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New Dammarane-Type Saponins from the Galls of *Sapindus mukorossi*

YAO-HAUR KUO,† HUI-CHI HUANG,‡,§ LI-MING YANG KUO,† YA-WEN HSU,† KUO-HSIUNG LEE,# FANG-RONG CHANG,‡ AND YANG-CHANG WU*,‡

National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China; Graduate Institute of Natural Products and Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China; and Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

Five new dammarane-type saponins, 3β,7β,20(*S*),22-tetrahydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, 3β,7β,20(*S*),22,23-pentahydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, 3β,7β,20(*S*),22,25-pentahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, 25-methoxy-3β,7β,20(*S*),22-tetrahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, and 25-methoxy-3β,7β,20(*R*)-trihydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, named sapinmusaponins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), respectively, together with three known phenylpropanoid glycosides (**6**−**8**), were isolated from the galls of *Sapindus mukorossi*. The structures of these saponins were elucidated on the basis of spectroscopic analyses and chemical methods. Preliminary bioassay data revealed that saponins **1** and **3**−**5** showed moderate cytotoxic activity ($ED_{50} \sim 9-18$ μg/mL) against human tumor cell lines (Hepa59T/VGH, NCI, HeLa, and Med) and that **1**−**5** were inactive in vitro against HIV replication in H9 lymphocytes.

KEYWORDS: Dammarane; saponin; sapinmusaponins A−E; *Sapindus mukorossi*; Sapindaceae; cytotoxicity

INTRODUCTION

Sapindus mukorossi (Sapindaceae), also known as soap-nut tree, is an important economic agricultural product in tropical and subtropical regions of Asia. The pericarp of *S. mukorossi* has been traditionally used as an expectorant, as well as a source of natural surfactants (*1*−*3*). Previous reports on *S. mukorossi* dealt with the isolation and identification of triterpenoids, saponins (*2*, *4*, *5*), fatty acids (*6*), and flavonoids (*7*), from the pericarp, stem, and fruit of the plant. Other literature reports discussed the biological activities, including molluscicidal (*8*), anti-inflammatory (*2*), and cytotoxic (*9*) effects, for this plant. We reported previously that extracts of *S. mukorossi* showed molluscicidal effects against the golden apple snail and isolated a series of hederagenin-based acetylated saponins from the plant pericarp. Recently, we found the ethanol extract of galls from the stems of *S. mukorossi* exhibited marginal cytotoxicity against human liver carcinoma (Hepa59T/VGH), human large lung cell carcinoma (NCI), cervical epitheloid carcinoma (HeLa), and medulloblastoma (Med) tumor cells. Usually, galls grow on the stems or leaves of the tree and are caused by parasite organisms such as insects, mites, mistletoes, fungi, and bacteria (*10*). Different kinds of galls occur in many plant families, including Anacardiaceae, Fagaceae, Leguminosae, Myrtaceae, Lauraceae, and Fagaceae (*11*). Wu-Bei-Zi, a gall that grows on *Rhus chinensis* (Anacardiaceae), is a famous Chinese medicine, used as an astringent, insecticide, anti-inflammatory agent, and antibacterial drug (*12*, *13*). However, the chemical constituents and pharmacological effects of the gall of *S. mukorossi* have not been reported. Thus, we were prompted to investigate the bioactive constituents from collected galls. We report herein on the isolation and structural elucidation of **1**−**5** using spectroscopic analyses, including 1D and 2D NMR techniques (COSY, HMQC, HMBC, and NOESY), and chemical methods. Biological evaluation of the newly isolated saponins in cytotoxicity and anti-HIV assays is also described.

MATERIALS AND METHODS

General Experimental Procedures. Infrared (IR) spectra were measured on a Mattson Genesis II spectrophotometer (Thermo Nicolet, Madison, WI) using a KBr matrix. FABMS data were obtained on a

* Author to whom correspondence should be addressed (telephone +886-2-28201999, ext. 7051, or +886-7-3121101, ext. 2197; fax +886-2-28236150 or +886-7-3114773; e-mail kuoyh@nricm.edu.tw or yachwu@kmu.edu.tw).
† National Research Institute of Chinese Medicine.
‡ Graduate Institute of Natural Products, Kaohsiung Medical University.
§ Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University.
# University of North Carolina.

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Table 1. Selected ^{1}H NMR Data for the Aglycon Moieties of Compounds **1**–**5**[a] (δ, in CD_3OD)

no.	1	2	3	4	5
1	1.66 m, 0.93 m	1.66 m, 0.94 m	1.63 m, 0.94 m	1.61 m, 0.93 m	1.63 m, 0.96 m
2	1.98 m, 1.66 m	1.98 m, 1.66 m	1.98 m, 1.66 m	1.98 m, 1.66m	1.90 m, 1.65 m
3	3.17 dd (11.2, 4.0)	3.17 dd (11.8., 4.0)	3.17 dd (11.2, 4.0)	3.16 m	3.17 dd (11.0, 4.0)
5	0.81 m	0.81 m	0.81 m	0.81 m	0.81 m
6	1.63 m, 1.55 m	1.63 m, 1.52m	1.63 m, 1.55 m	1.63 m, 1.55 m	1.62 m, 1.55 m
7	3.70 dd (10.0, 4.8)	3.72 d (4.8)	3.70 m	3.70 m	3.69 dd (11.2, 4.4)
9	1.24 m	1.25 m	1.22 m	1.22 m	1.24 m
11	1.52 m, 1.32m	1.49 m, 1.31m	1.53 m, 1.32 m	1.52 m, 1.31 m	1.52 m, 1.30 m
12	1.71 m, 1.35 m	1.68 m, 1.32 m	1.71 m, 1.33 m	1.70 m, 1.33 m	1.68 m, 1.33 m
13	1.75 m	1.73m	1.73 m	1.73 m	1.70 m
15	1.66 m, 1.35 m	1.66 m, 1.35 m	1.69 m, 1.36 m	1.69 m, 1.34 m	1.67 m, 1.35 m
16	1.98 m, 1.27 m	1.98 m, 1.25 m	1.98 m, 1.26 m	1.99 m, 1.26 m	1.96 m, 1.26 m
17	1.92 m	2.14 dd (4.0, 10.0)	2.01 m	2.01 m	1.98 m
18	0.97 s	0.98 s	0.97 s	0.97 s	0.97 s
19	0.86 s	0.86 s	0.85 s	0.85 s	0.85 s
21	1.05 s	1.15 s	0.98 s	0.99 s	1.11 s
22	3.41 m	3.15 d (2.4)	3.82 m/4.28[c] d (7.2)	3.87 d (7.2)	2.23 m, 2.22 m
23	2.25 dd (14.8, 6.8), 2.05 m	4.67 dd (8.8, 2.0)	5.57 m/6.48[c] dd (15.6, 7.2)	5.76 dd (16.0, 7.2)	5.68 dt (16.0, 7.2)
24	5.24 t (6.8)	5.43 dt (8.8, 1.2)	5.74 m/6.29[c] d (15.6)	5.64 d (16.0)	5.45 d (16.0)
26	1.70 s	1.73 s	1.28 s	1.27 s	1.25 s
27	1.61 s	1.70 s	1.28 s	1.27 s	1.25 s
28	1.05 s	1.04 s	1.04 s	1.04 s	1.04 s
29	0.85 s	0.85 s	0.86 s	0.86 s	0.86 s
30	0.93 s	0.94 s	0.94 s	0.94 s	0.91 s
OCH_3				3.16 s	3.14 s

[a] Assignments confirmed by decoupling, ^{1}H–^{1}H COSY, TOCSY, NOESY, HMQC, and HMBC. [b] Overlapped signal. [c] Chemical shifts and coupling constants were measured in C_5D_5N.

JEOL SX-102A instrument (JEOL USA, Inc., Peabody, MA). High-resolution FABMS were measured on a Finnigan/Thermo Quest MAT mass spectrometer (Scientific Instrument Services, Inc., Ringoes, NJ). NMR spectra were performed on Bruker NMR spectrometers (Unity Plus 400 MHz) (Bruker BioSpin GmbH, Rheinstetten, Germany) using CD_3OD and C_5D_5N as solvent for measurement. Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20, and silica gel (Merck 70−230 and 230−400 mesh) (Merck & Co., Inc., Whitehouse Station, NJ) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 50% H_2SO_4 and then heating at 100 °C. HPLC separations were performed on a Shimadzu LC-6AD series apparatus with an RID-10A refractive index, equipped with a 250 × 20 mm i.d. preparative Cosmosil 5SL-II column (TimTec, Inc., Newark, DE).

Plant Materials. The galls of *S. mukorossi* were collected in October 2001 in Taipei County, Taiwan, and identified by Professor Muh-Tsuen Kao of the National Institute of Chinese Medicine.

Extraction and Isolation. The dried galls of *S. mukorossi* (8.5 kg) were extracted three times with ethanol (40 L). Removal of solvent in a vacuum gave the ethanol extract, which was partitioned with MeOH/$CHCl_3$/H_2O (7:10:3, 1000 mL × 3) to give $CHCl_3$ and H_2O layers. The $CHCl_3$ layer (190 g) was partitioned with methanol/*n*-hexane (1:3, 800 mL × 3) to give methanol and *n*-hexane layers. After the evaporation of methanol layer in a vacuum, the residue (100 g) was purified by chromatography on a silica gel column (41 × 10.5 cm) with a stepwise gradient of $CHCl_3$/MeOH (1:0, 40:1, 30:1, 20:1, 10:1, 8:1, 6:1, 4:1, 2:1, 1:1, 0:1, each 1 L) to afford 13 fractions (fr 1−13). Fraction 12 (18.8 g) was chromatographed on a Diaion HP-20 porous polymer resin column (23 × 4.5 cm), eluting with 10, 40, 60, 80, and 100% MeOH (each 2 L), respectively, to yield 5 fractions (fr 12.1−12.5). Fraction 12.3 (3.0 g) was further separated by chromatography on a Sephadex LH-20 column (30 × 2.8 cm) with MeOH (1.5 L) to yield 5 fractions (fr 12.3.1−12.3.5). Fraction 12.3.2 (2.0 g) was rechromatographed on a Sephadex LH-20 column (40 × 1.5 cm) with MeOH (1.0 L), and 4 fractions (fr 12.3.2.1−12.3.2.4) were obtained. Fraction 12.3.2.2 (1.1 g) was further purified by HPLC on an ODS column (250 × 20.0 mm i.d., flow rate = 5 min/mL, with 80% MeOH to afford **1** (17.0 mg), **4** (6.0 mg), and **5** (21.0 mg). Using the same column as that of fr 12.3.2.2 on HPLC with 75% MeOH, **6** (0.5 mg), **7** (1.3 mg), and **8** (1.0 mg) were isolated from fr 12.3.3 (0.3 g), and **2** (23.0 mg) and **3** (4.0 mg) were yielded from fr 12.3.4 (0.7 g) eluting with 60% MeOH.

3*β*,7*β*,20(*S*),22-Tetrahydroxydammar-24-ene-3-*O*-α-L-rhamnopyrnosyl-(1→2)-*β*-D-glucopyranoside (1) was obtained as a white amorphous powder: mp 169−171 °C; $[\alpha]_D^{24}$ +50.0° (*c* 0.3, MeOH); IR ν_{max} (KBr) 3387, 1629, 1047 cm^{-1}; FABMS, *m*/*z* 807 [M + Na]$^+$; HRFABMS, *m*/*z* 807.4864 (calcd, 807.4870, $C_{42}H_{72}O_{13}Na$). ^{1}H and ^{13}C NMR data are shown in **Tables 1**−**3**.

3*β*,7*β*,20(*S*),22,23-Pentahydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (2) was obtained as a white amorphous powder: mp 254−256 °C; $[\alpha]_D^{24}$ +22.0° (*c* 0.3, MeOH); IR ν_{max} (KBr) 3389, 1640, 1047 cm^{-1}; HRFABMS, *m*/*z* 823.4813 (calcd 823.4819, $C_{42}H_{72}O_{14}Na$). ^{1}H and ^{13}C NMR data are shown in **Tables 1**−**3**.

3*β*,7*β*,20(*S*),22,25-Pentahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (3) was obtained as a white amorphous powder: mp 198−200 °C; $[\alpha]_D^{24}$ +66.0° (*c* 0.3, MeOH); IR ν_{max} (KBr) 3382, 1641, 1045 cm^{-1}; HRFABMS, *m*/*z* 823.4824 (calcd 823.4819, $C_{42}H_{72}O_{14}Na$). ^{1}H and ^{13}C NMR data are shown in **Tables 1**−**3**.

25-Methoxy-3*β*,7*β*,20(*S*),22-tetrahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (4) was obtained as a white amorphous powder: mp 189−191 °C; $[\alpha]_D^{24}$ +46.6° (*c* 0.4, MeOH); IR ν_{max} (KBr) 3394, 1654, 1048 cm^{-1}; HRFABMS, *m*/*z* 837.4982 (calcd 837.4976, $C_{43}H_{74}O_{14}Na$). ^{1}H and ^{13}C NMR data are shown in **Tables 1**−**3**.

25-Methoxy-3*β*,7*β*,20(*R*)-trihydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (5) was obtained as a white amorphous powder: mp 190−192 °C; $[\alpha]_D^{24}$ +10.2° (*c* 0.3, MeOH); IR ν_{max} (KBr) 3409, 1597, 1049 cm^{-1}; HRFABMS, *m*/*z* 821.5016 (calcd 821.5027, $C_{43}H_{74}O_{13}Na$). ^{1}H and ^{13}C NMR data are shown in **Tables 1**−**3**.

4-Allyl-2-methoxyphenyl-6-*O*-*β*-D-apiosyl-(1→6)-*β*-D-glucoside (6) was obtained as an amorphous powder; IR ν_{max} (KBr) 3370, 1640, 1599, 1500 cm^{-1}; FABMS, *m*/*z* 481 [M + Na]$^+$ (*14*).

4-Allyl-2-methoxyphenyl-3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (7) was obtained as a white amorphous powder: IR ν_{max} (KBr) 3450, 1630, 1600, 1490 cm^{-1}; FABMS, *m*/*z* 495 [M + Na]$^+$ (*15*).

Table 2. ^{13}C and ^{1}H NMR Data for the Sugar Moieties of Compounds **1**–**5** (δ, in CD_3OD)

	1		2		3		4		5	
no.	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1′	105.57	4.39 d (7.6)	105.56	4.39 d (7.6)	105.59	4.39 d (7.3)	105.56	4.40 d (7.6)	105.58	4.40 d (7.2)
2′	78.96	3.39 m	78.97	3.39 dd (8.4, 7.6)	78.76	3.39 m	78.94	3.39 m	78.93	3.39 d (8.0, 7.2)
3′	79.50	3.45 t (8.8)	79.48	3.43 t (8.4)	79.53	3.44 t (8.4)	79.48	3.45 t (8.4)	79.51	3.45 t (8.0)
4′	72.17	3.27 t (8.8)	72.15	3.28 t (8.4)	72.15	3.26 t (8.4)	72.15	3.27 t (8.4)	72.04	3.27 t (8.0)
5′	77.61	3.23 m	77.58	3.23 dd (5.6, 2.0)	77.62	3.23 m	77.58	3.23 dd (5.2, 2.0)	77.61	3.23 dd (5.2, 2.0)
6′	62.79	3.83 dd (11.6, 1.8)	62.76	3.84 dd (12.0, 2.0)	62.78	3.83 dd (11.0, 1.2)	62.76	3.84 dd (12.0, 2.0)	62.78	3.84 dd (12.0, 2.0)
		3.65 dd (11.6, 5.2)		3.65 dd (12.0, 5.6)		3.64 dd (11.0, 5.6)		3.65 dd (12.0, 5.2)		3.65 dd (12.0, 5.2)
1″	101.85	5.35 br s	101.85	5.35 d (1.2)	101.85	5.35 br s	101.84	5.35 d (1.6)	101.84	5.36 d (1.6)
2″	72.08	3.95 m	72.03	3.94 dd (3.2, 1.6)	72.06	3.94 m	72.02	3.94 dd (2.8, 1.6)	72.13	3.94 dd (3.2, 1.6)
3″	72.04	3.74 dd (9.2, 3.6)	72.03	3.74 dd (9.6, 3.2)	72.06	3.72 dd (9.2, 3.2)	72.02	3.72 dd (9.2, 3.2)	72.08	3.74 dd (9.6, 3.2)
4″	73.97	3.35 t (9.2)	73.95	3.37 t (9.6)	73.97	3.35 t (9.2)	73.95	3.35 t (9.2)	73.96	3.35 t (9.6)
5″	69.98	3.98 m	69.98	3.96 m	69.98	3.97 m	69.97	3.96 dd (6.0, 3.2)	69.96	3.97 dd (6.4, 3.2)
6″	17.98	1.19 d (6.4)	17.98	1.19 d (6.0)	17.98	1.20 d (6.4)	17.98	1.20 d (6.0)	17.97	1.21 d (6.4)

Table 3. ^{13}C NMR Data for the Aglycon Moieties of Compounds **1**–**5** (δ, in CD_3OD)

no.	1	2	3	4	5
1	40.40	40.40	40.41	40.39	40.39
2	27.45	27.45	27.46	27.44	27.45
3	90.03	90.04	90.03	90.03	90.00
4	40.26	40.25	40.26	40.25	40.26
5	55.47	55.45	55.46	55.45	55.45
6	29.10	29.08	29.10	29.08	29.11
7	75.94	75.93	75.95	75.94	75.89
8	47.37	47.38	47.35	47.34	47.33
9	51.89	51.89	51.89	51.87	51.84
10	37.88	37.89	37.89	37.88	37.87
11	22.63	22.57	22.59	22.58	22.63
12	26.34	26.64	26.44	26.41	26.31
13	43.88	44.07	44.35	44.35	44.28
14	50.62	50.45	50.45	50.43	50.97
15	35.54	35.42	35.42	35.39	35.57
16	28.83	28.50	28.70	28.67	28.77
17	46.49	46.12	46.22	46.23	50.03
18	10.45	10.05	10.49	10.50	10.43
19	16.90	16.89	16.69	16.90	16.91
20	78.81	80.14	78.76	78.59	76.09
21	19.15	19.03	18.73	18.86	26.02
22	77.61	77.58	78.49	78.52	45.29
23	31.05	69.33	126.97	130.85	128.12
24	123.21	126.99	142.01	138.83	138.73
25	133.59	135.54	71.17	76.43	76.54
26	26.03	26.02	29.78	26.10	26.31
27	18.12	18.33	29.84	26.19	26.41
28	28.39	28.39	28.38	28.39	28.36
29	17.10	17.11	17.11	17.11	17.07
30	16.69	16.67	16.64	16.62	16.65
OCH_3				50.78	50.03

4-Allyl-2-methoxyphenyl-6-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (8) was obtained as a white amorphous powder: $[\alpha]_D^{24}$ +30° (*c* 0.2, MeOH); IR ν_{max} (KBr) 3470, 1720, 1600, 1430 cm^{-1}; FABMS, *m*/*z* 481 $[M + Na]^+$ (*16*).

Acid Hydrolysis of 1. Compound **1** (6.0 mg) was treated with 2 N methanolic HCl (2 mL) under reflux at 90 °C for 1 h. The mixture was extracted with CH_2Cl_2, and the aqueous layer was neutralized with Na_2CO_3 and filtered. The dried filtrate was acetylated with pyridine−Ac_2O. GC-MS analysis showed peracetylrhamnose and peracetylglucose (1:1), in comparison with reference compounds.

Cytotoxicity Assay. The assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) against Hepa59T/VGH (human liver carcinoma), NCI (human large lung cell carcinoma), HeLa (human cervical epitheloid carcinoma), and Med (human medulloblastoma) tumor cells was based on the reported methods (*17*). The tumor cells were purchased from the American Type Culture Collection (ATCC). In brief, the cells were cultured in RPMI-1640 medium supplemented with serum in 5% CO_2 and incubated at 37 °C. Test samples and control drug standard were prepared at concentrations of 1, 10, 20, and 40 μg/mL. After seeding of 2880 cells/well in a 96-well microplate for 4 h, 20 μL of sample or standard agent was placed in each well and incubated at 37 °C for 3 days, and then 20 μL of MTT was added for 5 h. After removal of the medium and addition of DMSO (200 μL/well) into the microplate with shaking for 10 min, the formazan crystals (the product of MTT reacting with dehydrogenase existing in mitochondria) were redissolved and their absorbance was measured on a model MR 7000 microtiter plate reader (Dynatech International Corp., Edgewood, NY) at a wavelength of 550 nm. The ED_{50} was defined by comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

HIV Inhibition Assay. HIV inhibition was measured as described previously (*18*).

RESULTS AND DISCUSSION

Bioassay-directed fractionation of the EtOH extract of galls from *S. mukorossi* resulted in the isolation of dammarane-type saponins **1**−**5** and phenylpropanoid glycosides **6**−**8**.

The HRFABMS of compound **1** showed a pseudomolecular ion at 807.4864 $[M + Na]^+$, consistent with the formula of $C_{42}H_{72}O_{13}$. The IR spectrum showed absorption bands for hydroxyl groups at 3387 cm^{-1} and olefinic groups at 1629 and 1047 cm^{-1}. The ^1H NMR spectrum (**Tables 1** and **2**) displayed signals for six tertiary methyls (δ_H 0.85, 0.86, 0.93, 0.97, and 1.05 × 2), two allylic methyls (δ_H 1.61 and 1.70), three oxygenated methines [δ_H 3.17 (dd, J = 11.2, 4.0 Hz, H-3), 3.70 (dd, J = 10.0, 4.8 Hz, H-7), and 3.41 (m, H-22)], and one olefinic proton [δ_H 5.24 (t, J = 6.8 Hz, H-24)], as well as two anomeric protons [δ_H 4.39 (d, J = 7.6 Hz, H-1′) and 5.35 (br s, H-1″)]. The signals for eight tertiary methyls, eight methylenes, eight methines (including an olefinic carbon signal at δ 133.59 and 123.21 and three oxygenated carbons at δ 75.94, 77.61, and 90.03), and one oxygenated quaternary carbon at δ_C 78.81 were found in the ^{13}C NMR (**Table 3**) and DEPT spectra of **1**. After subtraction of the 12 carbon resonances of the sugar moieties (**Table 2**), the remaining 30 signals were attributable to a dammarane-type triterpene aglycon (*19*−*24*). On the basis of the triplet coupling pattern of the olefinic proton in the ^1H NMR spectrum and the long-range correlation in the HMBC spectrum, the double bond was likely substituted with one methylene and two geminal methyl groups, suggesting the partial structure of the side chain (*25*).

The planar structure of **1** was further clarified by $^1H-^1H$ COSY, TOCSY, and HMBC experiments. The TOCSY spectrum (**Figure 1**) indicated the four partial structures for a dammarane-type triterpene as drawn with bold lines (C_1-C_3, C_5-C_7, C_9-C_{17}, and $C_{22}-C_{24}$). Detailed inspection of the HMBC spectrum showed correlations between the following

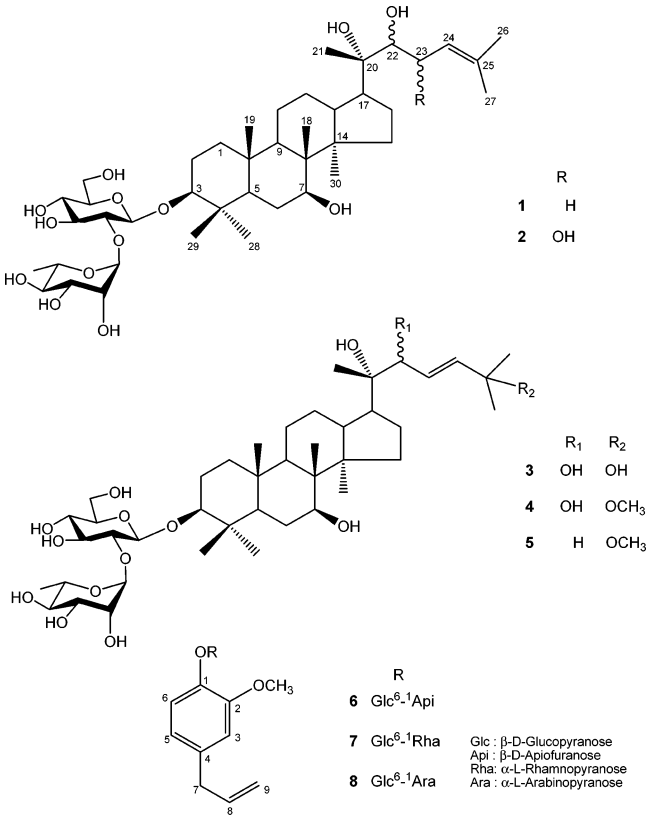

Figure 1. Structures of dammarane saponins and phenylpropanoid glycosides isolated from the galls of *S. mukorossi*.

proton and carbon pairs: H_3-18/C-7, 8, 9; H_3-19/C-1, 5, 9, 10; H_3-21/C-17, 22; H_3-26, 27/C-24, 25; H_3-28, 29/C-3, 4, 5. These data confirmed the positions of the seven methyl groups of **1** as shown in **Figure 1**. Moreover, the secondary hydroxy groups were located at C-7 and C-22 on the basis of the correlations between H-7 and C-6, 14, 18 and between H-22 and C-21, 23, 24, respectively.

Acid hydrolysis (*26*) of **1** gave D-glucose and L-rhamnose as the component sugars, which was confirmed by GLC analysis. HMBC spectroscopic data were used to assign the linkage positions of these sugars in **1**. Thus, the C-1 of rhamnose was linked to the C-2 of glucose on the basis of correlations between C-1 (δ_C 101.85) of the terminal rhamnose and H-2′ of glucose (δ_H 3.39). Also, the glucose was linked at C-3 of the triterpene, due to a cross-peak between C-1′ (δ_C 105.57) of glucose and H-3 (δ_H 3.17) of the aglycon. Moreover, the coupling pattern (dd, J = 11.2, 4.0 Hz) of H-3 indicated that the glucose−aglycon linkage has the β orientation. Coupling constants of the H-1 protons for the glucose (d, J = 7.6 Hz) and rhamnose (br s) (*22*) fully established the sugars of **1** as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The relative stereochemistries of **1** were deduced by NOESY experiments. As shown in **Figure 2**, correlations between the proton pairs, H-3/H-5 with H_3-28 and H-7/H-5 with H_3-30, indicated that the configurations of the oxygenated geminal protons are H-3α and H-7α. Moreover, H-17 showed NOESY correlations with H_3-30 and H-16α; therefore, the side chain at C-17 was β-oriented. These data together with 2D-NOE correlation between H_3-21 and H-13 established **1** as 3β,7β,20(*S*),22-tetrahydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. This compound has been named sapinmusaponin A (**1**).

The molecular formula of **2** was deduced as $C_{42}H_{72}O_{14}$ from a pseudomolecular ion at 823.4813 $[M + Na]^+$ in the HR-FABMS. The IR spectrum showed absorption bands for hydroxyl (3389 cm^{-1}) and olefinic (1640, 1047 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** showed signal patterns similar to those of **1**, except for the appearance of signals for an oxymethine [δ_H 4.67 (dd, J = 8.8, 2.0 Hz), δ_C 69.33] in **2**. Further detailed analysis of **2** using a TOCSY experiment suggested the same four partial structures of **1** as shown in **Figure 1**. The side chains of **1** and **2** differ by an additional hydroxyl group found in **2**. Moreover, correlations between δ_H 4.67 (H-23) and δ_C 126.99 (C-24) and 135.54 (C-25) were found in the HMBC spectrum, suggesting that the hydroxyl group is attached to C-23. After acid hydrolysis, compound **2**, as well as the isolates **3**−**5** described subsequently, also gave D-glucose and L-rhamnose as component sugars.

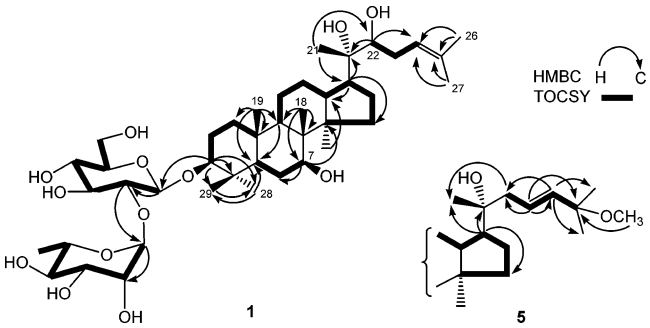

Figure 2. Key TOCSY and HMBC correlations of **1** and **5**.

The stereochemistry at C-20 was assigned as *S*, due to 2D-NOE correlations between H_3-21 and H-13/H-16β, as well as between H-17 and H_3-30 as shown in **Figure 2**. Thus, **2** was elucidated as 3β,7β,20(*S*),22,23-pentahydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside and named sapinmusaponin B.

Sapinmusaponin C (**3**) had a molecular formula of $C_{42}H_{72}O_{14}$ based on a pseudomolecular ion at 823.4824 $[M + Na]^+$ in its HRFABMS. The IR spectrum showed the absorption bands for hydroxyl (3382 cm^{-1}) and olefinic (1641 and 1045 cm^{-1}) groups. As found in **1** and **2**, compound **3** possessed two sugars and a dammarane triterpene skeleton based on their similar ^1H and ^{13}C NMR spectra. Comparison of the ^1H spectra of **1** and **3** showed that the H_3-26 and H_3-27 signals of **3** were found at higher field (δ_H 1.70 and 1.61 in **1**; δ_H 1.28 × 2 in **3**) and that the vinyl quaternary carbon at C-25 in **1** was replaced by an oxygenated carbon in **3**. Also, two vinyl carbons signals at δ_C 126.97 and 142.01 were observed in **3** and assigned as C-23 and 24, respectively, due to the long-range correlations between H-24 and C-26/C-27 and between H-22 and C-23/C-24 in the HMBC spectrum. Furthermore, when the ^1H NMR spectrum of **3** was measured in C_5D_5N rather than CD_3OD, the coupling constants (J = 15.6 Hz) for H-23 and H-24 were clearly observed and, therefore, *trans*-geometry was assigned to the double bond (*27*). On the basis of the HMBC spectrum (**Figure 1**), the five hydroxyl groups (δ_C 90.03, 75.95, 78.76, 78.49, and 71.17) in **3** were assigned at C-3, C-7, C-20, C-22, and C-25, respectively.

The stereochemistry of each of the hydroxyl groups was based on a NOESY experiment (**Figure 3**). H-3 was correlated with H-5 and H_3-28 and H-7 with H-5 and H_3-30, indicating their orientations as H-3α and H-7α, respectively. Moreover, correlations of H_3-21 with H-13 and H_3-18 determined that C-20 has an *S* configuration. Together with above data, **3** was tentatively elucidated as 3β,7β,20(*S*),22,25-pentahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

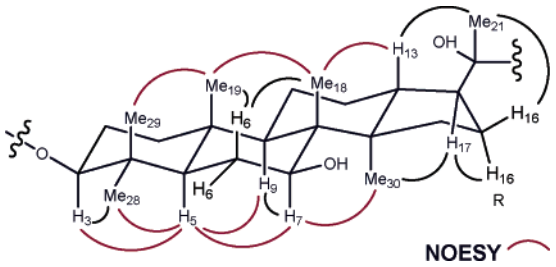

Figure 3. Main NOESY correlations for sapinmusaponins A–E (**1**–**5**).

The molecular formula of sapinmusaponin D (**4**) was determined as $C_{43}H_{74}O_{14}$ from the HRFABMS, which exhibited a pseudomolecular ion peak at 837.4982 $[M + Na]^+$. The IR spectrum showed absorption bands for hydroxyl (3394 cm^{-1}) and olefinic (1654 and 1048 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **4** contained similar patterns as found in **3**, expect for the appearance of an additional methoxy signal (δ_H 3.16 and δ_C 50.78) in **4**. Consequently, C-25 (δ_C 76.43) in **4** was shifted to lower field (+5.26), and C-26 (δ_C 26.10) and C-27 (δ_C 26.19) were shifted to higher field (−3.68 and −3.65, respectively), compared with **3**. In detailed inspection of the HMBC spectrum, a cross-peak between δ_H 3.16 (OCH_3) and δ_C 76.43 (C-25) placed the methoxyl group at the C-25 position. Like **3**, the configurations of **4** were based on NOE studies and, therefore, **4** was tentatively identified as 25-methoxy-3β,7β,20-(*S*),22-tetrahydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Sapinmusaponin E (**5**) was obtained as a white powder. Its HRFABMS spectrum revealed the molecular formula of $C_{43}H_{74}O_{13}$ from a pseudomolecular ion at 821.5016 $[M + Na]^+$. As found in **4**, the ^1H and ^{13}C NMR spectra of **5** indicated a dammarane-type triterpene with glucose and rhamnose moieties. The NMR spectra of **4** and **5** revealed similar structures, expect that **5** had only a methylene group at C-22 rather than the hydroxymethine found in **4**. Other obvious carbon shifts included a downfield shift of the C-21 signal in **5** (δ_C 18.86 in **4** and δ_C 26.02 in **5**) and upfield shifts of C-20 (δ_C 76.09) and C-22 (δ_C 45.29), compared with **4**. On the basis of the HMBC spectrum (**Figure 1**), cross-peaks between δ_H 2.23, 2.22 (H-22) and δ_C 76.09 (C-20), 128.12 (C-23), and 138.73 (C-24) further demonstrated the presence of a methylene group at C-22. Together with the NOE correlations similar to those of **4**, **5** was thus determined to be 25-methoxy-3β,7β,20(*R*)-trihydroxydammar-23-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compounds **6**−**8** were identified as known phenylpropanoid glycosides, 4-allyl-2-methoxyphenyl-6-*O*-β-D-apiosyl-(1→6)-β-D-glucoside (**6**) (*14*), 4-allyl-2-methoxyphenyl-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**7**) (*15*), and 4-allyl-2-methoxyphenyl-6-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**8**) (*16*), respectively, by analysis of their spectroscopic data (FAB-MS, ^1H NMR, and ^{13}C NMR spectra) and comparison with literature values.

The isolated dammarane-type saponins, **1**−**5**, were evaluated in a cytotoxicity assay (Hepa59T/VGH, NCI, HeLa, and Med). The quantities of **6**−**8** were insufficient for the bioasssay. The cytotoxicity data are shown in **Table 4** and show that **3** and **4** exhibited weak cytotoxic effects against all four tested cell lines (ED_{50} ∼ 9−17 μg/mL). Compound **1** had a weak response against Hepa59T/VGH and Med tumor cells, and **5** demonstrated weak cytotoxicity against HeLa and Med tumor cells. These results were consistent with literature data that some dammarane glycosides from processed ginseng had moderate cytotoxicity against SK-Hep-1 hepatoma cancer cells (*28*). Notably, compound **2**, which has three rather than one or two hydroxyl groups in the dammarane side chain, had the lowest inhibitory effects (ED_{50} > 20 μg/mL).

Table 4. Cytotoxic Activity of Compounds **1**–**5**

	ED_{50} (μg/mL) for cell line[a]			
compd	Hepa59T/VGH	NCI	HeLa	Med
1	15.2	–[b]	–	14.9
2	–	–	–	–
3	17.3	12.7	17.1	9.9
4	12.1	9.1	10.3	10.8
5	–	–	16.9	18.5

[a] Hepa59T/VGH, human liver carcinoma; NCI, human large lung cell carcinoma; HeLa, human cervical epitheloid carcinoma; Med, human medulloblastoma. [b] –, inactive, ED_{50} > 20 μg/mL.

Compounds **1**−**5** were also evaluated for in vitro inhibitory effects against HIV replication in H9 lymphocytes. None of these dammarane-type saponins suppressed HIV replication.

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