Four New Saponins from the Starfish *Certonardoa semiregularis*

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Four new saponins, designated as certonardosides K—N (1—3, 5), were isolated, along with culcitoside C_6 (4), from the brine shrimp active fraction of the starfish *Certonardoa semiregularis*. The structures were determined on the basis of spectral analysis and chemical derivatization. These compounds were evaluated for cytotoxicity and antibacterial activity.

Key words starfish; *Certonardoa semiregularis*; saponin; cytotoxicity; antibacterial activity

Starfish saponins, composed of a polyhydroxysterol aglycone and a monosaccharide or disaccharide unit, are an increasingly studied subgroup of bioactive marine saponins. In the preceding study, ten new saponins (certonardosides A—J) were isolated from the starfish *Certonardoa semiregularis*.[1)] The disaccharide 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-β-D-xylopyranosyl unit in certonardosides A—E and 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl unit in certonardosides F—H were unprecedented in literature.[1)] In our continuing search for bioactive metabolites from the same starfish, we have isolated four new saponins (**1**—**3**, **5**) along with culcitoside C_6 (**4**), which was previously isolated from the starfish *Culcita novaeguineae*.[2)] Three saponins (**1**—**3**) are characterized by the presence of the 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl sugar unit. Compound **5** contains a non-substituted xylofuranosyl unit, which has not previously been encountered in starfish saponins.

Results and Discussion

Certonardoside K (**1**) was isolated as colorless needles. The molecular formula of **1** was established as $C_{39}H_{68}O_{14}$ on the basis of the pseudomolecular ion peak at *m/z* 783.4515 $[M+Na]^+$ (Calcd for $C_{39}H_{68}NaO_{14}$, 783.4507) and the NMR data. An examination of its spectral data (Tables 1—3) indicated that **1** contains the same 24-hydroxylated side chain and the same 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl sugar unit as observed in certonardosides F—H.[1)] The ^{1}H-NMR spectrum suggested the presence of the 3β,4β,6α,8,15β-pentahydroxy steroidal nucleus. The location of the sugar moiety at C-24 was confirmed by the correlation between C-24 and H-1′ in the heteronuclear multiple bond correlation (HMBC) experiment. The common D configuration was assumed for xylose. Methanolysis (4.5% HCl in MeOH) of **1** gave 5α-cholesta-8,14-diene-3β,4β,6α,24-tetrol (**1a**), which was esterified with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride in dry pyridine. The ^{1}H-NMR spectrum of the resulting (*R*)-MTPA ester showed two doublets of the isopropyl methyl protons at δ 0.83 and 0.86, which matched well with those of the (*R*)-MTPA ester of the (24*S*)-24-hydroxy steroid (δ 0.84 and 0.86), while the (*R*)-MTPA ester of the 24*R* isomer would display a single 6H doublet at δ 0.92.[3)] Therefore, the structure of certonardoside K (**1**) was defined as (24*S*)-24-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl]-5α-cholestane-3β,4β,6α,8,15β,24-hexol.

Comparison of the ^{1}H-NMR spectra of **2** and **3** with those of **1** showed that they share the same sugar moiety (Table 3). Certonardoside L (**2**) was isolated as colorless needles. The pseudomolecular ion peak at *m/z* 751.4624 $[M+Na]^+$ (Calcd for $C_{39}H_{68}NaO_{12}$, 751.4608), as well as the NMR spectral data (Tables 1—3), indicated the molecular formula $C_{39}H_{68}O_{12}$. The ^{1}H-NMR data revealed the lack of the broad signal at δ 4.25 assigned to H-4α in **1**, and the presence of the triplet of doublets at δ 0.71 instead of the doublet of doublets at δ 0.82 assigned to H-9 in **1**. In the correlation spectroscopy (COSY) spectrum, the H-3 oxymethine proton signal (δ 3.48) was coupled to four methylene proton signals at δ 2.19, 1.76, 1.43, and 1.17. The H-9 methine proton signal (δ 0.71) showed coupling with two methylene proton signals

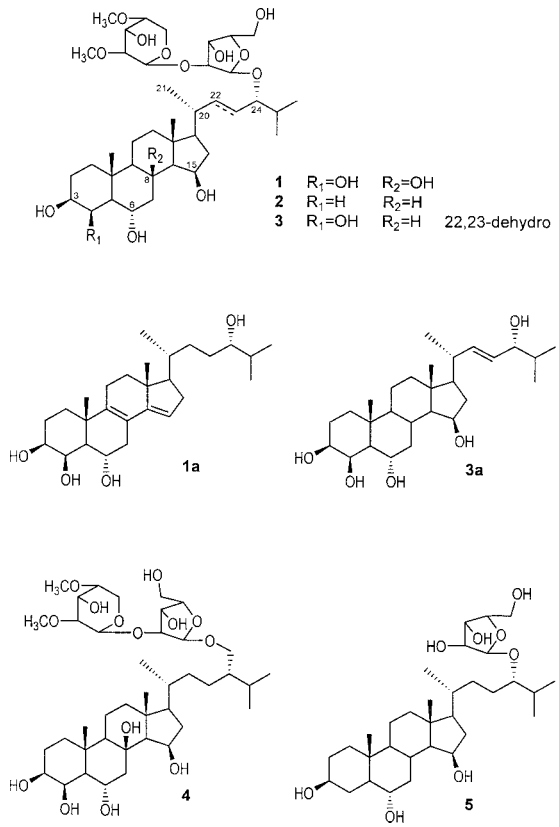

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Table 1. ^{1}H-NMR Data of the Aglycones of **1**—**5** in CD_3OD (500 MHz)[a)]

	1	2	3	4	5
1	1.71 dt (13.0, 3.5)	1.72 dt (13.0, 3.3)	1.70 dt (13.2, 3.3)	1.70 dt (12.5, 3.3)	1.71 dt (13.0, 3.3)
	0.98 m	1.03 m	1.03 m	0.98 m	1.02 m
2	1.83 m	1.76 m	1.81 qd (12.6, 3.0)	1.82 m	1.74 m
	1.56 m	1.43 m	1.57 m	1.55 m	1.43 m
3	3.43 m	3.48 m	3.43 dt (11.3, 4.0)	3.43 m	3.47 m
4	4.25 br s	2.19 dt (12.5, 2.0)	4.22 br s	4.25 br s	2.18 dt (12.0, 2.1)
		1.17 m			1.17 m
5	0.94 m	1.02 m	0.91 m	0.94 m	1.02 m
6	4.16 td (11.0, 3.6)	3.39 m	3.91 td (10.9, 4.6)	4.16 td (11.0, 4.0)	3.38 td (11.0, 4.5)
7	2.45 dd (12.5, 4.0)	2.28 dt (11.8, 3.9)	2.36 dt (11.8, 4.1)	2.44 dd (12.3, 3.8)	2.27 dt (12.0, 4.0)
	1.30 t (12.5)	0.92 m	0.93 m	1.31 t (12.3)	0.92 m
8		1.87 m	1.90 qd (11.3, 3.5)		1.87 m
9	0.82 dd (12.5, 2.8)	0.71 td (11.8, 4.5)	0.69 td (11.4, 3.6)	0.82 dd (12.5, 3.0)	0.71 td (12.0, 4.0)
11	1.79 m	1.53 m	1.46 m	1.78 m	1.52 m
	1.44 m	1.33 m	1.33 m	1.43 m	1.33 m
12	1.98 dt (13.0, 3.0)	1.96 dt (12.5, 3.0)	1.94 dt (12.7, 3.0)	1.98 dt (13.0, 3.5)	1.95 dt (12.0, 2.8)
	1.16 m	1.12 m	1.14 m	1.15 m	1.11 m
14	1.02 m	0.92 m	0.91 m	1.00 d (5.5)	0.91 m
15	4.42 br t (5.7)	4.17 td (6.2, 1.8)	4.15 td (6.6, 2.0)	4.42 td (6.5, 1.8)	4.16 td (6.5, 1.8)
16	2.39 dt (14.0, 8.3)	2.43 dt (14.8, 8.0)	2.31 dt (15.3, 8.2)	2.36 dt (14.5, 8.0)	2.42 dt (15.0, 8.3)
	1.39 m	1.36 m	1.39 m	1.39 m	1.35 m
17	1.01 m	1.08 m	1.14 m	1.01 m	1.06 m
18	1.26 s	0.95 s	0.97 s	1.26 s	0.94 s
19	1.15 s	0.87 s	1.07 s	1.15 s	0.86 s
20	1.51 m	1.50 m	2.24 m	1.51 m	1.48 m
21	0.93 d (7.0)	0.96 d (6.5)	1.05 d (6.6)	0.94 d (6.0)	0.95 d (7.0)
22	1.60 m	1.61 m	5.48 dd (15.3, 8.8)	1.46 m	1.60 m
	0.99 m	1.01 m		1.04 m	0.99 m
23	1.59 m	1.59 m	5.33 dd (15.3, 8.0)	1.39 m	1.58 m
	1.39 m	1.39 m		1.19 m	1.36 m
24	3.34 m	3.35 m	3.66 dd (7.4, 6.7)	1.35 m	3.35 m
25	1.85 m	1.86 m	1.77 m	1.80 m	1.85 m
26	0.90 d (7.0)	0.91 d (6.8)	0.92 d (6.6)	0.90 d (7.0)	0.90 d (7.5)
27	0.88 d (7.0)	0.90 d (6.8)	0.86 d (6.8)	0.89 d (7.5)	0.89 d (7.0)
24[1]				3.72 dd (9.5, 5.5)	
				3.24 dd (9.5, 5.5)	

a) δ values in ppm and coupling constants (in parentheses) in Hz.

at δ 1.53 and 1.33, and with one methine proton signal at δ 1.87. Thus, the structure of certonardoside L (**2**) was established as the 4,8-dideoxy derivative of **1**. The stereochemistry at C-24 was assumed to be *S* by analogy with the co-occurring saponin **1**.

Certonardoside M (**3**) was isolated as colorless needles. The molecular formula of **3** was deduced to be $C_{39}H_{66}O_{13}$ on the basis of the pseudomolecular ion peak at *m/z* 765.4400 $[M+Na]^+$ (Calcd for $C_{39}H_{66}NaO_{13}$, 765.4401) and the NMR data. The ^{1}H-NMR spectrum showed signals of a 3β,4β,6α,15β-tetrahydroxy steroidal nucleus. In addition, the two doublets of doublets resonating at δ 5.48 (J=15.3, 8.8 Hz) and 5.33 (J=15.3, 8.0 Hz) suggested a *trans* double bond at C-22, which was further confirmed by the downfield shifts of H-21 to δ 1.05 (δ 0.95 in compounds with saturated side chain) and H-24 to δ 3.66 (δ 3.34 in compounds with saturated side chain). Methanolysis of **3** gave (22*E*)-5α-cholest-22-ene-3β,4β,6α,15β,24-pentol (**3a**). The (*R*)- and (*S*)-MTPA esters of **3a** were prepared to determine the absolute configuration at C-24 by modified Mosher's method. A 24*R* configuration was suggested on the basis of the $\Delta\delta$ ($\delta_S-\delta_R$) value (−0.13 ppm) of H-23. In addition, it is known that the isopropyl methyl signals appear at δ 0.87—0.90 for the (*R*)-MTPA ester of the (22*E*, 24*R*) model compounds, while they shift to δ 0.95—0.98 for that of the (22*E*, 24*S*) isomer.[4)] The isopropyl methyl signals were observed to shift upfield to δ 0.84 and 0.83 in the spectrum of the (*R*)-MTPA ester of **3a**, but shift downfield to δ 0.94 and 0.92 in that of the (*S*)-MTPA ester. Thus, the structure of certonardoside M (**3**) was determined as (*E*)-(24*R*)-24-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl]-5α-cholest-22-ene-3β,4β,6α,15β,24-pentol.

Compound **4** was identified as culcitoside C_6 by comparison of the NMR data with those reported.[2)]

Certonardoside N (**5**) was isolated as light yellow needles. The molecular formula of **5** was established as $C_{32}H_{56}O_8$ on the basis of the pseudomolecular ion peak at *m/z* 591.3879 $[M+Na]^+$ (Calcd for $C_{32}H_{56}NaO_8$, 591.3873) and the NMR data. The NMR data revealed that it contains the same 5α-cholestane-3β,6α,15β,24-tetrol aglycone as that of **2**. The ^{1}H-NMR data of the sugar moiety showed four oxymethine proton signals at δ 4.94, 4.02, 4.03, and 4.19. The last methine proton signal was coupled to methylene proton signals at δ 3.86 and 3.75. The chemical shifts of H-1′—4′ and the long-range correlation between C-1′ and H-4′ in the HMBC experiment indicated that the sugar is in furanose form. These suggested the presence of a β-D-xylofuranosyl unit in **5**, which was confirmed by comparison of the ^{1}H-NMR data of the sugar moiety with those of desulfated scopariioside B.[3)] The 24*S* stereochemistry was proposed by analogy

with the co-occurring saponin **1**. Thus, the structure of **5** was defined as (24*S*)-24-*O*-β-D-xylofuranosyl-5α-cholestane-3β,6α,15β,24-tetrol.

Compounds **1**—**5** and the previously isolated compounds (certonardosides A—J)[1] were evaluated for cytotoxicity against a small panel of human solid tumor cell lines (Table 4). Among these saponins, certonardoside C was most active against the SK-MEL-2 skin cancer cell line (ED_{50}, 3.8 μg/ml). Certonardosides L (**2**) and N (**5**) showed considerable cytotoxicity against all five cell lines, while certonardosides A, H, K (**1**), and M (**3**) were weakly active only against the skin cancer cell line. Other congeners did not show any activity against the five human solid tumor cell lines.

Compounds **1**—**3** and **5** were also evaluated for antibacterial activity against 20 clinically isolated strains. Compounds **1**—**3** showed weak antibacterial activity against *Streptococcus pyogenes* 308A, *Pseudomonas aeruginosa* 1771, and *Pseudomonas aeruginosa* 1771M (MIC, 25.0 μg/ml). Compound **5** displayed weak antibacterial activity against *Pseudomonas aeruginosa* 1771 and *Pseudomonas aeruginosa* 1771M (MIC, 25.0 μg/ml). Compounds **1**—**3** and **5** were inactive (MIC, >25.0 μg/ml) against the rest of the strains.[5]

Table 2. ^{13}C-NMR Data of the Aglycones of **1**—**3** and **5** in CD_3OD (50 MHz)

	1	**2**	**3**	**5**
1	39.7	38.6	38.8	38.6
2	26.2	32.0	26.3	31.6
3	73.7	72.0	73.7	72.0
4	69.1	33.0	69.1	33.0
5	57.3	53.1	56.5[a)]	53.1
6	64.8	70.0	66.6	70.0
7	49.7	41.8	41.9	41.8
8	77.4	31.6[a)]	31.5	31.6[a)]
9	58.4	55.7	56.6[a)]	55.7
10	38.1	37.5	37.5	37.5
11	19.2	22.2	21.5	22.2
12	43.3	42.7	42.5	42.7
13	44.4	43.4	43.4	43.4
14	62.7	62.1	62.4	62.1
15	71.1	70.6	70.7	70.6
16	42.5	42.3	42.9	42.3
17	58.0	57.8	57.4	57.8
18	16.5	15.2	15.3	15.2
19	17.0	13.8	16.1	13.8
20	36.5	37.1	41.3	37.1
21	19.0	19.3	21.2	19.3
22	32.7	33.0	142.0	33.0
23	28.7	28.8	127.5	28.8
24	85.8	85.8	86.0	85.5
25	31.4	31.4[a)]	33.4	31.3[a)]
26	18.5	18.5	19.2	18.4
27	18.1	18.1	18.5	18.1

a) Assignments with the same superscript in the same column may be interchanged.

Experimental

General Optical rotation was recorded with a JASCO DIP-370 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC200 and Varian Inova 500 instruments. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FAB-MS data were obtained on a JEOL JMS-700 double focusing (B/E configuration) instrument. HPLC was performed with a YMC-Pack ODS column (250×10 mm i.d., 5 μm, 120 Å), a C18-5E Shodex packed column (250×10 mm i.d., 5 μm, 100 Å), and a Vydac column (250×10 mm i.d., 5 μm, 90 Å) using a Shodex RI-71 detector.

Animal Material The starfish was collected in July 2000, off the coast of Komun Island, Korea.[1] The specimen was identified by Prof. Sook Shin, Sahmyook University, Seoul, Korea. The voucher specimen (J00K-4) of the starfish was deposited at the Marine Natural Product Chemistry Laboratory, Pusan National University, Busan, Korea.

Extraction and Isolation The frozen starfish (9 kg) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay, the MeOH extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to afford aqueous MeOH-soluble (14 g) and *n*-hexane-soluble (39 g) fractions. The aqueous MeOH fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), and eluted with a step gradient solvent system of 33 to 0% H_2O/MeOH to afford 13 fractions (1—13). Fraction 6 (0.84 g) was very active in the brine shrimp assay (LD_{50}, 38 μg/ml) and was further separated by normal-phase MPLC (Silica gel 60, 400/230 mesh), eluted with a solvent system of 20 to 100% MeOH/$CHCl_3$ to afford 20 fractions. Compounds **1** (2.5 mg), **3** (3.3 mg), **4** (1.0 mg), and **5** (2.8 mg) were obtained by the separation of subfraction 6—12 on a reversed-phase HPLC (Vydac, 250×10 mm i.d., 5 μm, 90 Å) column eluting with 80% MeOH, followed by purification on a C18-5E Shodex packed (250×10 mm i.d., 5 μm, 100 Å) column eluting with 75% MeOH. Compound **2** (4.9 mg) was obtained by the separation of subfraction 6—9 on a reversed-phase HPLC (Vydac, 250×10 mm i.d., 5 μm, 90 Å) column eluting with 80% MeOH, followed by purification on the same column eluting with 75% MeOH.

Methanolysis of Saponins Each solution of **1** (1 mg) and **3** (1.5 mg) in

Table 3. NMR Data of the Sugar Residues of **1**—**5** in CD_3OD

	1—**3**		**4**	**5**	
	^{1}H[a)]	^{13}C	^{1}H[a)]	^{1}H[a)]	^{13}C
1′	5.10 br s	108.7	4.94 br s	4.94 s	109.6
2′	4.12 br s	89.6	4.04 dd (4.5, 1.5)	4.02 m	82.1
3′	4.20 dd (4.8, 2.0)	75.7	3.97 dd (7.5, 4.5)	4.03 m	77.3
4′	4.15 q (5.4)	83.9	3.87 ddd (7.5, 5.0, 3.0)	4.19 m	83.8
5′	3.87 dd (11.4, 5.3)	62.3	3.77 dd (12.5, 3.0)	3.86 dd (11.5, 5.5)	62.4
	3.77 dd (11.4, 6.2)		3.62 dd (12.5, 5.0)	3.75 dd (11.5, 6.0)	
1″	4.41 d (7.6)	104.8	4.41 d (7.5)		
2″	2.86 dd (9.1, 7.6)	84.8	2.86 dd (9.0, 7.5)		
3″	3.39 t (8.9)	76.5	3.38 t (9.0)		
4″	3.19 td (9.2, 5.0)	80.8	3.18 td (9.5, 5.0)		
5″	4.02 dd (11.2, 4.9)	64.4	4.01 dd (11.3, 4.8)		
	3.13 t (11.2)		3.11 dd (11.3, 10.0)		
2″-OMe	3.54 s	61.2	3.57 s		
4″-OMe	3.46 s	59.1	3.46 s		

a) δ values in ppm and coupling constants (in parentheses) in Hz.

Table 4. Cytotoxicity Data of Certonardosides A—N and Culcitoside C_6 against Human Solid Tumor Cells[a)]

Compound[b)]	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
Certonardoside A	>30	>30	6.7	>30	>30
Certonardoside C	25.0	25.9	3.8	19.4	15.8
Certonardoside D	>30	>30	>30	>30	>30
Doxorubicin	0.02	0.16	0.02	0.08	0.06
Certonardoside B	>30	>30	>30	>30	>30
Certonardoside E	>30	>30	>30	>30	>30
Certonardoside F	>30	>30	>30	>30	>30
Certonardoside G	>30	>30	>30	>30	>30
Certonardoside H	>30	>30	16.1	>30	>30
Certonardoside I	>30	>30	>30	>30	>30
Certonardoside J	>30	>30	>30	>30	>30
Certonardoside K (**1**)	>30	>30	10.6	>30	24.5
Certonardoside L (**2**)	7.5	6.8	5.8	6.4	3.9
Certonardoside M (**3**)	>30	>30	9.7	25.4	43.4
Culcitoside C_6 (**4**)	>30	>30	16.3	>30	>30
Certonardoside N (**5**)	8.0	8.4	7.7	7.2	8.2
Doxorubicin	0.02	0.17	0.02	0.06	0.03

a) Data as expressed in ED_{50} values (μg/ml). A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human central nervous systems (CNS) cancer; HCT15: human colon cancer. *b*) Compounds were assayed in two separate batches.

anhydrous 4.5% HCl in MeOH (0.5 ml) was heated at 80 °C in a stoppered reaction vial. After 30 min, TLC analysis [ODS plate, MeOH–H_2O (9 : 1)] showed that the starting material had disappeared. The reaction mixture was cooled, neutralized with Ag_2CO_3, and centrifuged. The supernatants were taken to dryness under N_2. The residues were purified by HPLC [YMC-Pack ODS column, C-18, 250×10 mm i.d., 5 μm, 120 Å, MeOH–H_2O (9 : 1)] to give **1a** and **3a**, respectively.

Preparation of MTPA Esters Compound **1a** (0.5 mg) was treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (2 μl) in dry pyridine (25 μl) at room temperature for 24 h to afford (*R*)-MTPA ester. The reaction was monitored by TLC (ODS, MeOH) and stopped when the original spot had disappeared. After removal of the solvent, the product was purified by reversed-phase HPLC on a YMC-Pack ODS column (250×10 mm i.d., 5 μm, 120 Å), and analyzed by ^{1}H-NMR. Compound **3a** (0.4 mg) was treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (2 μl) in dry pyridine (25 μl) at room temperature for 24 h, respectively. The subsequent procedure was the same as that for compound **1**.

Certonardoside K (**1**): Colorless needles, $[\alpha]_D^{21}$ −24.2° (*c*=0.12, MeOH). ^{1}H-NMR data: see Tables 1 and 3. ^{13}C-NMR data: see Tables 2 and 3. FAB-MS (+ve) *m/z*: 783 $[M+Na]^+$. FAB-MS (−ve) *m/z*: 759 $[M-H]^-$ (100), 581 $[M-H-C_7H_{13}O_5-H]^-$ (7), 449 $[M-H-C_7H_{13}O_5-C_5H_8O_4-H]^-$ (2). HR-FAB-MS (+ve) *m/z*: 783.4515 ($[M+Na]^+$, Calcd for $C_{39}H_{68}NaO_{14}$: 783.4507).

Compound **1a**: White amorphous powder. ^{1}H-NMR (CD_3OD) δ: 5.36 (br s, H-15), 4.26 (br s, H-4), 4.15 (td, *J*=9.5, 4.0 Hz, H-6), 3.45 (m, H-3), 3.23 (m, H-24), 1.20 (3H, s, H-19), 0.97 (d, *J*=7.0 Hz, H-21), 0.91 (d, *J*=7.0 Hz, H-26), 0.89 (d, *J*=6.5 Hz, H-27), 0.81 (s, H-18).

(*R*)-MTPA ester of compound **1a**: ^{1}H-NMR (CD_3OD) δ: 0.96 (d, *J*=6.0 Hz, H-21), 0.86 (d, *J*=6.0 Hz, H-26 or H-27), 0.83 (d, *J*=7.0 Hz, H-26 or H-27).

Certonardoside L (**2**): Colorless needles. ^{1}H-NMR data: see Tables 1 and 3. ^{13}C-NMR data: see Tables 2 and 3. FAB-MS (+ve) *m/z*: 751 $[M+Na]^+$. FAB-MS (−ve) *m/z*: 727 $[M-H]^-$. HR-FAB-MS (+ve) *m/z*: 751.4624 ($[M+Na]^+$, Calcd for $C_{39}H_{68}NaO_{12}$: 751.4608).

Certonardoside M (**3**): Colorless needles. ^{1}H-NMR data: see Tables 1 and 3. ^{13}C-NMR data: see Tables 2 and 3. FAB-MS (+ve) *m/z*: 765 $[M+Na]^+$. FAB-MS (−ve) *m/z*: 741 $[M-H]^-$. HR-FAB-MS (+ve) *m/z*: 765.4400 ($[M+Na]^+$, Calcd for $C_{39}H_{66}NaO_{13}$: 765.4401).

Compound **3a**: White amorphous powder. ^{1}H-NMR (CD_3OD) δ: 5.47 (dd, *J*=15.5, 8.0 Hz, H-22), 5.17 (dd, *J*=15.5, 8.5 Hz, H-23), 4.21 (br s, H-4), 4.14 (td, *J*=6.5, 2.0 Hz, H-15), 3.90 (td, *J*=11.0, 4.8 Hz, H-6), 3.45 (m, H-3), 1.07 (d, *J*=7.0 Hz, H-21), 1.06 (s, H-19), 0.96 (s, H-18), 0.91 (d, *J*=7.0 Hz, H-26), 0.85 (d, *J*=6.5 Hz, H-27), 0.67 (td, *J*=11.0, 2.5 Hz, H-9).

(*S*)-MTPA ester of compound **3a**: ^{1}H-NMR (CD_3OD) δ: 5.55 (dd, *J*=15.0, 9.0 Hz, H-22), 5.29 (dd, *J*=15.0, 8.0 Hz, H-23), 1.17 (s, H-19), 1.03 (d, *J*=6.0 Hz, H-21), 0.94 (d, *J*=6.5 Hz, H-26 or H-27), 0.93 (s, H-18), 0.92 (d, *J*=7.0 Hz, H-26 or H-27).

(*R*)-MTPA ester of compound **3a**: ^{1}H-NMR (CD_3OD) δ: 5.66 (dd, *J*=15.0, 9.0 Hz, H-22), 5.42 (dd, *J*=15.0, 8.0 Hz, H-23), 1.17 (s, H-19), 1.05 (d, *J*=6.0 Hz, H-21), 0.94 (s, H-18), 0.84 (d, *J*=6.5 Hz, H-26 or H-27), 0.83 (d, *J*=7.0 Hz, H-26 or H-27).

Compound **4**: Colorless needles. ^{1}H-NMR data: see Tables 1 and 3. FAB-MS (+ve) *m/z*: 797 $[M+Na]^+$. FAB-MS (−ve) *m/z*: 773 $[M-H]^-$. HR-FAB-MS (+ve) *m/z*: 797.4659 ($[M+Na]^+$, Calcd for $C_{40}H_{70}NaO_{14}$: 797.4663).

Certonardoside N (**5**): Colorless needles. ^{1}H-NMR data: see Tables 1 and 3. ^{13}C-NMR data: see Tables 2 and 3. FAB-MS (+ve) *m/z*: 591 $[M+Na]^+$. FAB-MS (−ve) *m/z*: 567 $[M-H]^-$. HR-FAB-MS (+ve) *m/z*: 591.3879 ($[M+Na]^+$, Calcd for $C_{32}H_{56}NaO_8$: 591.3873).

Evaluation of Cytotoxicity SRB (sulforhodamine B) assay, developed for measuring the cellular protein content of the cultures, is applied for the measurement of the cytotoxicity of the compounds against tumor cells. The rapidly growing cells were harvested, counted, and inoculated at the appropriate concentrations (1—2×10^4 cells/well) into 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate followed by incubation for 48 h at 37 °C under a 5% CO_2 atmosphere. The cultures fixed with cold TCA were stained by 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered tris base by a gyrotory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). Fifty percent inhibitory concentration (ED_{50}) was defined as the concentration which reduced absorbance by 50% compared to the control level in the untreated wells.

Evaluation of Antibacterial Activity The compounds were evaluated for antibacterial activity against 20 clinically isolated bacterial strains. The Mueller Hinton Agar plates were impregnated with 17 serial dilutions of the sample and standard (meropenem) to make a final concentration of 25 μg/ml to 0.002 μg/ml. The strains were inoculated into Fleisch extract broth (containing 10% horse serum depending on strains) and incubated for 18 h at 37 °C. The cultured strains were inoculated onto the Muller Hinton agar plates with 10^4 CFU per spot population by automatic inoculator (Dynatech, U.S.A.). The MIC was measured after 18 h of incubation.

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References and Notes

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5) Twenty clinically isolated strains, *Streptococcus pyogenes* 308A, *Streptococcus pyogenes* 77A, *Streptococcus faecium* MD8b, *Staphylococcus aureus* SG511, *Staphylococcus aureus* 285, *Staphylococcus aureus* 503, *Escherichia coli* 078, *Escherichia coli* DC0, *Escherichia coli* DC2, *Escherichia coli* TEM, *Escherichia coli* 1507E, *Pseudomonas aeruginosa* 9027, *Pseudomonas aeruginosa* 1592E, *Pseudomonas aeruginosa* 1771, *Pseudomonas aeruginosa* 1771M, *Salmonella typhimurium*, *Klebsiella oxytoca* 1082E, *Klebsiella aerogenes* 1522E, *Enterobacter cloacae* P99, and *Enterobacter cloacae* 1321E, were employed for antibacterial activity; Meropenem was employed as a positive control. Meropenem displayed MIC values of 6.3 μg/ml for *Streptococcus faecium* MD8b and <0.4 μg/ml for the rest of the strains.