity. However, detailed structure–activity relationships of the C_{18} dibenzocyclooctadiene lignans employed to inhibit the activity of human type B hepatitis still need to be explored.

Experimental

General Experimental Procedures ^{1}H- and ^{13}C-NMR spectra were recorded at 300.13 and 75.46 MHz, respectively, on a Bruker 300 AC spectrometer. The spectra of heteronuclear correlation, HMBC was established by the coupling of 8 Hz. EI-MS was performed on a JEOL SX-102A instrument. Silica gel (Merck 70—230 mesh) was used for column chromatography, and precoated silica gel (Merck 60F-254) plates were used for TLC. HPLC was accomplished on an SPD-6AV liquid chromatograph using a preparative C_{18} column. Melting points were determined on a Fisher–Johns apparatus and are uncorrected.

Plant Material The stems of *Kadsura matsudai* HAYATA were collected in July 2000 in Pi-Tong County, and the stems of *Schizandra arisanensis* were collected in May 2001 in Taipei County, Taiwan. Both voucher species (No 20000703 for *K. matsudai*, No. 20010501 for *S. arisanensis*) were 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) and the fruits of *S. arisanensis* (1.8 kg) were extracted exhaustively with ethanol. The crude ethanol syrup was extracted five times with hexane. The respective ethanol layer of *K. matsudai* and *S. arisanensis* were partitioned with EtOAc–H_2O (1 : 1) three times to give EtOAc and H_2O layers. The EtOAc extract of *K. matsudai* was evaporated *in vacuo* and 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. After recrystallization, compound **4** (194 mg) was furnished from fraction 6. Fraction 7 was further purified by column chromatography on silical gel eluting with *n*-hexane–CH_2Cl_2 (10 : 1, 8 : 1, 6 : 1, 4 : 1, 2 : 1, 1 : 1, CH_2Cl_2) to yield 14 fractions. Compounds **1** (4.7 mg) and **3** (3.2 mg) were yielded from fraction 7-7 by HPLC ($5C_{18}$, 250×10 mm, MeOH–H_2O=65 : 35). Compound **2** (4.9 mg) was obtained from fraction 7-8. The EtOAc extract (32 g) of *S. arisanensis* was chromatographed on a silica gel column with *n*-hexane–EtOAc (8 : 1, 6 : 1, 4 : 1, 2 : 1, 1 : 1, EtOAc) to give 14 fractions. Compounds **5** (6.7 mg), **6**[12] (10.2 mg) and **8**[14] (3.6 mg) were furnished from fraction 11 by HPLC ($5C_{18}$, 250×10 mm, MeOH–H_2O=70 : 30). Compound **7**[12] (25.3 mg) was obtained from fraction 7.

Schizanrin F (**1**): White solids; mp 141—143 °C; IR ν_{max} (KBr) 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm^{-1}; EI-MS *m/z* 594 (rel. intensity) (M^+, 15), 534 (100), 412 (40); high resolution (HR)-EI-MS *m/z* 594.2099 $[M]^+$ (Calcd for $C_{32}H_{34}O_{11}$: 594.2101); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Schizanrin G (**2**): White needles; mp 179—182 °C; IR ν_{max} (KBr) 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm^{-1}; EI-MS *m/z* 558 (rel. intensity) (M^+, 38), 498 (70), 398 (100); HR-EI-MS *m/z* 558.2097 $[M]^+$ (Calcd for $C_{29}H_{34}O_{11}$: 558.2101); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Schizanrin H (**3**): White needles; mp 193—195 °C; IR ν_{max} (KBr) 3400 (OH), 1715 (ester), 1610 and 1590 (aromatic) cm^{-1}; EI-MS *m/z* 610 (rel. intensity) (M^+, 15), 550 (100), 428 (30); HR-EI-MS *m/z* 610.2406 $[M]^+$ (Calcd for $C_{33}H_{38}O_{11}$: 610.2414); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Schiarisanrin E (**5**): Pale yellow solids; IR ν_{max} (KBr) 1725 (C=O), 1650, 1590 , 715 (aromatic) cm^{-1}; EI-MS *m/z* 482 (rel. intensity) (M^+, 67), 382 (100); HR-EI-MS *m/z* 482.1940 $[M]^+$ (Calcd for $C_{27}H_{30}O_8$: 482.1941); ^{1}H- and ^{13}C-NMR, see Table 3.

Anti-HBsAg and Anti-HBeAg Test The assays for *in vitro* antiviral activity against hepatitis B virus (HBV) were performed according to our previously described procedure.[15] Briefly, the HBV-producing cell line MS-G2 was plated into 24-well flat-bottomed tissue culture plates at a density of 3×10^5 cells/ml/well. After an overnight stay to ensure that the cells had been properly attached, cells were challenged by test compounds. DMSO alone was added to each culture as solvent control. All tested pure compounds were dissolved in DMSO at a concentration of 1, 5, 10, and 20 μg/ml, respectively. The concentration of DMSO in the media was maintained at no more than 2.5 μl/ml, to ensure that it did not affect the growth of MS-G2 cells. Subsequently, the culture media were collected after 3 d for anti-viral assay. Then, we analyzed the HBsAg and HBeAg values as anti-viral indicators using the ELISA assay (enzyme-linked immunosorbent assay) (Instrument: DYNATECH MR 7000 at 490 nm) to evaluate the anti-viral effects for the test samples. The percentage inhibition (%) was calculated by comparing with the control group. Inhibition between 25—35% was defined as moderate inhibition, 35—45% as medium, and >45% as strong, while an inhibition percentage below 25% was defined as inactive.

(1) Cell Line and Cell Culture: A HBV DNA integrated HCC cell line, MS-G2, kindly provided by Dr. Max Essex,[15] was established from a hepatoblastoma derived cell line, HepG2, by transfection with two copies of the entire HBV genome. The MS-G2 cell line secreted HBV containing viral DNA and a DNA polymerase activity. The MS-G2 cells were cultured in RPMI-1640 (GIBCO, BRL, Grand Island, NY, U.S.A.) medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 2 mmol/l L-glutamine, 1% nonessential amino acids, and 2.5 mg/ml fungizone. Exponentially growing cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Under these conditions the plating efficiency was above 95%.

(2) Cytotoxic Assay: Cell damage was tested by an AST (aspartate transaminase) Fuji kit. AST values higher than 25 I.U./l served to indicate of cell damage or lysis, as described previously.[15]

Acknowledgments The authors thank the National Science Council, R.O.C. (NSC 88-2314-B-077-007) and the National Research Institute of Chinese Medicine for their financial support and many assistance to Y. H. Kuo. They also appreciate Mr. Shih-Jen Wang, NSC Regional Instrument Center of HSIN-CHU, for measuring the HRMS data.

References

1) Bao T. T., Xu G. F., Liu T. G., Sun R. H., Song Z. Y., *Acta Pharmaceut. Sinica*, **14**, 1—7 (1979).
2) Lu H., Liu G. T., *Planta Med.*, **58**, 311—313 (1992).
3) Hikino H., Kiso Y., Taguchi H., Ikeya Y., *Planta Med.*, **50**, 213—218 (1984).
4) Yang X. W., Hattori M., Namba T., Chen D. F., Xu G. J., *Chem. Pharm. Bull.*, **40**, 406—409 (1992).
5) Yang X. W., Miyashiro H., Hattori M., Namba T., Tezuka Y., Kikuchi T., Chen D. F., Xu G. J., Hori T., Extine M., Mizuno H., *Chem. Pharm. Bull.*, **40**, 1510—1516 (1992).
6) Kuo Y. H., Kuo L. M. Y., Chen C. F., *J. Org. Chem.*, **62**, 3242—3245 (1997).
7) Kuo Y. H., Huang H. C., Kuo L. M. Y., Chen C. F., *J. Org. Chem.*, **64**, 7023—7027 (1999).
8) Kuo Y. H., Li S. Y., Huang R. L., Wu M. D., Huang H. C., Lee K. H., *J. Nat. Prod.*, **64**, 487—490 (2001).
9) Chen D. F., Zhang S. X., Chen K., Zhou B. N., Wang P., Cosentino L. M., Lee K. H., *J. Nat. Prod.*, **59**, 1066—1068 (1996).
10) Chen Y. G., Wang P., Lin Z. W., Sun H. D., Qin G. W., Xie Y. Y., *Phytochemistry*, **48**, 1059—1062 (1998).
11) Taguchi H., Ikeya Y., *Chem. Pharm. Bull.*, **23**, 3296—3298 (1975).
12) Ikeya Y., Taguchi H., Yosioka I., Kobayashi H., *Chem. Pharm. Bull.*, **27**, 1383—1394 (1979).
13) Ikeya Y., Taguchi H., Yosioka I., Kobayashi H., *Chem. Pharm. Bull.*, **28**, 3357—3361 (1980).
14) Ikeya Y., Taguchi H., Yosioka I., *Chem. Pharm. Bull.*, **28**, 2422—2427 (1980).
15) Huang R. L., Chen C. C., Huang Y. L., Hsieh D. J., Hu C. P., Chen C. F., Chang C. M., *Hepatology*, **24**, 508—515 (1996).