Two New Dammarane-Type Triterpenoid Saponins from *Gynostemma pentaphyllum*

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Two new dammarane-type triterpenoid saponins were isolated from the EtOH extract of *Gynostemma pentaphyllum* (THUNB.) MAKINO. Their structural elucidations were accomplished mainly on the basis of the interpretation of spectroscopic data, such as IR, NMR, and HR-TOF-MS. Their liver fibrosis inhibitory activities were evaluated against hepatic stellate cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Thus, *G. pentaphyllum* can be used as ingredient for ancillary drugs or functional food.

Introduction. – '*Jiao-Gu-Lan*', *Gynostemma pentaphyllum* (THUNB.) MAKINO is a traditional Chinese medicine used for a variety of conditions. It is widely used in China, Japan, and Korea as a herbal medicine or tea. The biologically active constituents are dammarane-type glycosides, called gypenosides [1], with beneficial effects reported against numerous diseases, including treatment of hepatitis, inflammation, cancer, and atherosclerosis, although the mechanisms underlying these therapeutic effects are unknown [2]. In our series of studies on the anticancer natural medicines, we have found some active compounds before [3–5].

Our previous study demonstrated that the total saponins of *G. pentaphyllum* inhibited hepatic stellate cells (HSC) proliferation, with almost no toxicity to human normal cells (L02). As a continuation of this research, two new triterpene saponins were isolated from this plant. They were characterized as (21*R*,23*R*)-3β,20ξ,21,26-tetrahydroxy-21,23-epoxy-19-oxodammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**1**) and (20*S*)-3β,20,21-trihydroxy-19-oxodammara-23,25-diene 3-*O*-{[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (**2**; *Fig. 1*).

Herein, we report the structure elucidations of these two new dammarane-type saponins.

Results and Discussion. – Compound **1** was obtained as white amorphous powder, showing a peak at *m*/*z* 937.4792 ([*M* + Na]$^+$; calc. 937.4775) in the HR-TOF-MS, indicating the molecular formula $C_{46}H_{74}O_{18}$. The IR spectrum (KBr) displayed absorptions at 3438 (OH), 1701 (C=O), and 1645 cm^{-1} (C=C). The ^{1}H-NMR spectrum

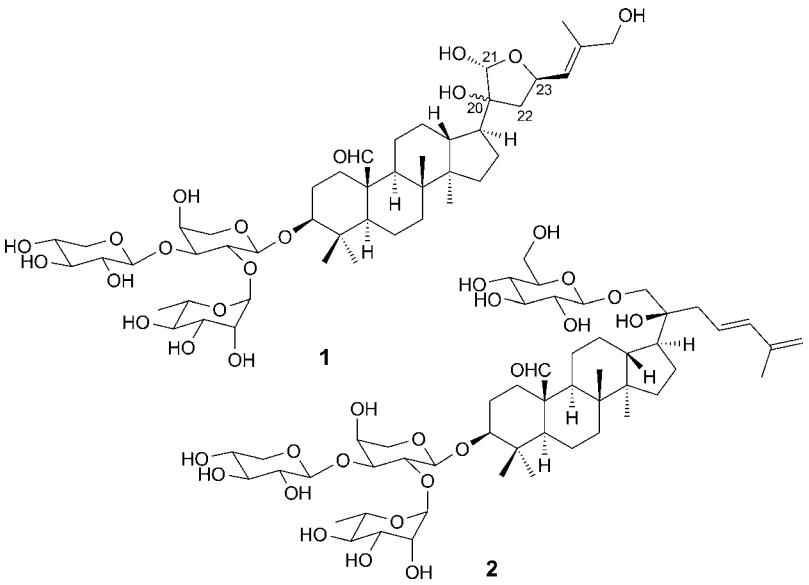

Fig. 1. *Chemical structures of compounds* **1** *and* **2**

(*Table 1*) exhibited signals of five Me groups (δ(H) 0.87 (*s*), 1.07 (*s*), 1.16 (*s*), 1.24 (*s*), 1.81 (*s*)), of an olefinic H-atom at δ(H) 6.44 (*d*, $J = 8.4$), of a CHO group at δ(H) 10.26 (*s*), and signals due to an α-L-arabinopyranoside moiety (δ(H) 4.87 (*s*, 1 H)), a β-D-xylopyranosyl moiety (δ(H) 5.00 (*d*, $J = 4.8$, 1 H)), and of an α-L-rhamnopyranosyl moiety (δ(H) 6.14 (*s*, 1 H), 1.56 (*s*, 3 H)). Analysis of the ^{1}H- and ^{13}C-NMR spectra established **1** as a triterpene saponin with an O-bridge between C(21) and C(23), and showing a close resemblance with (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)][β-D-xylopyranosyl-(1 $\rightarrow$ 3)]-α-L-arabinopyranoside [6] with respect to ^{13}C-NMR spectra, except for the upfield shifts of C(20) ($\Delta\delta$ -2.1), C(21) ($\Delta\delta$ -2.8), C(22) ($\Delta\delta$ -0.8), C(24) ($\Delta\delta$ -0.9), and the downfield shifts of C(23) ($\Delta\delta$ $+1.2$) and C(25) ($\Delta\delta$ $+1.0$). These differences can most readily be reconciled by assuming different relative configurations at C(21) and C(23). Furthermore, both absolute configurations at C(21) and C(23) of **1** have been determined as (*R*), according to the pertinent NOESY interactions (*Fig. 2*). In the NOESY spectrum, NOE cross-peaks were observed between the olefinic H-atom signal at δ(H) 6.44 (H–C(24)) and the signal at δ(H) 4.22–4.25 (CH_2(26)), between the signals at δ(H) 5.47–5.50 (H–C(23)) and δ(H) 1.81 (Me(27)). So, the aglycone part of **1** was determined as (21*R*,23*R*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene. The C-atom signals assignable to the sugar moieties and C(3) in the ^{13}C-NMR spectrum were superimposable with those of (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)][β-D-xylopyranosyl-(1 $\rightarrow$ 3)]-α-L-arabinopyranoside. The linkage sites, and sequences with the trisaccharide and its attachment to the aglycone were confirmed by the 2D-NMR experiments. In the HMBC (*Fig. 3*), the cross peaks between H–C(1′) of the arabinose and C(3) of the aglycone, H–C(1″) of the rhamnose

Table 1. *^{1}H- and ^{13}C-NMR* (600, 150 MHz, resp., in C_5D_5N) *Data for* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC (H → C)
CH_2(1)	2.56–2.60 (*m*), 0.69–0.72 (*m*)	33.7	
CH_2(2)	2.04–2.08 (*m*), 1.66–1.69 (*m*)	27.7	
H–C(3)	3.30–3.34 (*m*)	87.2	1′
C(4)		40.1	
H–C(5)	1.14–1.16 (*m*)	54.9	3, 4, 6, 10
CH_2(6)	1.85–1.88 (*m*), 1.62–1.65 (*m*)	17.8	
CH_2(7)	1.67–1.70 (*m*), 1.34–1.37 (*m*)	34.9	
C(8)		40.5	
H–C(9)	1.69–1.71 (*m*)	53.0	
C(10)		52.9	
CH_2(11)	1.70–1.73 (*m*), 1.09–1.12 (*m*)	22.3	
CH_2(12)	2.08–2.11 (*m*), 1.94–1.97 (*m*)	25.1	
H–C(13)	2.23–2.26 (*m*)	41.4	
C(14)		50.2	
CH_2(15)	1.66–1.74 (*m*), 1.17–1.23 (*m*)	32.2	
CH_2(16)	2.16–2.19 (*m*), 1.58–1.61 (*m*)	27.1	
H–C(17)	2.07–2.08 (*m*)	45.1	20
Me(18)	1.16 (*s*)	16.7	8, 14, 15, 30
H–C(19)	10.26 (*s*)	205.5	
C(20)		82.6	
H–C(21)	5.88 (*s*)	100.2	17, 23
CH_2(22)	2.64–2.67 (*m*), 2.67–2.71 (*m*)	44.3	24
H–C(23)	5.47–5.50 (*m*)	74.5	
H–C(24)	6.44 (*d*, J = 8.4)	128.2	26, 27
C(25)		138.1	
CH_2(26)	4.22–4.25 (*m*)	67.6	24, 25, 27
Me(27)	1.81 (*s*)	14.2	24, 25
Me(28)	1.24 (*s*)	26.5	3, 4, 5, 29
Me(29)	1.07 (*s*)	16.1	3, 4, 5, 28
Me(30)	0.87 (*s*)	17.5	7, 8, 9, 14
Ara–O–C(3)			
H–C(1′)	4.87 (*s*)	104.8	3
H–C(2′)	4.62–4.64 (*m*)	74.5	1″
H–C(3′)	4.23–4.25 (*m*)	81.8	1‴
H–C(4′)	4.44–4.46 (*m*)	68.5	
CH_2(5′)	4.25–4.28 (*m*), 3.80 (*d*, J = 10.2)	65.2	
Rha–O–C(2′)			
H–C(1″)	6.14 (*s*)	102.1	2′
H–C(2″)	4.56–4.59 (*m*)	72.6	
H–C(3″)	4.74 (br. *s*)	72.5	
H–C(4″)	4.26–4.28 (*m*)	74.0	
H–C(5″)	4.54–4.57 (*m*)	70.1	
Me(6″)	1.56 (*s*)	18.6	
Xyl–O–C(3′)			
H–C(1‴)	5.00 (*d*, J = 4.8)	105.3	3′
H–C(2‴)	4.91–4.93 (*m*)	74.7	
H–C(3‴)	4.09–4.11 (*m*)	77.8	
H–C(4‴)	4.10–4.13 (*m*)	71.0	
CH_2(5‴)	4.28–4.31 (*m*), 3.63–3.66 (*m*)	67.1	

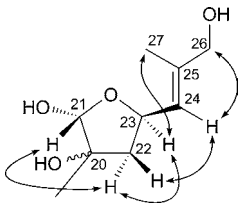

Fig. 2. *Key NOE correlations of* **1**

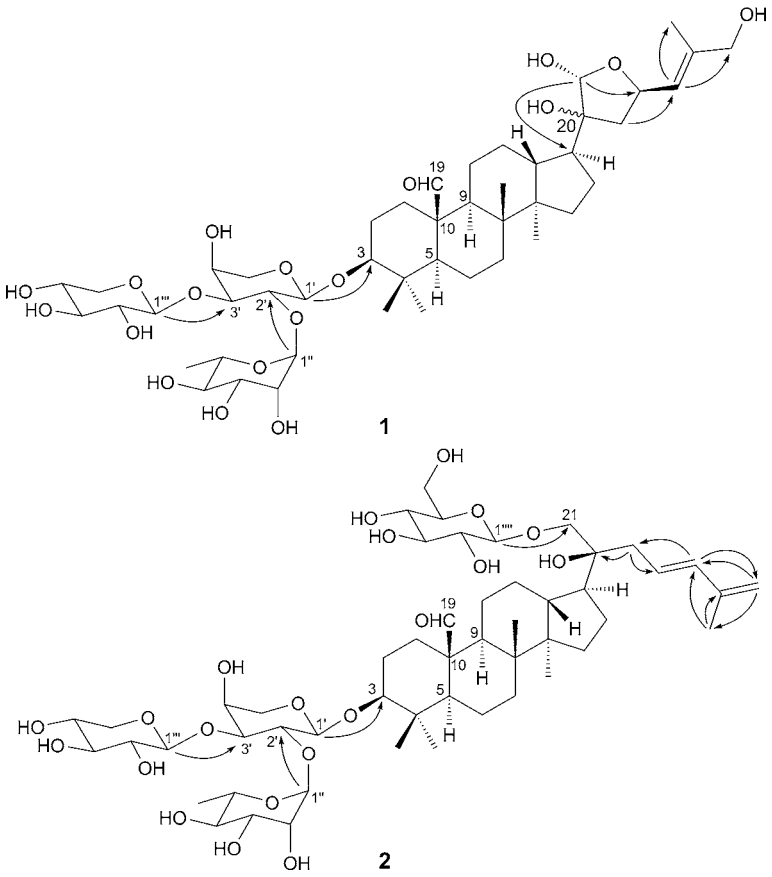

Fig. 3. *Key HMBCs of* **1** *and* **2**

and C(2′) of the arabinose, H–C(1′′′′) of the xylose and C(3′) of the arabinose were observed. Thus, **1** was deduced as (21*R*,23*R*)-3*β*,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 3)]-*α*-L-arabinopyranoside.

Compound **2**, a white amorphous powder, showed a peak at *m*/*z* 1067.5378 ([*M* + Na]$^+$, calc. 1067.5397) in the HR-TOF-MS, providing the molecular formula $C_{52}H_{84}O_{21}$. The IR spectrum (KBr) displayed absorptions at 3424 (OH), 1703 (C=O), and

Table 2. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp., in C_5D_5N) *Data for* **2**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (H → C)
CH_2(1)	2.59 – 2.62 (*m*), 0.65 – 0.67 (*m*)	33.3	
CH_2(2)	2.01 – 2.04 (*m*), 1.23 – 1.25 (*m*)	27.2	
H–C(3)	3.29 (*dd*, *J* = 11.8, 3.9)	86.8	1′
C(4)		39.7	
H–C(5)	1.10 – 1.15 (*m*)	54.5	10, 28
CH_2(6)	1.86 – 1.90 (*m*), 1.62 – 1.64 (*m*)	17.4	
CH_2(7)	1.60 – 1.62 (*m*), 1.29 – 1.32 (*m*)	34.3	
C(8)		40.1	
H–C(9)	1.63 – 1.65 (*m*)	52.4	
C(10)		52.4	
CH_2(11)	1.67 – 1.69 (*m*)	21.9	
CH_2(12)	1.89 – 1.91 (*m*)	24.3	
H–C(13)	2.01 – 2.03 (*m*)	41.4	
C(14)		49.9	
CH_2(15)	1.57 – 1.62 (*m*), 1.09 – 1.16 (*m*)	31.8	
CH_2(16)	2.05 – 2.08 (*m*), 1.65 – 1.67 (*m*)	27.2	
H–C(17)	2.16 – 2.19 (*m*)	46.0	
Me(18)	0.85 (*s*)	16.8	7, 8, 9, 14
H–C(19)	10.27 (*s*)	205.2	
C(20)		76.7	
H–C(21)	4.30 – 4.32 (*m*), 3.92 – 3.95 (*m*)	76.6	
CH_2(22)	2.88 (*dd*, *J* = 14.1, 6.0), 2.66 (*dd*, *J* = 14.1, 7.2)	39.4	20, 23, 24
H–C(23)	6.17 (*dd*, *J* = 15.6, 7.2)	127.7	25
H–C(24)	6.45 (*d*, *J* = 15.6)	135.3	22, 25, 26, 27
C(25)		142.8	
CH_2(26)	5.01 – 5.03 (*m*), 4.91 – 4.93 (*m*)	114.7	24, 27
Me(27)	1.84 (*s*)	18.5	24, 25, 26
Me(28)	1.23 (*s*)	26.1	3, 4, 5, 29
Me(29)	1.07 (*s*)	15.7	3, 4, 5, 28
Me(30)	0.90 (*s*)	16.1	8, 13, 14, 15
Ara–O–C(3)			
H–C(1′)	4.87 (*d*, *J* = 5.4)	104.5	3
H–C(2′)	4.61 – 4.64 (*m*)	74.2	1″, 1′
H–C(3′)	4.25 – 4.28 (*m*)	81.4	1‴′
H–C(4′)	4.46 (br. *s*)	68.1	
H–C(5′)	4.25 – 4.27 (*m*), 3.79 (*d*, *J* = 9.6)	64.8	
Rha–O–C(2′)			
H–C(1″)	6.13 (br. *s*)	101.7	2′, 2″
H–C(2″)	4.55 – 4.58 (*m*)	72.2	
H–C(3″)	4.71 – 4.73 (*m*)	72.1	
H–C(4″)	4.24 – 4.27 (*m*)	73.6	
H–C(5″)	4.53 – 4.56 (*m*)	69.7	
Me(6″)	1.59 (*d*, *J* = 6.0)	18.3	
Xyl–O–C(3′)			
H–C(1‴′)	5.01 (br. *s*)	105.0	3′
H–C(2‴′)	3.91 (*t*, *J* = 7.8)	74.2	
H–C(3‴′)	4.06 – 4.09 (*m*)	77.5	
H–C(4‴′)	4.09 – 4.11 (*m*)	70.6	
CH_2(5‴′)	4.26 – 4.29 (*m*), 3.64 (*t*, *J* = 9.6)	66.7	

Table 2 (cont.)

	δ(H)	δ(C)	HMBC (H → C)
Glc–O–C(21)			
H–C(1‴)	5.02 (*d*, *J* = 7.8)	106.1	21
H–C(2‴)	4.08 – 4.11 (*m*)	75.2	
H–C(3‴)	4.22 – 4.24 (*m*)	78.3	
H–C(4‴)	4.21 – 4.23 (*m*)	71.4	
H–C(5‴)	3.95 – 3.98 (*m*)	78.3	
CH_2(6‴)	4.51 – 4.54 (*m*), 4.34 – 4.37 (*m*)	62.4	

1635 cm^{-1} (C=C). The ^{1}H-NMR spectrum (*Table 2*) exhibited signals of five Me groups (δ(H) 0.85 (*s*), 0.90 (*s*), 1.07 (*s*), 1.23 (*s*), 1.84 (*s*)), of four olefinic H-atoms at δ(H) 6.17 (*dd*, *J* = 15.6, 7.2), 6.45 (*d*, *J* = 15.6), 5.01 – 5.03 (*m*), 4.91 – 4.93 (*m*), a CHO signal at δ(H) 10.27 (*s*), and signals due to a β-D-glucopyranosyl (δ(H) 5.02 (*d*, *J* = 7.8)), an α-L-arabinopyranosyl (δ(H) 4.87 (*d*, *J* = 5.4)), a β-D-xylopyranosyl (δ(H) 5.01 (*s*)), and an α-L-rhamnopyranosyl moiety (δ(H) 6.13 (br. *s*, 1 H), 1.59 (*d*, *J* = 6.0, 3 H)). The C-atom signals of the aglycon part in the ^{13}C-NMR spectra closely resembled those of gypenoside XLIX [7], except for a few signals stemming from the side chain. The structure of the side chain was determined by long-range HMBCs between the H–C(22) and C(20) and C(23); H–C(24), and C(22) and C(26); H–C(26), and C(24) and C(27); and Me(27), and C(24) and C(25) (*Fig. 3*). Up to this point, it could be assumed that the C=C bonds are located between C(23) and C(24), and C(25) and C(26) [6]. Therefore, the aglycone was identified as (20*S*)-3β,20,21-trihydroxy-19-oxodammara-23,25-diene. The ^{13}C-NMR signals assignable to the the sugar moieties, and to C(3) and C(21) of **2** were very similar to those of gypenoside XLIX. The linkage sites and sequences of the four sugar units and of the aglycon were confirmed by the 2D-NMR experiments. The HMBCs, between H–C(1′) of the arabinose and C(3) of the aglycon, H–C(1″) of the rhamnose and C(2′) of the arabinose, and H–C(1‴) of the xylose and C(3′) of the arabinose, H–C(1‴) of the glucose and C(21) were observed. Thus, **2** was elucidated as (20*S*)-3β,20,21-trihydroxy-19-oxodammara-23,25-diene 3-*O*-{[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh; *Qingdao Marine Chemical Group, Co.*); macroporous resin *D101* (*Hebei, Co.*); and *Sephadex LH-20* (*Pharmacia, Co.*). Prep. HPLC (*Beijing CXTH3000* system): *P3000* pump, *UV3000* spectrophotometric detector at 203 nm, *Daisogel* C_{18} reversed-phase (RP) column (10 μm, 30 × 250 nm; flow rate, 14.0 ml/min). Optical rotations: *PerkinElmer* polarimeter. UV Spectra : *Shimadzu UV-2201* spectrophotometer; MeOH soln.; in λ_{max} (log ε). IR Spectra: *Bruker IFS-55* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AV-600 and ARX-300* spectrometer; δ in ppm rel. to Me_4Si as internal standard; *J* in Hz. HR-TOF-MS: *BIC micro TOF-Q* mass spectrometer; in *m*/*z* (rel.%). GC: *Agilent Technologies 6890N* apparatus, *OV-17* (30 m × 0.32 mm) column.

Plant Material. The aerial parts of *Gynostemma pentaphyllum* (THUNB.) MAKINO were collected from Shaanxi Province in P. R. China by *Xi'an Tianyi Co., Ltd*. A voucher specimen of the plant

(No. 2007016) was deposited with our laboratory and was identified by Prof. *Qishi Sun* of Shenyang Pharmaceutical University.

Extraction and Isolation. Dried aerial parts of *G. pentaphyllum* (8.0 kg) were extracted with 75% EtOH (× 3), and the H_2O-soluble extract of the plant was separated using a macroporous resin column to obtain the 70% EtOH eluates, which, upon drying, afforded the total saponins (80 g). The total saponins were subjected repeatedly to CC (silica gel) to provide five fractions, *Frs. A – E*. *Fr. E* was separated into eight fractions, *Frs. C_a – C_e*, by HPLC (*ODS*, 78% MeOH). From *Fr. C_a*, **1** (20 mg) was obtained as white amorphous powder. *Fr. C_e* was then subjected to prep. RP-HPLC (73% MeOH) to yield **2** (t_R 25 min; 25 mg).

*(3β,20ξ,21*R*,23*R*,24*E*)-20,21,26-Trihydroxy-19-oxo-21,23-epoxydammar-24-en-3-yl α-*L*-Rhamnopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranoside* (**1**). White amorphous powder. $[\alpha]_D^{28} = -5.4$ ($c = 0.18$, MeOH). UV: 213 (1.08). IR: 3438, 2931, 1701, 1645, 1384, 1042. *Liebermann–Burchard* and *Molish* reactions were positive. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-TOF-MS: 937.4792 ($[M + Na]^+$, calc. 937.4775).

*(3β,23*E*)-3-{[α-*L*-Rhamnopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranosyl]oxy}-20-hydroxy-19-oxodammara-23,25-dien-21-yl β-*D*-Glucopyranoside* (**2**). White amorphous powder. $[\alpha]_D^{28} = -35.2$ ($c = 0.25$, MeOH). UV: 230 (2.60), 282 (0.38). IR: 3424, 2940, 1703, 1635, 1402, 1043, 618. *Liebermann–Burchard* and *Molish* reactions were positive. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-TOF-MS: 1067.5378 ($[M + Na]^+$, calc. 1067.5397).

Acid Hydrolysis of **1** *and* **2**. Each compounds (4 mg) was heated in 5 ml of 2M HCl/MeOH 4 : 1 at 90° for 6 h in a H_2O bath. After cooling, the mixture was diluted to 20 ml with H_2O and then extracted with $CHCl_3$ (20 ml × 3). After concentration, each aq. layer was examined by TLC ($CHCl_3$/MeOH/H_2O 55 : 45 : 10) and compared with authentic samples.

Determination of Sugar Components. The monosaccharide uints were obtained by HCl hydrolysis as described above. The aq. layer was concentrated to dryness to give a residue, which was dissolved in pyridine (1 ml), and then hexamethyldisilazane (0.4 ml) and Me_3SiCl (0.2 ml) were added to the soln. to form the trimethylsilyl (TMS) ethers. The mixture was stirred at 20° for 15 min, and extracted with H_2O (1 ml). Each aq. layer was examined by GC (H_2 flame ionization detector, column temp., 100 – 280°; programmed increase, 10°/min; carrier gas, N_2 (1.5 ml/min); injector and detector temp., 280°; injection volume, 1 μl; split ratio, 10: 1). The derivatives of L-arabinose, D-xylose, L-rhamnose, and D-glucose were detected. t_R [min]: 6.20, 8.84, 9.76, and 26.59, resp. The standard monosaccharides were subjected to the same reaction and GC analysis under the same conditions.

The Liver Fiberosis Inhibitory Activity Evaluation. Inhibitions of hepatic stellate cells (HSCs) were evaluated by MTT assay. Compound **1** and **2** showed moderate cytotoxicities against HSC-T6 with the IC_{50} values of 31.5 μM, 47.2 μM, resp.

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