New Saponins from the Starfish *Certonardoa semiregularis*

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Ten new saponins designated as certonardosides A−J (**1**−**5**, **7**−**11**) and the known halityloside D (**6**) were isolated from the brine shrimp active fraction of the MeOH extract of the starfish *Certonardoa semiregularis*. The structures were determined on the basis of spectral analysis and chemical manipulation. The compounds were evaluated for antiviral activity against HIV, HSV, CoxB, EMCV, and VSV and displayed insignificant activity within the range of noncytotoxic concentrations.

Various secondary metabolites including steroidal glycosides,[1−3] steroids,[1,2] anthraquinones,[3] alkaloids,[4,5] and phospholipids[6,7] have been reported from starfish. Steroidal glycosides are the predominant metabolites of starfish and have been reported to exhibit cytotoxic, hemolytic, antiviral, antifungal, and antimicrobial activities.[8]

In our search for bioactive metabolites from the starfish *Certonardoa semiregularis* (family Linckiidae), nine sulfated and two nonsulfated saponins were isolated from the brine shrimp active fraction of the MeOH extract. The MeOH extract of the frozen starfish was partitioned between H_2O and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 layer between aqueous MeOH and *n*-hexane. The aqueous MeOH layer was then subjected to successive reversed-phase flash column chromatography, MPLC, and HPLC to afford certonardosides A−J (**1**−**5**, **7**−**11**) and the known halityloside D (**6**). The gross structures of the compounds were elucidated with the aid of COSY, HMQC, and HMBC experiments. The absolute configurations of the side chains were defined by the ^{1}H NMR analysis of the MTPA esters. Compounds **1**−**5** contain a previously undescribed 2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl unit as a sugar moiety. The sugar moiety 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-*β*-D-xylofuranosyl in compounds **7**−**9** was also unprecedented.

Results and Discussion

Certonardoside A (**1**) was isolated as colorless needles. The HRFABMS spectrum of **1** showed a pseudomolecular ion peak at *m*/*z* 883.3736 $[M + Na]^+$ (calcd 883.3738, Δ −0.2 mmu) for the monosodium salt ($C_{39}H_{65}NaO_{17}S$) of the molecule. The fragment ion peak at *m*/*z* 763 $[(M + Na) - NaHSO_4]^+$ indicated the presence of a sulfate group. Fragment ion peaks at *m*/*z* 737 [(M + Na) − 147 + H] and *m*/*z* 503 [(M + Na) − 147−234 + H] corresponded to the loss of a methoxylated pentose and further loss of a sulfated pentose, respectively. The negative ion FABMS spectrum of **1** corroborated the above results, giving the $[M - Na]^-$ ion peak at *m*/*z* 837. The presence of the sulfate group was also supported by the absorption bands at 1254 and 1227 cm^{-1} in the IR spectrum. The ^{1}H NMR spectrum of **1** showed the presence of a 3*β*,4*β*,6α,8,15*β*-pentahydroxy steroidal nucleus, and proton signals at *δ* 0.95 and 1.09 (methyl doublets), *δ* 3.78 and 3.32 (oxymethylene), and *δ* 4.78 and 4.77 (olefinic methylene) were reminiscent of a 26-hydroxy-24-methylcholest-24(24^1)-ene side chain. The inter-glycosidic linkages were defined on the basis of the long-range correlations between C-1′ and H-26 and between C-2′ and H-1″. Additional signals attributable to two pentose residues were assigned on the basis of COSY and HMBC data.

Solvolysis of **1** in dioxane−pyridine afforded the desulfonated derivative **1a**, whose negative ion FABMS exhibited a pseudomolecular ion peak at *m*/*z* 757 $[M - H]^-$, accompanied by fragment ion peaks at *m*/*z* 611 and 479, due to the sequential loss of a methoxylated pentose unit (147 amu) and a pentose unit (132 amu). Solvolysis of **1** to the desulfonated derivative (**1a**) was further confirmed by upfield shifts of H-3′ (*δ* 4.39 → 3.50), 2′ (*δ* 3.70 → 3.43), 4′ (*δ* 3.80 → 3.48), and 1′ (*δ* 4.54 → 4.36) of the internal xylopyranosyl unit. The signals of C-3′, 2′, and 4′ were shifted by -4.2, +4.4, and +1.3 ppm, respectively. Thus, it was deduced that the sulfate group was located at C-3′. The chemical shifts of the anomeric carbons at *δ* 103.8 and 104.5 and the coupling constants of the anomeric protons at *δ* 4.36 (*J* = 7.0 Hz, H-1′) and 4.71 (*J* = 7.3 Hz, H-1″) indicated that the glycosidic linkages in **1a** are *β* and the sugar units are in their pyranose form (Tables 4 and 5). Direct comparison of the NMR data for **1a** with those reported for acodontasteroid G from the antarctic starfish *Acodontaster conspicuus* indicated they were the same.[9] The common 20*R* configuration was assumed on the basis of the chemical shift of H-21 (ca. *δ* 0.95 in compounds with a saturated side chain).[10] The stereochemistry at C-25 remains unassigned. The common D configuration was assumed for xylose. Thus, the structure of certonardoside A (**1**) was assigned as the 3′-*O*-sulfonated derivative of acodontasteroid G. The sugar moiety 2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl was unprecedented.

Comparison of the ^{1}H NMR spectra of **2**−**5** with those of **1** revealed the same 2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl sugar moiety (Table 4). Certonardoside B (**2**) was isolated as colorless needles. It was a 4,8-dideoxy derivative of **1**. The FABMS gave a pseudomolecular ion peak at *m*/*z* 851 $[M + Na]^+$, 32 amu less than that of **1**. The ^{1}H NMR data revealed the lack of the broad singlet at *δ* 4.27 assigned to H-4α in **1** and

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Table 1. ^{1}H NMR Data of the Aglycones of **1**−**5** and **1a** (CD_3OD, 500 MHz)[a]

position	**1**, **1a**	**2**	**3**	**4**	**5**
1	1.71 (dt, 13.1, 3.3)	1.72 (dt, 13.2, 3.2)	1.72 (m)	1.70 (dt, 13.0, 3.3)	1.71 (dt, 9.5, 3.5)
	0.98 (m)	1.04 (m)	0.99 (m)	0.99 (m)	0.97 (m)
2	1.83 (m)	1.74 (m)	1.74 (m)	1.82 (m)	1.74 (m)
	1.56 (m)	1.44 (m)	1.48 (m)	1.55 (m)	1.47 (m)
3	3.45 (m)	3.49 (m)	3.49 (m)	3.45 (m)	3.49 (m)
4	4.27 (br s)	2.19 (dt, 12.8, 2.0)	2.20 (dt, 12.4, 2.1)	4.26 (br s)	2.18 (m)
		1.17 (m)	1.22 (m)		1.20 (m)
5	0.95 (m)	1.03 (m)	1.05 (m)	0.95 (m)	1.04 (m)
6	4.16 (td, 10.9, 4.2)	3.38 (td, 10.9, 4.4)	3.70 (td, 10.8, 4.2)	4.15 (td, 11.0, 4.4)	3.69 (td, 10.8, 3.5)
7	2.46 (dd, 11.7, 4.2)	2.29 (dt, 11.8, 3.7)	2.39 (m)	2.45 (dd, 12.5, 4.5)	2.37 (dd, 12.0, 4.3)
	1.33 (t, 11.7)	0.93 (m)	1.30 (t, 12.1)	1.31 (t, 12.5)	1.25 (m)
8		1.87 (qd, 11.2, 2.9)			
9	0.83 (dd, 12.5, 2.6)	0.73 (td, 11.4, 3.6)	0.85 (dd, 12.3, 2.6)	0.82 (dd, 12.5, 2.7)	0.83 (dd, 12.3, 3.0)
11	1.79 (m)	1.53 (m)	1.82 (m)	1.78 (m)	1.80 (m)
	1.44 (m)	1.33 (m)	1.50 (m)	1.43 (m)	1.50 (m)
12	1.98 (dt, 12.8, 3.4)	1.97 (m)	1.98 (m)	1.96 (dt, 12.5, 3.3)	1.96 (dt, 12.5, 3.0)
	1.18 (m)	1.13 (m)	1.19 (m)	1.17 (m)	1.18 (m)
14	1.02 (d, 5.5)	0.93 (m)	1.03 (m)	1.01 (d, 5.7)	1.02 (d, 5.3)
15	4.44 (td, 6.5, 1.9)	4.19 (br t, 5.6)	4.45 (br t, 5.5)	4.40 (br t, 6.0)	4.39 (br t, 6.3)
16	2.38 (dt, 14.3, 8.0)	2.41 (m)	2.39 (m)	2.21 (dt, 14.5, 7.0)	2.21 (dt, 14.5, 8.5)
	1.39 (m)	1.37 (m)	1.39 (m)	1.35 (m)	1.36 (m)
17	1.03 (m)	1.11 (m)	1.03 (m)	1.01 (m)	1.01 (m)
18	1.26 (s)	0.95 (s)	1.27 (s)	1.28 (s)	1.28 (s)
19	1.16 (s)	0.87 (s)	0.99 (s)	1.15 (s)	0.98 (s)
20	1.58 (m)	1.57 (m)	1.58 (m)	2.13 (m)	2.13 (m)
21	0.95 (d, 6.4)	0.97 (d, 6.5)	0.96 (d, 6.5)	0.99 (d, 6.5)	0.99 (d, 7.5)
22	1.58 (m)	1.57 (m)	1.58 (m)	5.19 (dd, 15.0, 8.0)	5.19 (dd, 15.0, 8.3)
	1.21 (m)	1.21 (m)	1.21 (m)		
23	2.14 (m)	2.14(m)	2.14 (m)	5.24 (dd, 15.0, 7.6)	5.24 (dd, 15.0, 8.0)
	1.96 (m)	1.96 (m)	1.95 (m)		
24				2.15 (m)	2.15 (m)
25	2.43 (m)	2.43 (m)	2.43 (m)	1.61 (m)	1.62 (m)
26	3.78 (dd, 9.4, 6.1)	3.78 (dd, 9.2, 6.0)	3.79 (dd, 9.5, 6.0)	3.74 (m)	3.75 (m)
	3.32 (m)[b]	3.31 (m)[b]	3.30 (m)[b]	3.25 (dd, 9.7, 6.7)	3.25 (dd, 9.5, 6.5)
27	1.09 (d, 6.9)	1.10 (d, 6.8)	1.11 (d, 6.9)	0.91 (d, 7.5)	0.91 (d, 7.0)
24^1	4.78 (br s)	4.78 (br s)	4.78 (br s)	0.93 (d, 7.5)	0.93 (d, 7.5)
	4.77 (br s)	4.77 (br s)	4.77 (br s)		

[a] Multiplicities and coupling constants are in parentheses. [b] Overlapped with the solvent signal.

showed significant upfield shifts of the signals of H-6, 7, 11, 15, 18, and 19 (Table 1). In the ^{13}C NMR spectrum, the absence of two oxygenated carbon signals and significant upfield shifts of signals C-5, 7, 9, and 19 and downfield shifts of signals C-2, 6, and 11 were observed. Thus, the structure of certonardoside B (**2**) was established as the 4,8-dideoxy derivative of **1**, that is, a sodium salt of 26-*O*-[2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl]-24-methyl-5α,25ξ-cholest-24(24^1)-ene-3*β*,6α,-15*β*,26-tetrol.

Certonardoside C (**3**) was isolated as light yellow needles. The positive and negative ion FABMS gave ion peaks at *m*/*z* 867 $[M + Na]^+$ and 821 $[M - Na]^-$, respectively, 16 amu less than those of **1**. It was an 8-hydroxy derivative of **2**, as determined by comparison of the ^{1}H and ^{13}C NMR data with those of **1** and **2** (Tables 1 and 3). Compared to compound **2**, the introduction of the 8-hydroxyl group caused downfield shifts of H-11 (*δ* 1.53, 1.33 → 1.82, 1.50), H-15 (*δ* 4.19 → 4.45), H_3-18 (*δ* 0.95 → 1.27), and H_3-19 (*δ* 0.87 → 0.99). The ^{13}C NMR data showed a smaller *γ*-gauche effect at C-11 (−2.5 ppm) than that expected.[11] An unexpected deshielding effect at the *γ*-carbon C-15 (+0.5 ppm) was also observed. These deviations from the additivity rule of substituent effects might be associated with the deformations that took place to relieve the 1,3-diaxial steric hindrance between the angular methyl groups and the 8-hydroxyl group.[12]

Certonardoside D (**4**) was isolated as colorless needles. The ^{1}H and ^{13}C NMR shifts of the aglycone were virtually identical to those of **1** (Tables 1 and 3) and suggested the presence of a 3*β*,4*β*,6α,8,15*β*-pentahydroxylated moiety. Compound **4**, with a pseudomolecular cation peak at *m*/*z* 883 $[M + Na]^+$ and an anion peak at *m*/*z* 837 $[M - Na]^-$, was isomeric with compound **1**. The only difference was the presence of a 26-hydroxy-24-methylcholest-22-ene side chain instead of the 26-hydroxy-24-methylcholest-24(24^1)-ene side chain as in **1**. The ^{1}H NMR spectrum of **4** showed two doublets of doublets centered at *δ* 5.19 and 5.24, which could be assigned to the Δ^{22E} protons (J = 15 Hz). The common 20*R* configuration was proposed on the basis of the H-21 signal at *δ* 0.99 (J = 6.5 Hz) and supported by comparison with the data of the starfish-derived saponins with a 26-hydroxy-24-methylcholest-22-ene side chain.[9,13] Methanolysis of **4** gave (22*E*)-24-methyl-5α-cholesta-8,14,-22-triene-3*β*,4*β*,6α,26-tetrol (**4a**), whose NMR data of H-26 (*δ* 3.57, 3.27), H-27 (*δ* 0.88), and H-24^1 (*δ* 0.94) were indicative of the *threo* configuration at C-24 and C-25,[14] while the *erythro* analogue would show the corresponding signals at *δ* 3.53, 3.34, 0.87, and 1.02, respectively.[14] The absolute configuration was then derived by ^{1}H NMR analysis of the MTPA derivatives. It was reported that in the (*S*)-(−)-MTPA esters of the *threo* pair [(24*R*,25*S*) and (24*S*,25*R*)], the H-26 of the 25*R* isomer displays a smaller $\Delta\delta_H$ than that of the 25*S* isomer, which usually appears as two doublets of doublets separated by 0.25 ppm or more.[14] Such behavior is reversed in the (*R*)-(+)-MTPA esters; the H-26 of the 25*S* isomer displays a smaller $\Delta\delta_H$. The ^{1}H NMR spectrum of the 3,4,6,26-(*S*)-(−)-MTPA ester of **4a** showed the H-26 methylene proton signals as two well-separated doublets of doublets at *δ* 4.09 and 4.39, while that of the 3,4,6,26-(*R*)-(+)-MTPA ester showed two close doublets of doublets at *δ* 4.17 and 4.28 (Table 6).

Table 2. ^{1}H NMR Data of the Aglycones of **6**−**11** (CD_3OD, 500 MHz)[a]

position	6	7	8	9	10	11
1	1.71 (dt, 13.0, 3.5)	1.73 (br d, 13.6)	1.72 (br d, 12.4)	1.68 (dt, 13.5, 3.5)	1.72 (m)	1.72 (m)
	0.98 (m)	1.02 (m)	1.08 (m)	1.03 (m)	0.97 (m)	0.98 (m)
2	1.82 (m)	1.83 (m)	1.84 (m)	1.81 (m)	1.83 (m)	1.76 (m)
	1.54 (m)	1.57 (m)	1.58 (m)	1.56 (m)	1.56 (m)	1.51 (m)
3	3.43 (m)	3.34 (m)[b]	3.35 (m)[b]	3.43 (m)	3.43 (m)	3.43 (m)
4	4.25 (br s)	4.31 (br s)	4.26 (br s)	4.21 (br s)	4.31 (br s)	4.27 (br s)
5	0.93 (m)	1.16 (m)	1.13 (m)	0.91 (m)	1.15 (m)	1.14 (m)
6	4.16 (td, 11.0, 4.0)	4.91 (td, 11.9, 4.2)	4.59 (td, 11.2, 4.5)	3.90 (td, 11.9, 4.5)	4.92 (br t, 12.0)	4.60 (td, 11.0, 4.0)
7	2.44 (dd, 12.3, 4.3)	2.70 (dd, 12.2, 4.3)	2.56 (dt, 11.9, 3.9)	2.35 (dt, 12.0, 4.0)	2.72 (dd, 12.0, 4.0)	2.65 (dt, 12.0, 4.3)
	1.29 (t, 12.3)	1.50 (t, 12.2)	1.13 (t, 11.9)	0.93 (m)	1.54 (t, 12.0)	1.13 (m)
8			1.94 (m)	1.88 (m)		1.94 (m)
9	0.81 (dd, 12.5, 3.0)	0.85 (dd, 12.6, 2.6)	0.73 (td, 11.5, 3.8)	0.67 (td, 11.5, 3.5)	0.84 (m)	0.72 (td, 11.5, 3.8)
11	1.78 (m)	1.80 (m)	1.46 (m)	1.43 (m)	1.77 (m)	1.56 (m)
	1.43 (m)	1.44 (m)	1.32 (m)	1.30 (m)	1.40 (m)	1.33 (m)
12	1.97 (dt, 13.0, 3.5)	1.98 (br d, 12.6)	1.95 (br d, 12.1)	1.94 (dt, 13.0, 3.5)	1.93 (dt, 13.0, 3.0)	1.90 (dt, 12.0, 2.8)
	1.14 (m)	1.17 (m)	1.13 (m)	1.09 (m)	1.13 (m)	1.05 (m)
14	0.99 (d, 5.5)	1.02 (m)	0.91 (m)	0.88 (m)	1.03 (d, 6.5)	0.90 (m)
15	4.40 (td, 6.1, 1.5)	4.42 (br t, 6.0)	4.17 (m)	4.16 (br t, 6.5)	4.38 (t, 6.0)	4.13 (dd, 12.0, 6.5)
16	2.38 (dt, 14.5, 8.0)	2.39 (dt, 14.4, 8.0)	2.42 (dt, 14.6, 8.2)	2.42 (dt, 14.5, 8.0)	4.19 (t, 6.8)	4.21 (dd, 14.0, 7.5)
	1.38 (m)	1.38 (m)	1.34 (m)	1.33 (m)		
17	0.97 (m)	0.98 (m)	1.07 (m)	1.06 (m)	0.93 (m)	0.99 (m)
18	1.25 (s)	1.26 (s)	0.93 (s)	0.93 (s)	1.23 (s)	0.91 (s)
19	1.15 (s)	1.24 (s)	1.14 (s)	1.06 (s)	1.23 (s)	1.13 (s)
20	1.49 (m)	1.52 (m)	1.51 (m)	1.47 (m)	1.90 (m)	1.89 (m)
21	0.93 (d, 7.0)	0.94 (d, 6.5)	0.95 (d, 6.5)	0.95 (d, 7.0)	0.94 (d, 6.0)	0.94 (d, 6.5)
22	1.62 (m)	1.58 (m)	1.63 (m)	1.59 (m)	1.71 (m)	1.70 (m)
	0.97 (m)	0.99 (m)	1.03 (m)	0.98 (m)	1.13 (m)	1.14 (m)
23	1.57 (m)	1.60 (m)	1.59 (m)	1.59 (m)	1.40 (m)	1.40 (m)
	1.30 (m)	1.38 (m)	1.38 (m)	1.38 (m)	1.11 (m)	1.11 (m)
24	3.32 (m)	3.34 (m)[b]	3.36 (m)[b]	3.34 (m)	1.23 (m)	1.24 (m)
25	1.85 (m)	1.85 (m)	1.86 (m)	1.85 (m)	1.74 (m)	1.74 (m)
26	0.90 (d, 6.0)	0.91 (d, 6.8)	0.91 (d, 6.9)	0.90 (d, 7.0)	0.87 (d, 7.0)	0.87 (d, 7.0)
27	0.89 (d, 7.0)	0.89 (d, 6.8)	0.89 (d, 6.7)	0.89 (d, 7.0)	0.84 (d, 6.5)	0.84 (d, 7.0)
24^1					1.68 (m)	1.70 (m)
					1.43 (m)	1.45 (m)
24^2					3.85 (td, 9.3, 5.8)	3.85 (td, 9.0, 6.0)
					3.53 (q, 9.3)	3.53 (q, 8.0)

[a] Multiplicities and coupling constants are in parentheses. [b] Overlapped with the solvent signal and the assignment may be interchanged.

Accordingly, the (24*R*,25*S*) configuration was assigned to compound **4**, and the structure was established as a sodium salt of (*E*)-(24*R*,25*S*)-26-*O*-[2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl]-24-methyl-5α-cholest-22-ene-3*β*,4*β*,6α,8,15*β*,26-hexol.

Certonardoside E (**5**) was isolated as light yellow needles. The FABMS gave a pseudomolecular ion peak at *m*/*z* 867 $[M + Na]^+$, 16 amu less than that of **4**. Comparison of the ^{1}H and ^{13}C NMR spectra with those of **4** indicated that it was the 4-deoxy derivative of **4**.

Compound **6** was isolated as colorless needles and identified as halityloside D by comparison of the spectral data with those reported.[10] The stereochemistry at C-24 was proposed as *S* by analogy with the co-occurring compound **7** (vide infra).

Certonardoside F (**7**) was isolated as colorless needles. The FABMS spectrum of **7** showed a pseudomolecular ion peak at *m*/*z* 885 $[M + Na]^+$, which was in agreement with the monosodium salt formula $C_{39}H_{67}NaO_{17}S$. The ^{1}H and ^{13}C NMR data implied that **7** has a 3*β*,4*β*,6α,8,15*β*-pentahydroxysteroid nucleus sulfonated at C-6, as deduced from the downfield shifts of the signals of H-6 and C-6 to δ_H 4.91 and δ_C 74.5 relative to those of **6** (Tables 2 and 3). The ^{1}H and ^{13}C NMR spectra of **7** (Tables 4 and 5) showed the presence of a 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-*β*-D-xylofuranosyl unit as a sugar moiety. The internal *β*-xylose residue was speculated to be in its furanose form since the signals of H-1′−4′ were shifted downfield compared to those of the pyranose form in **1a**, due to the stronger steric hindrance in the furanose form. In particular, we noted that the signal of H-4′ in **7** was shifted downfield to *δ* 4.15 (*δ* 3.93 in **6**) because of the 1,3-*syn* interaction between H-4′/2′-OH in the xylofuranose structure as compared to the *anti* arrangement in the arabinofuranose structure of **6**.[15] The chemical shift of the anomeric carbon (*δ* 108.7) was also characteristic of the *β*-xylofuranosyl form. Acid hydrolysis (9% HCl in H_2O) of **7**, followed by TLC comparison of the hydrolysate with standard sugars, affirmed the presence of a nonsubstituted xylose. The cross-peaks in the HMBC experiment between C-2′ and H-1″ and between C-24 and H-1′ indicated that the disaccharide moiety 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-*β*-D-xylofuranose is linked to the aglycone at C-24. Methanolysis (4.5% HCl in MeOH) of **7** gave 5α-cholesta-8,14-diene-3*β*,4*β*,6α,24-tetrol (**7a**), 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* 0.84 and 0.87, 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.[16] Therefore, the structure of certonardoside F (**7**) was defined as a sodium salt of (24*S*)-24-*O*-[2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-*β*-D-xylofuranosyl]-6-*O*-sulfonato-5α-cholestane-3*β*,4*β*,6α,8,15*β*,24-hexol.

Certonardoside G (**8**) was isolated as colorless needles. The FABMS gave a pseudomolecular ion peak at *m*/*z* 869 $[M + Na]^+$, 16 amu less than that of **7**. Comparison of its

Table 3. ^{13}C NMR Data of the Aglycones of **1**−**11** and **1a** (CD_3OD, 50 MHz)

position	**1**, **1a**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	39.7	38.6	39.4	39.7[a]	39.4[a]	39.7	39.4	38.6	38.8	39.4	38.5
2	26.1	31.9	31.5	26.2	31.5	26.1	26.5	26.7	26.4	26.5	26.7
3	73.6	72.0	72.2	73.7[b]	72.2	73.7	72.7	72.7	73.7	73.0	72.0
4	69.0	33.0	32.4[a]	69.1	32.4	69.1	68.9	69.0	69.1	68.9	69.0
5	57.2	53.1	53.8	57.2	53.8	57.3	56.1	55.4	56.6	55.9	55.0
6	64.8	70.0	67.7	64.8	67.7	64.8	74.5	75.8	66.6	74.5	75.8
7	49.7	41.8	49.6	49.6	49.8	49.6	47.6	39.6	41.9	47.8	39.6
8	77.4	31.6	77.5	77.4	77.5	77.4	77.5	31.7	31.4	77.2	31.7
9	58.3	55.7	57.4	58.4	57.4	58.4	58.1	56.1	56.5	58.1	56.0
10	38.1	37.5	38.0	38.2	38.0	38.1	38.7	38.1	37.5	38.7	38.0
11	19.2	22.2	19.7	19.2	19.7	19.2	19.1	21.4	21.5	18.8	21.2
12	43.3	42.6	43.4	43.2	43.3	43.3	43.2	42.5	42.6	43.4	42.4
13	44.4	43.5	44.4	44.2	44.2	44.4	44.4	43.4	43.4	44.6	43.5
14	62.7	62.1	62.6	62.8	62.7	62.7	62.5	62.2	62.3	61.0	60.3
15	71.0	70.6	71.1	71.1	71.1	71.1	71.0	70.5	70.7	71.0	70.1
16	42.5	42.4	42.6	43.7	43.7	42.6	42.5	41.7	42.3	72.9	73.2
17	57.6	57.6	57.7	57.7	57.7	58.0	57.9	57.7	57.8	63.0	62.8
18	16.5	15.2	16.5	16.6	16.7	16.5	16.5	15.1	15.2	17.9	16.2
19	17.0	13.8	14.1	17.0	14.1	17.0	16.9	16.0	16.1	16.9	15.9
20	35.8	36.5	35.9	41.2	41.2	36.5	36.4	37.1	37.1	31.4	31.9
21	18.9	19.2	18.9	21.2	21.2	19.0	19.0	19.3	19.4	18.6	18.9
22	35.3	35.5	35.3	137.3	137.3	32.8	32.7	32.9	33.0	34.7	34.9
23	32.2	32.4	32.3[a]	133.8	133.9	28.5	28.7	28.7	28.8	28.6	28.6
24	153.7	153.7	153.7	39.7[a]	39.7[a]	84.5	85.7	85.7	85.8	42.3	42.3
25	40.7	40.7	40.7	39.9[a]	39.9[a]	31.5	31.4	31.4	31.5	30.6	30.6
26	74.6	74.6	74.6	73.5[b]	73.5	18.3	18.5	18.5	18.5	20.1	20.1
27	17.7	17.7	17.7	14.6	14.6	18.2	18.1	18.1	18.1	19.0	19.0
24^1	109.6	109.5	109.6	17.4	17.4					31.8	31.7
24^2										70.1	70.1

[a,b] Assignments with the same superscript in the same column may be interchanged.

Table 4. ^1H NMR Data (CD_3OD, 500 MHz) of the Sugar Residues in **1**−**11** and **1a**

position	**1**−**5**	**1a**	**6**	**7**−**9**	**10**, **11**
1′	4.54 (d, 5.5)	4.36 (d, 7.0)	5.07 (br s)	5.10 (br s)	4.18 (d, 7.5)
2′	3.70 (dd, 6.8, 5.5)	3.43 (m)	4.04 (dd, 3.5, 1.0)	4.12 (br s)	3.15 (m)
3′	4.39 (t, 6.9)	3.50 (m)	3.97 (m)	4.20 (dd, 4.8, 2.0)	3.35 (m)[a]
4′	3.80 (td, 7.2, 4.4)	3.48 (m)	3.93 (m)	4.15 (q, 5.4)	3.17 (m)
5′	3.97 (dd, 11.8, 4.4)	3.85 (dd, 11.5, 4.0)	3.75 (dd, 11.8, 3.3)	3.87 (dd, 11.4, 5.3)	4.03 (dd, 9.0, 3.0)
	3.34 (m)[a]	3.16 (dd, 11.5, 9.5)	3.63 (dd, 11.8, 6.8)	3.77 (dd, 11.4, 6.2)	3.13 (m)
4′-OMe					3.46 (s)
1″	4.85 (d, 6.3)	4.71 (d, 7.3)	4.40 (d, 7.5)	4.41 (d, 7.6)	
2″	3.05 (t, 6.3)	2.92 (dd, 8.4, 7.3)	2.86 (dd, 9.0, 7.5)	2.86 (dd, 9.1, 7.6)	
3″	3.46 (t, 7.1)	3.37 (m)	3.38 (t, 9.0)	3.39 (t, 8.9)	
4″	3.49 (td, 7.4, 4.3)	3.48 (m)	3.17 (td, 9.5, 5.0)	3.19 (td, 9.2, 5.0)	
5″	4.01 (dd, 11.6, 4.2)	3.88 (dd, 11.5, 5.5)	3.98 (dd, 11.3, 5.3)	4.02 (dd, 11.2, 4.9)	
	3.24 (dd, 11.6, 7.5)	3.16 (dd, 11.5, 9.5)	3.09 (t, 11.3)	3.13 (t, 11.2)	
2″-OMe	3.58 (s)	3.60 (s)	3.57 (s)	3.54 (s)	
4″-OMe			3.46 (s)	3.46 (s)	

[a] Overlapped with the solvent signal.

Table 5. ^{13}C NMR Data (CD_3OD, 50 MHz) of the Sugar Residues in **1**−**11** and **1a**

position	**1**−**5**	**1a**	**6**	**7**−**9**	**10**, **11**
1′	102.9	103.8	107.8	108.7	105.1
2′	76.9	81.3	92.7	89.6	75.0
3′	81.9	77.7	77.8	75.7	76.9
4′	70.0	71.3	83.9	83.9	80.9
5′	64.4	66.5	62.5	62.3	64.3
4′-OMe					59.0
1″	102.8	104.5	105.2	104.8	
2″	83.2	84.9	84.9	84.8	
3″	74.9	77.0	76.5	76.5	
4″	70.8	71.1	80.8	80.8	
5″	65.5	66.6	64.4	64.4	64.4
2″-OMe	60.5	60.9	61.1	61.2	61.2
4″-OMe			59.1	59.1	59.1

NMR spectra with those of **7** showed that it is the 8-deoxy derivative of **7**.

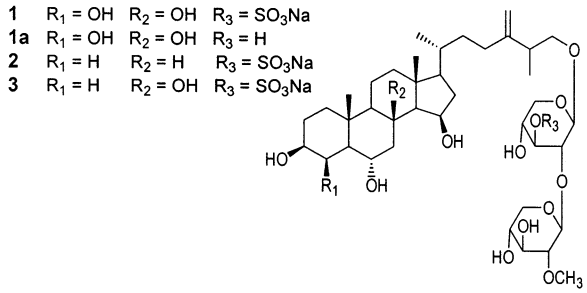
Certonardoside H (**9**) was isolated as colorless needles. The FABMS showed a pseudomolecular ion peak at *m*/*z* 767 $[M + Na]^+$, 102 amu less than that of **8**. Analysis of the spectral data indicated that **9** is the desulfonated form of **8**. Methanolysis of **9** gave 5α-cholest-14-ene-3*β*,4*β*,6α,-24-tetrol (**9a**). The ^1H NMR spectrum of the (*R*)-(+)-MTPA ester of **9a** showed two methyl doublets of the isopropyl group at *δ* 0.82 and 0.86, and accordingly the 24*S* configuration was assigned.[16]

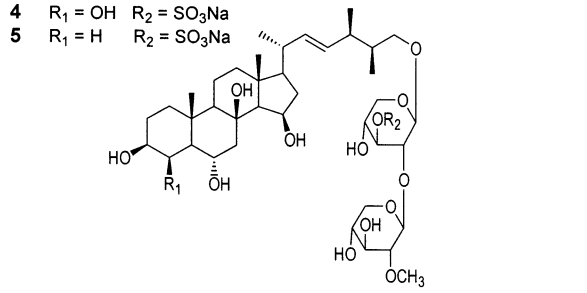
Table 6. Selected ^1H NMR Data of the MTPA Esters of **4a**, **7a**, and **9a** (CD_3OD, 500 MHz)[a]

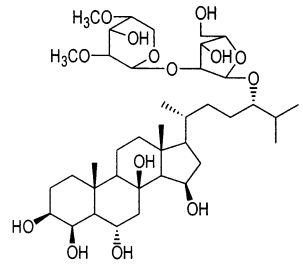
MTPA esters	H-26	H-26, H-27
(*S*)-(−)-MTPA ester of **4a**	4.39 (dd, 10.8, 4.8)	
	4.09 (dd, 10.8, 6.5)	
(*R*)-(+)-MTPA ester of **4a**	4.28 (dd, 11.3, 4.8)	
	4.17 (dd, 11.3, 6.3)	
(*R*)-(+)-MTPA ester of **7a**		0.87 (d, 6.0)
		0.84 (d, 7.0)
(*R*)-(+)-MTPA ester of **9a**		0.86 (d, 6.5)
		0.82 (d, 7.0)

[a] Multiplicities and coupling constants are in parentheses.

Certonardosides I and J (**10**, **11**) possessed a sulfated sterol nucleus and shared the same sugar moiety. Com-







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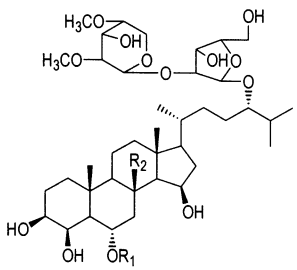

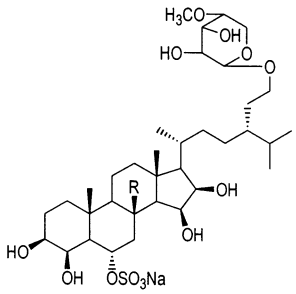

pound **10** was isolated as light yellow needles. The FABMS gave a pseudomolecular ion peak at *m*/*z* 783 $[M + Na]^+$, accompanied by a fragment ion peak at *m*/*z* 663, corresponding to the loss of $NaHSO_4$. Examination of the ^1H and ^{13}C NMR spectral data suggested the presence of a 3*β*,4*β*,6α,8,15*β*,16*β*-hexahydroxylation pattern. In the ^1H NMR spectrum, two coupled oxymethine signals were observed at *δ* 4.38 and 4.19, which could be assigned to H-15 and H-16, respectively. The chemical shifts of C-15 (*δ* 71.0) and C-16 (*δ* 72.9) further suggested the 15*β*,16*β*-dihydroxy configuration because they were close to those of 15*β*,16*β*-dihydroxy steroids but dissimilar to those of 15α,16*β*-dihydroxy steroids (ca. 80−83 ppm).[10] The 15α,-16α configuration could be eliminated by the downfield shifts of the H-18 methyl proton signal and the C-18 carbon signal.[10] The upfield shift of the H-4′ to *δ* 3.17 (*δ* 3.50 in nonsubstituted *β*-D-xylopyranoside) along with the downfield shift of H_{eq}-5′ to *δ* 4.03 (*δ* 3.89 in nonsubstituted *β*-D-xylopyranoside) indicated the location of the methoxy group at C-4′ of the xylopyranose. This was confirmed by the cross-peaks in the HMBC experiment between the methoxy group and C-4′ or H-4′. The chemical shift of the anomeric carbon (*δ* 105.1) and the coupling constant of the anomeric proton (*δ* 4.18, $J = 7.5$ Hz) suggested that the glycosidic linkage in **10** is *β*. The location of the sugar residue was established as shown on the basis of the long-range correlation between C-24[2] and H-1′. The 24*R* configuration was assigned on the basis of comparison of the ^1H and ^{13}C NMR spectral data with those of 24[2]-hydroxylated model compounds.[17] The $\Delta\delta_H$ of the isopropyl methyl proton signals of (24*R*)-24[2]-hydroxy steroids ranged from 0.03 to 0.06 ppm, while that of the 24*S* isomer was always under 0.03 ppm. Likewise, the $\Delta\delta_C$ of the isopropyl methyl carbon signals of (24*R*)-24[2]-hydroxy steroids ranged from 1.1 to 1.4 ppm, while that of the 24*S* isomer ranged between 0.1 and 0.4 ppm. The $\Delta\delta$ of the isopropyl methyl proton and carbon signals of **10** were 0.03 and 1.1 ppm, respectively, which were close to those of the 24*R* isomer.[17] Thus, the structure of certonardoside I (**10**) was defined as a sodium salt of (24*R*)-24[2]-*O*-(4-*O*-methyl-*β*-D-xylopyranosyl)-6-*O*-sulfonato-24-ethyl-5α-cholestane-3*β*,4*β*,6α,8,15*β*,16*β*,24[2]-heptol.

Certonardoside J (**11**) was isolated also as light yellow needles. Analysis of the FABMS data (*m*/*z* 767 $[M + Na]^+$) and NMR data (Tables 2−5) indicated that it is the 8-deoxy derivative of **10**. As in the case of **10**, the 24*R* stereochemistry of **11** was proposed by comparison of its NMR data with those of the model compounds.[17]

The isolated compounds were assayed for antiviral activity since some sulfated sterols from marine invertebrates have been reported to be active.[18] It was reported that sterols sulfated exclusively on the A and B rings were much more potent than other sulfated sterols.[18] However, the antiviral activity of our compounds was insignificant within the range of noncytotoxic concentrations (Table 7). Only weak antiviral activity against HSV was observed in compounds **1a**, **10**, and **11**. Compounds **1** and **3** exhibited rather substantial cytotoxicity against the MT-4 cell line.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a JASCO DIP-370 digital polarimeter. The IR spectrum was measured by a JASCO FT/IR-410 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AC200, DMX600, 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). FABMS data were obtained on a JEOL JMS-SX-102A double-focusing spectometer. HPLC was performed with a YMC-Pack ODS column (250 × 20 mm i.d., 4 *μ*m, 80 Å), a C18-5E Shodex packed column (250 × 10 mm i.d., 5 *μ*m, 100 Å), and a Vydac column (250 × 10 mm i.d., 5 *μ*m, 90 Å) using a Shodex RI-71 detector.

Animal Material. The animals were collected in July 2000, at depths of 5−10 m off the coast of Komun Island, Korea. The specimen was identified by Prof. Sook Shin, Sahmyook University, Seoul, Korea. The starfish was moderately sized (10 cm in diameter), five-armed species. Arms were slender, broad at base, cylindrical in cross-section, and tapering evenly to rounded tips. The dorsal surface is a shade of red and the ventral surface was pale. The dorsal surface was covered in plates that are arranged in regular longitudinal and transverse rows up to one-third of its arms. The voucher specimen (J00K-4) of the starfish was deposited in the Marine Natural Product 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

Table 7. Evaluation of Antiviral Activity and Cytotoxicity[a]

	cytotoxicity[c]	antiviral activity (EC_{50})		cytotoxicity[d]	antiviral activity (EC_{50})		cytotoxicity[e]	antiviral activity (EC_{50})		
compd[b]	CC_{50}	HIV-1	HIV-2	CC_{50}	HSV-1	HSV-2	CC_{50}	EMCV	Cox B3	VSV
1	6.8	>6.8	>6.8	>100	>100	>100	>100	>100	>100	>100
1a	42.5	>42.5	>42.5	>100	86.1	94.5	51.5	>51.5	>51.5	>51.5
3	1.4	>1.4	>1.4	97.5	>97.5	>97.5	>100	>100	>100	>100
4	>100	>100	>100	79.9	>79.9	>79.9	>100	>100	>100	>100
6	39.7	>39.7	>39.7	>100	>100	>100	64.8	>64.8	>64.8	>64.8
7	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9	42.5	>42.5	>42.5	>100	>100	>100	59.9	>59.9	>59.9	>59.9
10	67.4	>67.4	>67.4	>100	81.1	82.3	58.8	>58.8	>58.8	>58.8
11	>100	>100	>100	>100	97.8	82.1	57.9	>57.9	>57.9	>57.9
heparin	>1000	0.9	>1000							
PS	>1000	0.9	2.3							
AZT	1.9	0.001	0.007							
ddC	11.0	0.1	0.1							
ddI	>100	2.1	11.0							
ACV				>10.0	0.8	2.4				
Ara-C				4.8	1.6	3.0				
Rib							300	27.2	236.4	13.7

[a] Data as expressed in μg/mL. Human immunodeficiency virus, herpes simplex virus, encephalomyocarditis virus, coxsackievirus B, and vesicular stomatitis virus were employed for antiviral assay. [b] PS: pentosan polysulfate; ddC: dideoxycytidine; ddI: dideoxyinosine; ACV: acyclovir; Rib: ribavirin. [c] MT-4 was employed as a host cell to HIV. MT-4: human T-cell transformed by co-cultivating with leukemia lymphocytes harboring human T-cell leukemia virus type 1 (HTLV-1). [d] Vero was employed as a host cell to HSV. [e] HeLa was employed as a host cell.

aqueous MeOH- (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), eluting with a step gradient solvent system of 33 to 0% H_2O−MeOH to obtain 13 fractions (1−13). Fraction 3 (0.98 g) was very active in the brine shrimp assay and was further purified by repeated HPLC (YMC ODS-H80, 250 × 20 mm i.d., 4 μm, 80 Å) using 60% MeOH−H_2O as the solvent system to give compounds **1** (30.2 mg) and **3** (7.8 mg). Fractions 4 (1.19 g) and 6 (0.84 g) were also very active in the brine shrimp test. Fraction 4 was subjected to a chromatotron (silica gel 60 PF_{254}), eluting with a solvent system of 17 to 100% MeOH−$CHCl_3$, to afford 60 fractions (1−60). The subfractions were repeatedly chromatographed on HPLC (C18-5E Shodex packed column, 250 × 10 mm i.d., 5 μm, 100 Å), eluting with 80% MeOH (containing 0.05% TFA) to yield compounds **4** (6.3 mg, from subfractions 45−60), **2** (1.0 mg) and **5** (1.0 mg, from subfractions 41−49), and **7** (3.8 mg), **8** (2.0 mg), **10** (4.0 mg), and **11** (4.1 mg, from subfractions 35−40). Fraction 6 was separated by MPLC (silica gel 60, 400/230 mesh) to afford 20 fractions. Subfractions 12−14 were combined and further purified to afford compounds **6** (2.8 mg) and **9** (36.5 mg).

Solvolysis of 1. A solution of **1** (2 mg) in a mixture of pyridine (0.5 mL) and dioxane (0.5 mL) was heated in a stoppered reaction vial. After 2 h, TLC analysis [ODS plate with MeOH−H_2O (3:1)] showed that the starting material had disappeared. The residue was evaporated to dryness and purified by HPLC (C18-5E Shodex packed column, 250 × 10 mm i.d., 5 μm, 100 Å) with MeOH−H_2O (80:20) as eluant.

Methanolysis of Saponins. Each solution of **4** (3.0 mg), **7** (0.7 mg), and **9** (2.0 mg) in 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 with MeOH−H_2O (9:1)] showed that the starting material had disappeared and a UV-active spot had appeared. 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 [Vydac column, C-18, 250 × 10 mm i.d., 5 μm, 90 Å, MeOH−H_2O (9:1)] to give **4a**, **7a**, and **9a**, respectively.

Acid Hydrolysis of Saponins. A solution of **9** (4.0 mg) in 9% HCl (1.0 mL) was heated at 80 °C in a stoppered reaction vial. After 2 h, TLC analysis [ODS with MeOH−H_2O (8:2)] showed that the starting material has disappeared. The hydrolysate was cooled, neutralized with Ag_2CO_3, and filtered. The filtrate was washed with EtOAc and then concentrated under vacuum. Examination of the residue by co-TLC [*n*-BuOH−HOAc−H_2O (4:1:5) and $CHCl_3$−MeOH−H_2O (8:5:1)] with standard sugars (xylose, arabinose, and ribose) indicated the presence of xylose.

Preparation of MTPA Esters. Compound **4a** (ca. 1 mg) was treated with (*R*)-(−)-and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (4 μL) in dry pyridine (25 μL) for 24 h at room temperature to afford (*S*)-(−)-MTPA ester and (*R*)-(+)-MTPA ester, respectively. The reaction was monitored by TLC (ODS, MeOH) and stopped when the starting material spot disappeared. After removal of solvent, the product was purified by reversed-phase HPLC on a Vydac column (C-18, 250 × 10 mm i.d., 5 μm, 90 Å) and analyzed by ^{1}H NMR. Only (*R*)-(+)-MTPA ester was prepared for **7a** and **9a**. Compounds **7a** (ca. 0.4 mg) and **9a** (ca. 1.0 mg) were treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (2 μL) in dry pyridine (25 μL) for 24 h at room temperature, respectively. The following procedure was the same as that for compound **4a**.

Certonardoside A (1): colorless needles; $[\alpha]^{21}_D$ −19.5° (*c* 0.41, MeOH); IR (KBr disk) ν_{max} 3434, 2942, 1643, 1377, 1254, 1227, 1166, 1058 cm^{-1}; ^{1}H NMR data, see Tables 1 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 883 $[M + Na]^+$ (100), 763 $[M + Na - SO_4Na - H]^+$ (2.2), 737 $[M + Na - C_6H_{11}O_4 + H]^+$ (0.8), 617 $[M + Na - SO_4Na - C_6H_{11}O_4]^+$ (0.1), 600 $[M + Na - SO_4Na - C_6H_{11}O_4 - OH]^+$ (0.1), 531 (0.2), 515 (0.08), 503 $[M + Na - C_6H_{11}O_4 - C_5H_7O_7SNa + H]^+$ (0.08); FABMS (−ve) *m/z* 837 $[M - Na]^-$ (100), 719 $[M - SO_4Na]^-$ (1.7), 691 $[M - C_6H_{11}O_4]^-$ (1.4), 673 (1.0); HRFABMS (+ve) *m/z* 883.3736 (calcd for $C_{39}H_{65}Na_2O_{17}S$, 883.3738); HRFABMS (−ve) *m/z* 837.3930 (calcd for $C_{39}H_{65}O_{17}S$, 837.3942).

Compound 1a: colorless needles; $[\alpha]^{21}_D$ −14.6° (*c* 0.14, MeOH); IR (KBr disk) ν_{max} 3419, 2940, 1641, 1418, 1374, 1165, 1051 cm^{-1}; ^{1}H NMR data, see Tables 1 and 4; ^{13}C NMR data, see Tables 2 and 5; FABMS (+ve) *m/z* 781 $[M + Na]^+$ (100), 693 (0.4), 663 (0.5), 635 $[M + Na - C_6H_{11}O_4 + H]^+$ (0.4), 617 $[M + Na - C_6H_{11}O_4 - OH]^+$ (0.5), 531 (0.4), 503 $[M + Na - C_6H_{11}O_4 - C_5H_8O_4 + H]^+$ (0.1); FABMS (−ve) *m/z* 757 $[M - H]^-$ (100), 611 $[M - C_6H_{11}O_4]^-$ (13), 479 $[M - C_6H_{11}O_4 - C_5H_8O_4]^-$ (7); HRFABMS (−ve) *m/z* 757.4368 (calcd for $C_{39}H_{65}O_{14}$, 757.4374).

Certonardoside B (2): colorless needles; ^{1}H NMR data, see Tables 1 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 851 $[M + Na]^+$ (100), 731 $[M + Na - SO_4Na - H]^+$ (1.9), 705 $[M + Na - C_6H_{11}O_4 + H]^+$ (1.1), 585 $[M + Na - SO_4Na - C_6H_{11}O_4]^+$ (0.5), 543 (1.1), 515 (1.7), 471 $[M + Na - C_6H_{11}O_4 - C_5H_7O_7SNa + H]^+$ (0.7).

Certonardoside C (3): light yellow needles; $[\alpha]^{21}_D$ −23.6° (*c* 0.13, MeOH); IR (KBr disk) ν_{max} 3434, 2938, 1641, 1376, 1249, 1224, 1166, 1062 cm^{-1}; ^{1}H NMR data, see Tables 1 and

4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 867 $[M + Na]^+$ (100), 747 $[M + Na - SO_4Na - H]^+$ (2.2), 721 $[M + Na - C_6H_{11}O_4 + H]^+$ (1.0), 601 $[M + Na - SO_4Na - C_6H_{11}O_4]^+$ (0.2), 583 (0.2), 515 (0.4), 487 $[M + Na - C_6H_{11}O_4 - C_5H_7O_7SNa + H]^+$ (0.1); FABMS (−ve) *m/z* 821 $[M - Na]^-$ (100), 703 $[M - SO_4Na]^-$ (2.8), 675 $[M - C_6H_{11}O_4]^-$ (3.6), 657 (1.7).

Certonardoside D (4): colorless needles; $[\alpha]^{21}_D$ −20.2° (*c* 0.60, MeOH); IR (KBr disk) ν_{max} 3434, 2943, 1641, 1379, 1256, 1227, 1165, 1057 cm^{-1}; ^{1}H NMR data, see Tables 1 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 883 $[M + Na]^+$ (100), 763 $[M + Na - SO_4Na - H]^+$ (6.0), 737 $[M + Na - C_6H_{11}O_4 + H]^+$ (2.0), 617 $[M + Na - SO_4Na - C_6H_{11}O_4]^+$ (0.5), 600 $[M + Na - SO_4Na - C_6H_{11}O_4 - OH]^+$ (0.3), 531 (1.0), 503 $[M + Na - C_6H_{11}O_4 - C_5H_7O_7SNa + H]^+$ (0.3); FABMS (-ve) *m/z* 837 $[M - Na]^-$ (100), 719 $[M - SO_4Na]^-$ (1.8), 691 $[M - C_6H_{11}O_4]^-$ (1.7), 673 (1.4).

Compound 4a: amorphous powder; ^{1}H NMR (CD_3OD), δ 5.35 (1H, br s, H-15), 5.30 (1H, dd, 15.5, 7.5, H-22), 5.25 (1H, dd, 15.5, 7.8, H-23), 4.26 (1H, br s, H-4), 4.17 (1H, td, 9.5, 4.0, H-6), 3.57 (1H, dd, 10.8, 5.3, H-26), 3.45 (1H, m, H-3), 3.27 (1H, dd, H-26, partially overlapped with other signals), 1.20 (3H, s, H_3-19), 1.03 (3H, d, 7.0, H_3-21), 0.94 (3H, d, 7.0, H_3-24^1), 0.88 (3H, d, 7.0, H_3-27), 0.83 (3H, s, H_3-18).

(*S*)-(−)-MTPA ester of compound 4a: ^{1}H NMR (CD_3OD), δ 4.39 (1H, dd, 10.8, 4.8, H-26), 4.09 (1H, dd, 10.8, 6.5, H-26), 1.28 (3H, s, H_3-19), 1.02 (3H, d, 6.0, H_3-21), 0.95 (3H, d, 7.0, H_3-24^1), 0.91 (3H, d, 7.0, H_3-27), 0.84 (3H, s, H_3-18).

(*R*)-(+)-MTPA ester of compound 4a: ^{1}H NMR (CD_3OD), δ 4.28 (1H, dd, 11.3, 4.8, H-26), 4.17 (1H, dd, 11.3, 6.3, H-26), 1.29 (3H, s, H_3-19), 1.01 (3H, d, 6.0, H_3-21), 0.94 (3H, d, 6.5, H_3-24^1), 0.93 (3H, d, 6.5, H_3-27), 0.85 (3H, s, H_3-18).

Certonardoside E (5): light yellow needles; ^{1}H NMR data, see Tables 1 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 867 $[M + Na]^+$ (100), 747 $[M + Na - SO_4Na - H]^+$ (1.6), 721 $[M + Na - C_6H_{11}O_4 + H]^+$ (0.8), 601 $[M + Na - SO_4Na - C_6H_{11}O_4]^+$ (0.2), 583 (0.7), 515 (0.8).

Certonardoside F (6): colorless needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 783 $[M + Na]^+$ (100), 651 (2.4), 605 $[M + Na - C_7H_{13}O_5 - H]^+$ (0.8), 519 (1.6), 473 $[M + Na - C_7H_{13}O_5 - C_5H_8O_4 - H]^+$ (2.6).

Certonardoside G (7): colorless needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 885 $[M + Na]^+$ (100), 765 $[M + Na - SO_4Na - H]^+$ (28.0), 575 $[M + Na - C_7H_{13}O_5 - C_5H_8O_4 - H]^+$ (2.6), 491 (2.6), 407 (4.5).

Compound 7a: amorphous powder; ^{1}H NMR (CD_3OD), δ 5.37 (1H, br s, H-15), 4.26 (1H, br s, H-4), 4.17 (1H, td, 9.5, 4.0, H-6), 3.45 (1H, m, H-3), 3.23 (1H, m, H-24), 1.20 (3H, s, H_3-19), 0.97 (3H, d, 6.5, H_3-21), 0.91 (3H, d, 7.0, H_3-26), 0.89 (3H, d, 7.0, H_3-27), 0.82 (3H, s, H_3-18).

(*R*)-(+)-MTPA ester of Complound 7a: ^{1}H NMR (CD_3OD), δ 0.96 (3H, d, 6.0, H_3-21), 0.87 (3H, d, 6.0, H_3-26 or H_3-27), 0.84 (3H, d, 7.0, H_3-26 or H_3-27).

Certonardoside H (8): colorless needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 869 $[M + Na]^+$ (100), 749 $[M + Na - SO_4Na - H]^+$ (24.2), 559 $[M + Na - C_7H_{13}O_5 - C_5H_8O_4 - H]^+$ (3.1), 545 (2.3), 391 (4.5).

Certonardoside I (9): colorless needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 767 $[M + Na]^+$ (100), 635 (3.1), 589 $[M + Na - C_7H_{13}O_5 - H]^+$ (1.2), 503 (2.0), 457 $[M + Na - C_7H_{13}O_5 - C_5H_8O_4 - H]^+$ (3.3).

Compound 9a: amorphous powder; ^{1}H NMR (CD_3OD), δ 5.21 (1H, br s, H-15), 4.24 (1H, br s, H-4), 3.92 (1H, td, 11.0, 4.8, H-6), 3.45 (1H, m, H-3), 3.24 (1H, m, H-24), 1.08 (3H, s, H_3-19), 0.97 (3H, d, 6.5, H_3-21), 0.94 (3H, s, H_3-18), 0.93 (3H, d, 7.0, H_3-26), 0.91 (3H, d, 7.0, H_3-27), 0.65 (1H, td, 11.0, 2.5, H-9).

(*R*)-(+)-MTPA ester of compound 9a: ^{1}H NMR (CD_3OD), δ 0.95 (3H, d, 6.0, H_3-21), 0.86 (3H, d, 6.5, H_3-26 or H_3-27), 0.82 (3H, d, 7.0, H_3-26 or H_3-27).

Certonardoside J (10): light yellow needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 783 $[M + Na]^+$ (100), 663 $[M + Na - SO_4Na - H]^+$ (22.1), 635 $[M + Na - C_6H_{11}O_4 - H]^+$ (1.7), 507 (1.4), 407 (4.5).

Certonardoside K (11): light yellow needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 5; FABMS (+ve) *m/z* 767 $[M + Na]^+$ (100), 647 $[M + Na - SO_4Na - H]^+$ (22.1), 619 $[M + Na - C_6H_{11}O_4 - H]^+$ (1.2), 491 (1.0), 491 (3.2).

Evaluation of Anti-HIV Activity. Standard virus-induced cytopathic effect (CPE) inhibition assay was used.[19] MT-4 cells on log phase were pelleted and infected with virus at an MOI (multiplicity of infection) of 100 $CCID_{50}$ (50% cell culture inhibitory dose) per well. For mock infection, no virus was added. The cells were immediately resuspended to a concentration of 10^6 cells/mL with RPMI 1640/10% FBS. Assays were performed in 96-well microtiter plates. To each well, 100 μL of 2 × concentrated test samples and 100 μL of the cells were added. After 5 days incubation at 37 °C , the cells were observed microscopically and quantified by MTT assay. The liquid was aspirated until 50 μL of the liquid and all cells remained, then 20 μL of 7.5 mg/mL of MTT solution was added. The plates were further incubated for 1 h, and 100 μL of the acidified 2-propanol was added and shaken on a microplate shaker until the reduced MTT form completely dissolved. The absorbances were read in a computer-controlled microplate reader at 540 and 690 nm, as main and reference, respectively. From the absorbance values of the virus-infected wells, the effective antiviral concentration was calculated and expressed as the EC_{50} or concentration of the compound achieving 50% protection. With the absorbance values of the mock-infected wells, the concentration of the compound responsible for 50% reduction of cell viability (CC_{50}) was calculated.

Evaluation of Anti-HSV-1 and Anti-HSV-2 Activity. Standard virus-induced CPE inhibition assay was used. After removal of the culture medium, confluently grown Vero cells in 96-well plates were infected with each virus at an MOI of 100 $CCID_{50}$ per well. After 1 h adsorption at 37 °C, the liquid was aspirated off and 100 μL of DMEM/2% FBS containing a compound was applied to each well in duplicate for each concentration. Mock-infected cells were also prepared simultaneously. After 3 days incubation at 37 °C, the antiviral activity was measured by MTT assay. The EC_{50} values and the CC_{50} values expressing cytocidal effects were calculated.

Evaluation of Anti-CoxB-3, Anti-EMCV, and Anti-VSV Activity. The CPE inhibition assay was used for the evaluation. The procedures were similar to those for anti-HSV evaluation, except that HeLa cells were used. Virus adsorption time was 30 min, and the cells were incubated for 2 days instead of 3 days.

Cells. Vero (African green monkey kidney) cells and HeLa (human cervical carcinoma) cells were grown in Dulbecco's modified Eagle medium (DMEM) (GIBCO) with 5% heat-inactivated fetal bovine serum (FBS) (GIBCO) and 4 μg/mL of gentamycin (Gm) (Sigma). MT-4 cells (HTLV-1-transformed human T cells) were kindly provided by N. Yamamoto at Tokyo Medical and Dental University, Japan, and grown in RPMI 1640 medium with 10% FBS and 4 μg/mL of Gm. H9/NIH (human cutaneous T-cell lymphoma) cells were kindly provided by National Institute for Biological Standards and Control (NIBSC), UK, according to MRC AIDS reagent program (the original donor was Dr. R. Gallo according to the NIH NIAID Research and Reference Reagent Program).

Viruses. The following viruses were purchased from American Type Culture Collection, Rockville, MD: herpes simplex virus type 1 (HSV-1) strain F and type 2 (HSV-2) strain MS, coxsackievirus B type 3 virus (CoxB-3) strain Nancy, encephalomyocarditis virus (EMCV) strain EMC, and vesicular stomatitis virus (VSV) strain Indiana. Both human immunodeficiency virus type 1 (HIV-1) strain IIIB and type 2 (HIV-2) strain ROD (the original donor was L. Montagnier at Pasteur Institute, Paris, France) were kindly provided by NIBSC, UK, according to MRC AIDS reagent program. The stocks of HSV-1

and HSV-2 strains were prepared in Vero cells, CoxB-3, EMCV, and VSV in HeLa cells, and HIV-1 and HIV-2 in H9 cells.

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