Cytotoxic Sterols and Saponins from the Starfish *Certonardoa semiregularis*

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Eleven new polyhydroxysterols (**1**−**11**) and eight new saponins (**12**−**19**) were isolated from the brine shrimp active fraction of the starfish *Certonardoa semiregularis*. The structures were determined on the basis of spectral analysis and chemical derivatization. The structural variations of these compounds are due to the hydroxylation pattern of the sterol nucleus, the functionalization of the side chain, or the nature and location of the saccharide moiety. These compounds displayed considerable cytotoxicity against a small panel of human solid tumor cell lines.

Starfish are known to be a rich source of structurally unique and biologically active steroids, many of which have no counterpart within the entire animal kingdom. These steroids are always highly oxygenated and often occur as complex mixtures that are very difficult to separate into individual components.

Previous studies on the starfish *Certonardoa semiregularis* Muller & Troschel (family Linckiidae) collected from Korean waters have yielded 31 polar steroids.[1−3] In our continuing search for new bioactive metabolites from the same starfish, we obtained 11 new polyhydroxysterols (**1**−**11**) as well as eight new glycosides of polyhydroxysterols (**12**−**19**). Among these compounds, certonardosterol D_2 (**1**) was found to be most cytotoxic to the target human cancer cells with ED_{50} of 0.01−0.15 μg/mL, which was comparable to that of doxorubicin. The planar structures of these compounds were elucidated with the aid of COSY, HSQC, and HMBC experiments. The absolute configurations of the side chains were defined by the ^{1}H NMR analysis of the MTPA esters.

Results and Discussion

Certonardosterol D_2 (**1**) was isolated as colorless needles. The FABMS gave a pseudomolecular ion peak at *m*/*z* 471 $[M + Na]^+$. Analysis of the ^{1}H NMR data revealed the presence of a 3β,6α,15β-trihydroxy sterol nucleus.[2] In addition, the two doublets of doublets resonating at δ 5.30 (J = 15.5, 8.5 Hz) and 5.19 (J = 15.5, 9.5 Hz) suggested a *trans* double bond at C-22. In an HMBC experiment, the oxymethylene proton signals at δ 3.54 and 3.48 showed correlations to one of the olefinic carbon signals (δ 127.7) and two methine carbon signals (δ 52.8 and 29.1), and these two methine carbon signals showed further correlations to two methyl proton signals at δ 0.90 and 0.83. These data indicated that the oxymethylene protons are located at C-24^1. The common ring junctions (H-5α, H-8β, H-9α, CH_3-18β, CH_3-19β, and H-14α) and H-17α of sterols were assumed by comparison of the NMR data with those of **11** (vide infra). The *trans* A/B and C/D ring fusions were also supported by the upfield chemical shift of the C-19 and C-18 signals at δ 13.8 and 15.3, respectively.[4] The configuration at C-24 can be established by analysis of the ^{1}H NMR data of its (*R*)-MTPA ester. The (*R*)-MTPA ester of the 24*S* synthetic model compound was reported to show an upfield shift of the H-21 signal over 0.1 ppm from that of the underivatized sterol, while the change is insignificant for that of the 24*R* isomer.[5] The (*R*)-MTPA esters of 24*S* and 24*R* model compounds also showed significant differences in the shape and chemical shift of the H-24^1 signal. The (*R*)-MTPA ester of the 24*S* synthetic model compound showed the H-24^1 signals as two separate doublets of doublets (δ 4.39 and 4.25), while that of the 24*R* isomer was observed as a doublet (δ 4.34).[5] In addition, the signals of H-26 and H-27 of the (*R*)-MTPA ester of the 24*S* synthetic model compound were more separated ($\Delta\ \delta_H$ = 0.07 ppm) than those of the 24*R* isomer ($\Delta\ \delta_H$ = 0.04 ppm).[5] In the ^{1}H NMR spectrum of the (*R*)-MTPA ester of **1**, the signal of H-21 was observed at δ 1.01 (upfield shifted by 0.03 ppm from that of **1**), the signal of H-24^1 appeared as a broad doublet at δ 4.30, and the $\Delta\ \delta_H$ between H-26 and H-27 was 0.04 ppm. Accordingly, the 24*R* configuration was assigned and the structure of certonardosterol D_2 (**1**) was established as (*E*)-(24*R*)-24-methyl-5α-cholest-22-ene-3β,6α,15β,24^1-tetrol.

Certonardosterol D_3 (**2**) was isolated as colorless needles. The molecular formula of **2** was established as $C_{29}H_{52}O_4$ on the basis of the pseudomolecular ion peak at *m*/*z* 487.3762 $[M + Na]^+$ (calcd for $C_{29}H_{52}O_4Na$, 487.3763). The ^{1}H NMR data showed that **2** shares the same sterol nucleus as **1**. In addition to the signals attributable to the sterol nucleus, an obvious oxymethylene proton signal was observed at δ 3.55 (δ_C 62.0). The location of the hydroxyl group at C-24^2 was established by an HMBC experiment in which the proton signals at δ 1.58 and 1.33 (H-24^1) showed correlations with the carbon signals at δ 62.0 (C-24^2), 41.9 (C-24), 30.5 (C-25), and 28.3 (C-23). The 24*R* configuration was assigned on the basis of comparison of the ^{1}H and ^{13}C NMR data with those of 24^2-hydroxylated model compounds.[6] The $\Delta\ \delta_H$ of the isopropyl methyl proton signals of (24*R*)-24^2-hydroxy steroids was reported to be 0.03−0.06 ppm, while that of the 24*S* isomer was always less than 0.03 ppm. Likewise, the $\Delta\ \delta_C$ of the isopropyl methyl carbon signals of (24*R*)-24^2-hydroxy steroids were reported to be 1.1−1.4 ppm, while that of the 24*S* isomer was 0.1−0.4 ppm. The $\Delta\ \delta$ of the isopropyl methyl proton and carbon signals of **2** were 0.03 and 1.1 ppm, respectively, which were close to those of the 24*R* isomer.[6] Thus, the

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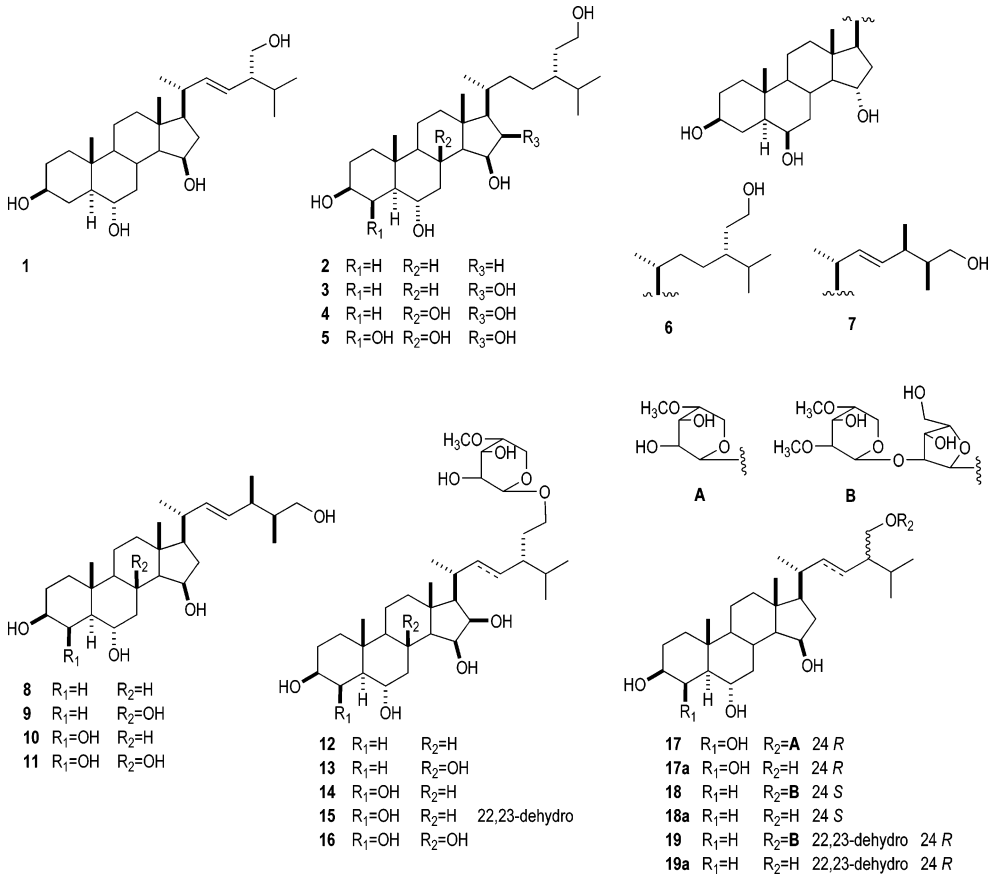
Chart 1



structure of certonardosterol D_3 (**2**) was defined as (24*R*)-24-ethyl-5α-cholestane-3*β*,6α,15*β*,24²-tetrol.

The ^{1}H NMR data of certonardosterols N_1−P_1 and E_2 (**3**−**6**) demonstrated that they share the same 24²-hydroxy-24-ethylcholestane side chain as **2**. Certonardosterol N_1 (**3**) was isolated as colorless needles and showed a pseudomolecular ion peak at *m*/*z* 503.3698 $[M + Na]^+$ (calcd for $C_{29}H_{52}O_5Na$, 503.3712) in the HRFABMS. Examination of its ^{1}H NMR data revealed the presence of two coupled broad triplets at *δ* 4.24 and 4.10 instead of the triplet of doublets at *δ* 4.16 assigned to H-15α in **2**. By the aid of ^{1}H−^{1}H COSY, HSQC, and HMBC experiments, these two signals could be assigned to H-16α and H-15α, respectively. As in the case of **2**, the 24*R* stereochemistry of **3** was proposed by comparison of its NMR data with those of the model compounds.[6] Thus, the structure of certonardosterol N_1 (**3**) was established as (24*R*)-24-ethyl-5α-cholestane-3*β*,6α,15*β*,16*β*,24²-pentol.

Certonardosterol O_1 (**4**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 519.3663 $[M + Na]^+$ (calcd for $C_{29}H_{52}O_6Na$, 519.3662). It was an 8-oxy derivative of **3**, as determined by comparison of the ^{1}H and ^{13}C NMR data with those of **3**. Compared to compound **3**, the introduction of the 8-hydroxyl group caused significant downfield shifts of the H-11, -15, -18, and -19 signals. In the ^{13}C NMR spectrum, the upfield shift of the C-11 signal and the downfield shifts of the C-15 and C-18 signals were observed. This pattern of shifts might be a result of a combination of the substituent effect and the 1,3-diaxial steric effect.[7]

Certonardosterol P_1 (**5**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 535.3616 $[M + Na]^+$ (calcd for $C_{29}H_{52}O_7Na$, 535.3611). Comparison of the ^{1}H NMR data with those of **4** revealed the presence of a broad singlet at *δ* 4.25 (H-4) and downfield shifts of the signals of H-6*β* (*δ* 3.71 → 4.18) and H-19 (*δ* 0.98 → 1.15), which indicated that **5** is a 4-oxy derivative of **4**.

Certonardosterol E_2 (**6**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 487.3756 $[M + Na]^+$ (calcd for $C_{29}H_{52}O_4Na$, 487.3763). The ^{1}H NMR signals, especially two methyl singlets at *δ* 0.74 (H-18) and 1.03 (H-19), a quartet at *δ* 3.74 (H-6, J = 2.8 Hz), and a triplet of doublets at *δ* 3.87 (H-15, J = 9.3, 3.3 Hz), and the carbon chemical shifts of C-6 (*δ* 72.5), C-15 (*δ* 74.2), C-18 (*δ* 13.8), and C-19 (*δ* 16.3) were reminiscent of the 3*β*,6*β*,15α-trihydroxy sterol nucleus.[2,8,9] Although more than 100 polyhydroxysterols and more than 150 glycosides of polyhydroxysterols have been previously reported from about 80 species of starfish, the 3*β*,6*β*,15α-trihydroxylation pattern is uncommon and only encountered in granulatoside B,[8] solasteroside S_1,[9] and certonardosterol E_1.[2]

Certonardosterol E_3 (**7**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 471.3461 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_4Na$, 471.3450). Comparison of its NMR data with those of **6** indicated that they share the same sterol nucleus. The ^{1}H NMR spectrum of **7** showed two doublets of doublets at *δ* 5.25 and 5.20, which could be assigned to the Δ^{22E} protons (J = 15 Hz). In the methyl region of the ^{1}H NMR spectrum, three doublets were observed. The doublet at *δ* 1.00 was assigned to H-21. The other two doublets at *δ* 0.92 and 0.87 showed correlations with the carbon signals at *δ* 42.1 and 39.6, respectively, and the latter showed correlation with the oxymethine carbon at *δ* 66.6. These implied the presence of the 26-hydroxy-24-methylcholest-22-ene side chain. The 24*R*,25*S* configuration was assumed by analogy with the co-occurring sterol **11** (vide infra). Thus, the structure of **7**

was established as (*E*)-(24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3*β*,6*β*,15α,26-tetrol.

Comparison of the ^{1}H NMR spectra of **8**−**11** with that of **7** revealed that they share the same 26-hydroxy-24-methylcholest-22-ene side chain. Certonardosterol D_4 (**8**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 471.3458 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_4Na$, 471.3450). The NMR data showed that **8** shares the same sterol nucleus as **2**. Thus, the structure of **8** was defined as (*E*)-(24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3*β*,6α,15*β*,26-tetrol.

Certonardosterol C_2 (**9**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 487.3388 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_5Na$, 487.3399). Analysis of the NMR data indicated that it is an 8-oxy derivative of **8**. The downfield shifts of H-11, -15, -18, and -19 were observed due to the introduction of the 8-hydroxyl group.

Certonardosterol B_2 (**10**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 487.3389 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_5Na$, 487.3399). It was postulated as a 4-oxy derivative of **8**. Comparison of the ^{1}H NMR spectrum with that of **8** revealed the presence of a broad singlet at *δ* 4.21 (H-4), which was coupled with the H-3 signal (*δ* 3.42) in the ^{1}H−^{1}H COSY spectrum. The downfield shifts of the signals of H-6*β* (*δ* 3.38 → 3.89) and H-19 (*δ* 0.87 → 1.06) were also observed.

Certonardosterol A_2 (**11**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 503.3337 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_6Na$, 503.3349). Analysis of its NMR data indicated that it is a 4,8-dioxy derivative of **8**. The upfield chemical shifts of the C-18 and C-19 signals (*δ* 16.6 and 17.0, respectively) indicated the *trans* C/D and A/B ring fusions, which was further supported by the NOE data. The 1,3-diaxial NOE correlations of H-9/H-5, H-14/H-7α (*δ* 1.29), and H-14/H-12α (*δ* 1.17) were observed. The NOE correlations of H-17 (*δ* 1.01)/H-12α (*δ* 1.17) and H-18 (*δ* 1.27)/H-20 (*δ* 2.13) were indicative of an α-oriented H-17, which was consistent with the coupling constant between H-16 and H-17 (*J* = 10.5, 7.8 Hz).[10] The NMR data of H-26 (*δ* 3.56, 3.27), H-27 (*δ* 0.87), and H-24^1 (*δ* 0.92) were indicative of a *threo* configuration at C-24 and C-25.[11] An *erythro* analogue would have shown the corresponding signals at *δ* 3.53, 3.34, 0.87, and 1.02, respectively.[11] The absolute configuration was established by analysis of the ^{1}H NMR data of its MTPA derivatives. It was reported that for the (*S*)-MTPA esters of the *threo* pair [(24*R*,25*S*) and (24*S*,25*R*)], the H-26 signals of the 25*R* isomer display smaller $\Delta\,\delta_H$ than those of the 25*S* isomer.[11] Such behavior is reversed in the (*R*)-MTPA esters, and the H-26 signals of the 25*S* isomer display smaller $\Delta\,\delta_H$. The H-26 signals of the (*S*)-MTPA ester of **11** appeared as two well-separated doublets of doublets at *δ* 4.37 and 4.10, while those of the (*R*)-MTPA ester appeared as two close doublets of doublets at *δ* 4.26 and 4.18. Accordingly, the 24*R*,25*S* configuration was assigned to **11**, and the structure was established as (*E*)-(24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3*β*,4*β*,6α,8,15*β*,26-hexol.

Certonardoside O_1 (**12**) was isolated as colorless needles. The molecular formula of **12** was deduced to be $C_{35}H_{62}O_9$ on the basis of the pseudomolecular ion peak at *m*/*z* 649.4289 $[M + Na]^+$ (Δ −0.3 mmu). The fragment ion peak at *m*/*z* 501 $[M + Na - C_6H_{11}O_4 - H]^+$ corresponded to the loss of a methoxylated pentose. Analysis of the ^{1}H NMR data indicated that the aglycon moiety of **12** corresponds to certonardosterol N_1 (**3**). In addition, a set of signals attributable to the sugar moiety were observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum showed a methoxy carbon signal at *δ* 59.0, one methylene carbon signal at *δ* 64.3, and four methine carbon signals at *δ* 105.1, 80.9, 77.0, and 75.0. Comparison of the above data with those of xylopyranoside suggested the presence of a 4-*O*-methyl-*β*-D-xylopyranosyl moiety.[1] The location of the methoxy group at C-4′ of the xylopyranose was confirmed by the reciprocal HMBC correlations between the methoxy group and C-4′. The chemical shift of the anomeric carbon (*δ* 105.1) and the coupling constant of the anomeric proton (*δ* 4.18, *J* = 7.0 Hz) suggested that the sugar has a *β*-configuration. The location of the sugar residue was established on the basis of the long-range correlation between C-24^2 and H-1′. As in the case of **2**, the 24*R* stereochemistry of **12** was proposed by comparison of its NMR data with those of the model compounds.[6] Thus, the structure of certonardoside O_1 (**12**) was defined as (24*R*)-24^2-*O*-*β*-(4-*O*-methyl-D-xylopyranosyl)-24-ethyl-5α-cholestane-3*β*,6α,15*β*,16*β*,24^2-pentol.

The NMR data indicated that compounds **12**−**17** possess identical sugar moieties. Certonardoside P_1 (**13**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 665.4246 $[M + Na]^+$ (calcd for $C_{35}H_{62}O_{10}Na$, 665.4241). Comparison of the ^{1}H and ^{13}C NMR data of **13** with those of **4** and **12** indicated that its aglycon corresponds to **4** and it is an 8-oxy derivative of **12**.

Certonardoside J_2 (**14**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 665.4245 $[M + Na]^+$ (calcd for $C_{35}H_{62}O_{10}Na$, 665.4241). The NMR data indicated that **14** is a 4-oxy derivative of **12**.

Certonardoside J_3 (**15**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 663.4092 $[M + Na]^+$ (calcd for $C_{35}H_{60}O_{10}Na$, 663.4084). Comparison of its NMR data with those of **14** indicated that they share the same sterol nucleus. The ^{1}H NMR spectrum of **15** showed two doublets of doublets at *δ* 5.56 and 5.21, which could be assigned to the Δ^{22E} protons (*J* = 15 Hz). A 24*R* configuration for **15** was assumed by analogy with Δ^{22E},(24*R*)-24-hydroxyethyl steroids occurring in starfish.[12,13] This was supported by the chemical shift difference between the H-26 and H-27 methyl signals ($\Delta\,\delta$ = 0.05 ppm), which matches well with that reported for the Δ^{22E},(24*R*)-24-hydroxyethyl steroids but differs from that of the 24*S* isomer ($\Delta\,\delta$ = 0.02 ppm).[6] Thus, the structure of certonardoside J_3 (**15**) was defined as (*E*)-(24*R*)-24^2-*O*-*β*-(4-*O*-methyl-D-xylopyranosyl)-24-ethyl-5α-cholest-22-ene-3*β*,4*β*,6α,15*β*,16*β*,24^2-hexol.

Certonardoside I_2 (**16**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 681.4185 $[M + Na]^+$ (calcd for $C_{35}H_{62}O_{11}Na$, 681.4190). The NMR data showed that **16** is a 4,8-dioxy derivative of **12** and its aglycon corresponds to **5**.

Certonardoside H_2 (**17**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 635.4130 $[M + Na]^+$ (calcd for $C_{34}H_{60}O_9Na$, 635.4135). The NMR data showed that **17** shares the same sterol nucleus with **10**. The NMR data of the side chain characterized three methyl groups and an oxymethylene group. The doublet at *δ* 0.93 was assigned to H-21. The other two doublets at *δ* 0.89 and 0.87 showed correlations to the methine carbon signals at *δ* 45.6 and 29.2, respectively, and these two carbon signals showed further correlations to the oxymethylene protons at *δ* 3.75 and 3.43. These implied that the oxymethylene protons are located at C-24^1. The glycosidic linkage was defined on the basis of the long-range correlation between C-1′ and H-24^1. The 24*R* configuration was assigned by comparison of the ^{1}H NMR data

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

position	1	2	3	4	5	6
1	1.71 (dt, 13.0, 3.3)	1.71 (m)	1.72 (m)	1.71 (dt, 12.5, 3.5)	1.70 (dt, 12.5, 2.5)	1.64 (dt, 13.5, 3.8)
	1.03 (m)	1.03 (m)	1.01 (m)	0.97 (m)	0.98 (m)	0.97 (m)
2	1.75 (m)	1.75 (m)	1.76 (m)	1.72 (m)	1.82 (qd, 13.0, 3.5)	1.73 (m)
	1.42 (m)	1.42 (m)	1.42 (m)	1.47 (m)	1.55 (m)	1.42 (m)
3	3.47 (m)	3.47 (m)	3.47 (m)	3.48 (m)	3.42 (ddd, 12.0, 5.0, 3.5)	3.53 (m)
4	2.18 (m)	2.18 (dt, 12.5, 2.5)	2.19 (dt, 12.5, 2.0)	2.19 (dt, 11.7, 2.3)	4.25 (br s)	1.74 (m)
	1.17 (m)	1.16 (m)	1.17 (m)	1.21 (m)		1.55 (m)
5	1.01 (m)	1.01 (m)	1.01 (m)	1.04 (m)	0.94 (m)	1.13 (dt, 13.5, 2.3)
6	3.38 (td, 10.8, 4.5)	3.38 (td, 10.8, 5.0)	3.39 (td, 10.5, 4.8)	3.71 (td, 11.0, 4.5)	4.18 (td, 11.0, 4.3)	3.74 (q, 2.8)
7	2.27 (m)	2.27 (dt, 12.5, 3.8)	2.32 (dt, 12.0, 4.0)	2.39 (dd, 12.0, 4.3)	2.46 (dd, 12.5, 4.0)	2.12 (dt, 14.0, 3.3)
	0.91 (m)	0.90 (m)	0.94 (m)	1.33 (m)	1.35 (m)	1.31 (m)
8	1.88 (m)	1.87 (m)	1.89 (m)			1.94 (m)
9	0.72 (td, 11.5, 3.5)	0.71 (td, 11.3, 3.5)	0.72 (td, 10.5, 3.0)	0.84 (dd, 11.0, 3.5)	0.82 (dd, 11.0, 3.0)	0.71 (td, 11.0, 3.3)
11	1.53 (m)	1.52 (m)	1.48 (m)	1.77 (m)	1.77 (m)	1.52 (m)
	1.33 (m)	1.33 (m)	1.30 (m)	1.47 (m)	1.42 (m)	1.37 (m)
12	1.94 (m)	1.95 (dt, 12.5, 3.0)	1.92 (m)	1.95 (dt, 12.5, 3.3)	1.93 (dt, 13.0, 3.5)	1.96 (m)
	1.15 (m)	1.11 (m)	1.05 (m)	1.13 (m)	1.11 (m)	1.24 (m)
14	0.92 (m)	0.89 (m)	0.88 (m)	1.02 (m)	1.01 (m)	1.06 (t, 9.3)
15	4.12 (td, 6.5, 2.0)	4.16 (td, 6.8, 2.0)	4.10 (br t, 6.5)	4.36 (dd, 7.0, 5.5)	4.37 (dd, 7.0, 5.5)	3.87 (td, 9.3, 3.3)
16	2.24 (m)	2.39 (dt, 14.5, 8.3)	4.24 (br t, 7.3)	4.21 (t, 7.0)	4.21 (t, 7.0)	1.87 (m)
	1.34 (m)	1.35 (m)				1.70 (m)
17	1.12 (m)	1.07 (m)	1.03 (m)	0.96 (m)	0.95 (m)	1.41 (m)
18	0.95 (s)	0.93 (s)	0.94 (s)	1.24 (s)	1.23 (s)	0.74 (s)
19	0.86 (s)	0.86 (s)	0.86 (s)	0.98 (s)	1.15 (s)	1.03 (s)
20	2.18 (m)	1.49 (m)	1.89 (m)	1.90 (m)	1.90 (m)	1.35 (m)
21	1.04 (d, 6.5)	0.95 (d, 7.0)	0.95 (d, 7.0)	0.95 (d, 6.5)	0.94 (d, 6.5)	0.94 (d, 5.5)
22	5.30 (dd, 15.5, 8.5)	1.41 (m)	1.68 (m)	1.69 (m)	1.68 (m)	1.38 (m)
		1.02 (m)	1.10 (m)	1.11 (m)	1.11 (m)	1.03 (m)
23	5.19 (dd, 15.5, 9.5)	1.38 (m)	1.41 (m)	1.41 (m)	1.41 (m)	1.37 (m)
		1.11 (m)	1.15 (m)	1.15 (m)	1.16 (m)	1.08 (m)
24	1.92 (m)	1.18 (m)	1.23 (m)	1.22 (m)	1.22 (m)	1.17 (m)
25	1.79 (m)	1.71 (m)	1.76 (m)	1.77 (m)	1.76 (m)	1.70 (m)
26	0.83 (d, 7.0)	0.84 (d, 7.0)	0.84 (d, 7.0)	0.84 (d, 7.0)	0.84 (d, 7.0)	0.84 (d, 7.0)
27	0.90 (d, 6.5)	0.87 (d, 7.0)	0.88 (d, 7.0)	0.88 (d, 7.0)	0.88 (d, 7.0)	0.87 (d, 7.0)
24^1	3.54 (dd, 10.8, 6.3)	1.58 (m)	1.58 (m)	1.58 (m)	1.58 (m)	1.57 (m)
	3.48 (dd, 10.8, 6.8)	1.33 (m)	1.34 (m)	1.35 (m)	1.35 (m)	1.32 (m)
24^2		3.55 (m)	3.55 (m)	3.57 (m)	3.57 (m)	3.55 (m)

[a] Multiplicities and coupling constants are in parentheses.

of 24-oxymethylene sterol **17a**, derived from **17** on acid treatment with anhydrous 4.5% HCl in MeOH, with those of the synthetic (24*R*)- and (24*S*)-3α,5-cyclo-6*β*-methoxy-24-methyl-5α-cholestane-24^1-ol.[5] The pattern of the H-24^1 signal (a 2H broad doublet at *δ* 3.48) of **17a** was similar to that of the synthetic 24*R* isomer (a 2H broad doublet at *δ* 3.52) but was distinct from those of the 24*S* isomer (two doublets of doublets at *δ* 3.47 and 3.56). Small but significant differences of the H-26 and H-27 proton signals were also reported (24*S* isomer: *δ* 0.92 and 0.93, Δ *δ* = 0.01 ppm; 24*R* isomer: *δ* 0.91 and 0.94, Δ *δ* = 0.03 ppm). The Δ *δ* of the isopropyl methyl proton signals of **17a** (*δ* 0.90 and 0.87) was 0.03 ppm, which was close to that of the 24*R* isomer.[5] Thus, the structure of certonardoside H_2 (**17**) was defined as (24*R*)-24^1-*O*-*β*-(4-*O*-methyl-D-xylopyranosyl)-24-methyl-5α-cholestane-3*β*,4*β*,6α,15*β*,24^1-pentol.

Certonardoside B_2 (**18**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 765.4766 [M + Na]$^+$ (calcd for $C_{40}H_{70}O_{12}Na$, 765.4765). Analysis of the ^{1}H NMR data revealed the presence of a 3*β*,6α,15*β*-trihydroxy sterol nucleus and a 24^1-hydroxylated side chain. In addition to the signals attributable to the aglycon, the ^{1}H NMR spectrum showed characteristic signals of the 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl unit, which were frequently encountered in the saponins from starfish.[14,15] The cross-peaks in the HMBC experiment between C-2′ and H-1″ and between C-24^1 and H-1′ indicated that the disaccharide moiety 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-α-L-arabinofuranose is linked to the aglycon at C-24^1. Methanolysis (4.5% HCl in MeOH) of **18** gave 24-methyl-5α-cholestane-3*β*,6α,-15*β*,24^1-tetrol (**18a**) as a major product and 24-methyl-5α-cholest-14-ene-3*β*,6α,24^1-triol as a minor product. In the ^{1}H NMR spectrum of **18a**, the signals of H-24^1 were observed at *δ* 3.55 and 3.46 and the isopropyl methyl proton signals were observed at *δ* 0.89 and 0.88 (Δ *δ* = 0.01 ppm), and accordingly the 24*S* configuration was assigned.[5] Thus, the structure of certonardoside B_2 (**18**) was established as (24*S*)-24^1-*O*-*β*-[2,4-di-*O*-methyl-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3*β*,6α,15*β*,24^1-tetrol. It is noteworthy that the stereochemistry at C-24 in **18** is opposite of that of **17**. The biosynthetic experiments on marine sterols suggest that the C-24 methyl cholesterols are transformed from C-24 methylene cholesterols, which makes understandable the diversity of the stereochemistry at C-24.[16,17]

Certonardoside B_3 (**19**) was isolated as colorless needles. It is the Δ^{22E} analogue of certonardoside B_2 (**18**). The planar structure was derived from HRFABMS (*m*/*z* 763.4598 [M + Na]$^+$) and NMR data. As in the case of **1**, the configuration of C-24 was defined by analysis of the (*R*)-MTPA ester of the aglycon. Methanolysis of **19** gave (*E*)-24-methyl-5α-cholest-22-ene-3*β*,6α,15*β*,24^1-tetrol (**19a**) as a major product, 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 the H-24^1 signal as a broad doublet at *δ* 4.30 and the H-21 signal as a doublet at *δ* 1.00 (shifted upfield by 0.03 ppm compared to that of **19a**), and the Δ δ_H of the isopropyl methyl proton signals was 0.04 ppm, which matched well with those of the (*R*)-MTPA ester of the (24*R*)-24^1-hydroxy

Table 2. ^{1}H NMR Data of Compounds **7**−**9** and **11** and the Aglycons of Compounds **14** and **15** (CD_3OD, 500 MHz)[a]

position	7	8	9	11	14	15
1	1.64 (dt, 13.0, 3.5)	1.71 (dt, 12.5, 3.3)	1.71 (m)	1.71 (dt, 12.5, 3.5)	1.69 (m)	1.69 (dt, 13.0, 3.2)
	0.97 (m)	1.01 (m)	0.96 (m)	0.99 (m)	1.02 (m)	1.02 (m)
2	1.74 (m)	1.75 (m)	1.73 (m)	1.83 (m)	1.80 (m)	1.81 (m)
	1.42 (m)	1.41 (m)	1.47 (m)	1.55 (m)	1.56 (m)	1.56 (m)
3	3.54 (m)	3.47 (m)	3.48 (m)	3.43 (m)	3.43 (m)	3.43 (m)
4	1.74 (m)	2.18 (m)	2.18 (dt, 12.5, 3.0)	4.25 (br s)	4.22 (br s)	4.22 (br s)
	1.54 (m)	1.17 (m)	1.19 (m)			
5	1.13 (dt, 13.0, 2.5)	1.01 (m)	1.02 (m)	0.94 (m)	0.91 (m)	0.91 (m)
6	3.73 (q, 2.8)	3.38 (td, 11.0, 4.5)	3.69 (td, 10.8, 4.0)	4.15 (td, 11.0, 4.3)	3.91 (td, 10.5, 5.0)	3.91 (td, 11.0, 5.0)
7	2.11 (dt, 14.5, 3.5)	2.26 (dt, 11.5, 4.3)	2.36 (dd, 12.0, 4.0)	2.43 (dd, 12.0, 4.5)	2.40 (dt, 12.0, 4.0)	2.41 (dt, 12.0, 4.0)
	1.30 (m)	0.91 (m)	1.25 (m)	1.29 (m)	0.95 (m)	0.95 (m)
8	1.95 (m)	1.86 (m)			1.89 (m)	1.91 (m)
9	0.72 (td, 11.3, 3.5)	0.72 (td, 11.0, 3.5)	0.83 (dd, 11.0, 3.0)	0.82 (dd, 12.0, 2.5)	0.68 (td, 11.5, 3.5)	0.69 (td, 11.8, 4.0)
11	1.55 (m)	1.52 (m)	1.80 (qd, 13.0, 3.0)	1.78 (m)	1.40 (m)	1.40 (m)
	1.39 (m)	1.31 (m)	1.50 (m)	1.43 (m)	1.29 (m)	1.29 (m)
12	1.94 (m)	1.93 (dt, 12.5, 3.3)	1.97 (dt, 12.5, 3.3)	1.96 (dt, 12.5, 3.5)	1.91 (m)	1.89 (m)
	1.24 (m)	1.12 (m)	1.17 (m)	1.17 (m)	1.04 (m)	1.07 (m)
14	1.07 (dd, 11.0, 9.5)	0.92 (m)	1.03 (m)	1.01 (d, 6.0)	0.86 (m)	0.87 (m)
15	3.85 (td, 9.5, 3.0)	4.12 (td, 6.5, 2.5)	4.37 (td, 6.5, 2.0)	4.38 (td, 6.5, 2.0)	4.10 (dd, 7.0, 6.0)	4.11 (dd, 7.0, 6.0)
16	1.87 (m)	2.21 (m)	2.18 (m)	2.19 (dt, 15.0, 7.8)	4.25 (t, 7.0)	4.18 (t, 6.7)
	1.57 (m)	1.31 (m)	1.36 (m)	1.35 (ddd, 15.0, 10.5, 2.5)		
17	1.45 (m)	1.11 (m)	1.02 (m)	1.01 (m)	1.01 (m)	1.04 (m)
18	0.76 (s)	0.95 (s)	1.28 (s)	1.27 (s)	0.91 (s)	0.95 (s)
19	1.03 (s)	0.87 (s)	0.98 (s)	1.15 (s)	1.06 (s)	1.06 (s)
20	2.00 (m)	2.13 (m)	2.13 (m)	2.13 (m)	1.89 (m)	2.63 (m)
21	1.00 (d, 6.0)	1.01 (d, 6.5)	0.99 (d, 7.0)	0.99 (d, 6.5)	0.95 (d, 7.0)	1.06 (d, 6.5)
22	5.20 (dd, 15.0, 8.0)	5.20 (dd, 15.0, 8.5)	5.18 (dd, 15.0, 8.0)	5.18 (dd, 15.0, 8.3)	1.71 (m)	5.56 (dd, 15.3, 7.3)
					1.09 (m)	
23	5.25 (dd, 15.0, 7.0)	5.26 (dd, 15.0, 7.3)	5.26 (dd, 15.0, 7.8)	5.26 (dd, 15.0, 8.0)	1.43 (m)	5.21 (dd, 15.3, 9.3)
					1.16 (m)	
24	2.07 (m)	2.08 (m)	2.08 (sextet, 6.8)	2.08 (m)	1.25 (m)	1.91 (m)
25	1.53 (m)	1.52 (m)	1.52 (m)	1.52 (m)	1.76 (m)	1.54 (m)
26	3.56 (dd, 11.0, 5.8)	3.57 (dd, 11.0, 5.5)	3.56 (dd, 11.0, 5.0)	3.56 (dd, 10.8, 5.3)	0.84 (d, 7.0)	0.84 (d, 7.0)
	3.26 (dd, 11.0, 8.0)	3.28 (m)	3.27 (dd, 11.0, 7.3)	3.27 (dd, 10.8, 7.5)		
27	0.87 (d, 6.5)	0.88 (d, 7.0)	0.87 (d, 7.0)	0.87 (d, 7.0)	0.87 (d, 7.0)	0.89 (d, 7.0)
24^1	0.92 (d, 6.5)	0.93 (d, 7.0)	0.92 (d, 7.0)	0.92 (d, 7.0)	1.69 (m)	1.78 (m)
					1.44 (m)	1.47 (m)
24^2					3.85 (td, 9.0, 5.7)	3.83 (ddd, 9.5, 7.5, 5.0)
					3.53 (q, 8.0)	3.49 (q, 8.3)

[a] Multiplicities and coupling constants are in parentheses.

steroid.[5] Thus, the structure of certonardoside B_3 (**19**) was established as (*E*)-(24*R*)-24^1-*O*-β-[2,4-di-*O*-methyl-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-24-methyl-5α-cholest-22-ene-3β,6α,15β,24^1-tetrol.

The isolated compounds were tested for cytotoxicity against a small panel of human solid tumor cell lines (Table 6) and were shown to exhibit moderate to significant cytotoxicity. Compound **1** was shown to be more potent than the positive control (doxorubicin) against the SK-OV-3, XF498, and HCT15 cancer cell lines. Analysis of the cytotoxicity data of **1**−**19** and comparison with those of our earlier study[1−3] showed that free sterols are generally more potent than their corresponding saponins except certonardosides P_1 (**13**) and J_3 (**15**). Saponins are usually considered to be responsible for the general toxicity of starfish.[14] However, our investigation of the chemical components of the starfish *Certonardoa semiregularis* showed that sterols and another class of minor chemical components[18] also play an important role in the general toxicity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a JASCO DIP-370 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and Varian Inova 500 instruments. Chemical shifts are reported with reference to the respective solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS 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, 5 μm, 120 Å), a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å), a Vydac column (250 × 10 mm, 5 μm, 90 Å), and a YMC-Pack C8 column (250 × 10 mm, 5 μm, 120 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 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 an aqueous MeOH-soluble fraction (14 g) and an *n*-hexane-soluble fraction (39 g). 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 afford 13 fractions (1−13). Fraction 7 (1.2 g) was very active in the brine shrimp assay (LD_{50} 5 μg/mL) and was further separated by normal-phase MPLC (silica gel 60, 400/230 mesh), eluting with a solvent system of 0 to 50% MeOH/$CHCl_3$, to afford 14 fractions. Compound **1** (3.6 mg) was obtained by separation of subfraction 7-7 on a reversed-phase HPLC column (Vydac, 250 × 10 mm, 5 μm, 90 Å) eluting with 80% MeOH, followed by purification on the same column eluting with 75% MeOH. Compounds **2** (1.3 mg), **6** (2.5 mg), **7** (0.7 mg), **9** (1.8 mg), **12** (3.4 mg), and **13** (3.1 mg) were obtained by separation of subfraction 7-9 on a C18-5E Shodex packed column (250 × 10

Table 3. ^{13}C NMR Data of Compounds **1**−**11** and the Aglycons of Compounds **14**, **15**, and **17** (CD_3OD, 50 MHz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**14**	**15**	**17**
1	38.6	38.6	38.6	39.4	39.7	39.9	39.9	38.6	39.4	38.8	39.6[a]	38.8	38.8	38.7
2	32.0	32.0	31.9	31.4	26.2	32.2	32.2	32.1	31.4	26.2	26.2	26.3	26.3	26.2
3	72.0	72.0	72.0	72.2	73.7	72.5	72.5	72.0	72.2	73.6	73.7	73.7	73.7	73.5
4	33.0	33.1	33.0	32.3	69.1	36.4	36.4	33.0	32.4	69.0	69.1	69.1	69.1	68.9
5	53.1	53.2	53.1	53.8	57.2	48.8	48.8	53.1	53.8	56.5[a]	57.3	56.5	56.5	56.4
6	70.0	70.0	70.0	67.6	64.7	72.5	72.5	70.0	67.6	66.6	64.7	66.7	66.6	66.4
7	41.8[a]	41.8	41.9	49.7	49.7	40.8	40.8	41.8	49.7	41.8	49.6	42.1	42.1	41.8
8	31.6	31.6	31.4	77.2	77.1	31.4	31.4	31.6	77.5	31.5	77.4	31.4[b]	31.4	31.3
9	55.7	55.7	55.6	57.4	58.4	55.8	55.8	55.7	57.4	56.6[a]	58.4	56.5	56.5	56.3
10	37.5	37.5	37.5	38.0	38.1	36.6	36.6	37.5	38.0	37.5	38.2	37.5	37.5	37.3
11	22.2	22.2	21.8	19.4	19.0	22.1	22.1	22.2	19.8	21.3	19.2	21.2	21.2[a]	21.3
12	42.5	42.7	42.6	43.5	43.4	41.6	41.5	42.5	43.3	42.4	43.2	42.6	42.4	42.4
13	43.3	43.4	43.5	44.5	44.5	44.9	44.8	43.3	44.2	43.5	44.2	43.5	43.5	43.2
14	62.2	62.2	60.5	61.0	61.1	63.8	64.0	62.2	62.7	62.1	62.9	60.6	60.7	62.1
15	70.6	70.6	70.4	71.2	71.2	74.2	74.2	70.6	71.2	70.6	71.1	70.5	70.6	70.5
16	42.9	42.3	73.1	72.8	72.8	41.9	42.2	42.9	43.6	43.0	43.5	73.1	73.5	42.1
17	57.6	57.7	62.8	63.0	63.0	54.9	54.9	57.6	57.7	57.5	57.7	62.8	63.4	57.5
18	15.3	15.2	16.2	17.9	17.9	13.8	14.0	15.3	16.5	15.0	16.6	16.2	16.2	15.0
19	13.8	13.8	13.8	14.0	17.0	16.3	16.3	13.8	14.1	16.1	17.0	16.0	16.1	15.9
20	41.6[a]	37.2	31.9	31.4	31.4	37.0	41.3	41.1	41.2	41.3	41.1	31.9	35.0	36.8
21	21.5[b]	19.4	18.9[a]	18.5	18.5	19.2	21.4	21.1	21.2	21.4	21.1	19.0[a]	20.7	19.1
22	140.6	34.9	34.9	34.6[a]	34.6[a]	34.9	137.0	137.1	137.2	137.1	137.1	34.9	140.1	34.7
23	127.7	28.3	28.8	28.8	28.8	28.2	133.8	133.8	133.8	133.7	133.8	28.7	130.4	24.8
24	52.8	41.9	42.1	42.1	42.1	42.0	39.6	39.7	39.7	39.7	39.7[a]	42.3	47.4	45.6
25	29.1	30.5	30.5	30.5	30.5	30.5	42.1	42.1	42.1	42.1	42.1	30.6	33.8	29.2
26	18.7	18.9	18.8[a]	18.8	18.8	18.9	66.6	66.6	66.6	66.6	66.6	18.9[a]	19.5	19.3
27	21.4[b]	20.0	20.2	20.1	20.1	20.0	14.0	14.0	14.0	14.0	14.0	20.1	21.3[a]	20.2
24^1	65.1	34.7	34.6	34.7[a]	34.7[a]	34.7	17.1	17.1	17.1	17.2	17.1	31.7[b]	33.5	71.5
24^2		62.0	62.1	62.1	62.1	62.0						70.1	69.9	

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

Table 4. ^1H and ^{13}C NMR Data of the Sugar Residues in Compounds **12**−**19**[a]

	12−**17**		**18**, **19**	
position	δ_H	δ_C	δ_H	δ_C
1′	4.18 (d, 7.0)	105.1	4.96 (br s)	108.1
2′	3.16 (m)	75.0	4.06 (dd, 3.8, 1.3)	91.6
3′	3.36 (m)	77.0	3.95 (dd, 7.8, 3.8)	77.7
4′	3.17 (m)	80.9	3.88 (ddd, 8.0, 5.5, 3.0)	84.3
$5'_{eq}$	4.03 (dd, 9.5, 3.0)	64.3	3.76 (dd, 12.0, 3.3)	62.7
$5'_{ax}$	3.13 (m)	59.0	3.62 (dd, 12.0, 5.5)	
4′-OMe	3.46 (s)			
1″			4.43 (d, 8.0)	104.5
2″			2.85 (dd, 9.3, 7.8)	84.9
3″			3.38 (t, 8.8)	76.5
4″			3.18 (ddd, 10.0, 8.3, 4.8)	80.8
$5''_{eq}$			4.01 (dd, 11.0, 4.5)	64.4
$5''_{ax}$			3.13 (dd, 11.0, 10.0)	
2″-OMe			3.56 (s)	61.2
4″-OMe			3.46 (s)	59.1

[a] Multiplicities and coupling constants are in parentheses.

mm, 5 μm, 100 Å) eluting with 80% MeOH, followed by purification on a YMC-Pack ODS column (250 × 10 mm, 5 μm, 120 Å) eluting with the same mobile phase. Compounds **8** (0.5 mg) and **17** (2.1 mg) were obtained by separation of subfraction 7-10 using the same purification system as for subfraction 7-9. Compounds **3** (1.3 mg), **4** (2.0 mg), **11** (3.1 mg), and **16** (1.1 mg) were obtained by separation of subfraction 7-11 on a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) eluting with 80% MeOH, followed by purification on a YMC-Pack C8 column eluting with 75% MeOH. Compounds **10** (0.6 mg), **14** (4.5 mg), and **15** (1.9 mg) were obtained by separation of subfraction 7-12 using the same purification system as for subfraction 7-11. Compounds **5** (4.0 mg), **18** (1.0 mg), and **19** (4.0 mg) were obtained by separation of subfraction 7-14 on a reversed-phase HPLC column (Vydac, 250 × 10 mm, 5 μm, 90 Å) eluting with 80% MeOH, followed by purification on a YMC-Pack C8 column eluting with 75% MeOH.

Certonardosterol D_2 (1): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 471 $[M + Na]^+$; HRFABMS (+ve) *m/z* 471.3456 (calcd for $C_{28}H_{48}O_4Na$, 471.3450).

(*R*)-MTPA ester of 1: ^1H NMR (CD_3OD, 500 MHz), δ 5.34 (1H, dd, J = 15.5, 8.0 Hz, H-22), 5.21 (1H, dd, J = 15.5, 9.0 Hz, H-23), 4.31 (2H, br d, J = 6.0 Hz, H-24^1), 1.01 (3H, d, J = 6.5 Hz, H-21), 0.96 (3H, s, H-19), 0.95 (3H, s, H-18), 0.87 (3H, d, J = 7.0 Hz, H-27), 0.83 (3H, d, J = 7.0 Hz, H-26).

Certonardosterol D_3 (2): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 487 $[M + Na]^+$ (100), 469 (2.0), 443 (1.5), 329 (0.5), 273 (1.0); HRFABMS (+ve) *m/z* 487.3762 (calcd for $C_{29}H_{52}O_4Na$, 487.3763).

Certonardosterol N_1 (3): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 503 $[M + Na]^+$ (100), 485 (2.0), 459 (1.0), 349 (3.2); HRFABMS (+ve) *m/z* 503.3698 (calcd for $C_{29}H_{52}O_5Na$, 503.3712).

Certonardosterol O_1 (4): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 519 $[M + Na]^+$ (100), 501 (2.0), 475 (1.2), 361 (0.3), 289 (0.5); HRFABMS (+ve) *m/z* 519.3663 (calcd for $C_{29}H_{52}O_6Na$, 519.3662).

Certonardosterol P_1 (5): colorless needles; $[\alpha]^{21}_D$ +36.4° (*c* 0.11, MeOH); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 535 $[M + Na]^+$ (100), 517 (1.1), 491 (0.9), 377 (0.5), 305 (1.0); HRFABMS (+ve) *m/z* 535.3616 (calcd for $C_{29}H_{52}O_7Na$, 535.3611).

Certonardosterol E_2 (6): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 487 $[M + Na]^+$ (100), 469 (1.5), 443 (1.0), 329 (0.5); HRFABMS (+ve) *m/z* 487.3756 (calcd for $C_{29}H_{52}O_4Na$, 487.3763).

Certonardosterol E_3 (7): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 471 $[M + Na]^+$ (100), 453 (1.2), 411 (1.0), 329 (0.4), 273 (0.5); HRFABMS (+ve) *m/z* 471.3461 (calcd for $C_{28}H_{48}O_4Na$, 471.3450).

Certonardosterol D_4 (8): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 471 $[M + Na]^+$ (100), 453 (2.0), 411 (0.5), 329 (0.5), 273 (0.5); HRFABMS (+ve) *m/z* 471.3458 (calcd for $C_{28}H_{48}O_4Na$, 471.3450).

Certonardosterol C_2 (9): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z*

Table 5. Selected ^{1}H NMR Data of the MTPA Esters of Compounds **1**, **11**, and **19a** (CD_3OD, 500 MHz)[a]

MTPA ester	H-21	H-26	H-26, H-27	H-24[1]
(*R*)-MTPA ester of **1**	1.01 (d, 6.5)		0.87 (d, 7.0) 0.83 (d, 7.0)	4.31 (br d, 6.0)
(*S*)-MTPA ester of **11**		4.37 (dd, 11.0, 4.5) 4.10 (dd, 11.0, 6.5)		
(*R*)-MTPA ester of **11**		4.26 (dd, 10.8, 4.5) 4.18 (dd, 10.8, 6.8)		
(*R*)-MTPA ester of **19a**	1.00 (d, 7.0)		0.87 (d, 6.5) 0.83 (d, 7.0)	4.30 (br d, 6.0)

[a] Multiplicities and coupling constants are in parentheses.

Table 6. Cytotoxicity Data of Compounds **1**−**19** against Human Solid Tumor Cells[a]

compound[b]	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	0.15	0.08	0.09	0.07	0.01
doxorubicin	0.02	0.17	0.02	0.06	0.06
2	1.36	1.33	0.68	0.84	2.48
3	0.82	0.90	0.40	0.43	1.25
4	8.32	6.59	5.32	6.92	17.7
5	3.30	3.50	2.50	3.48	4.39
6	0.48	0.83	0.28	0.40	1.26
7	0.54	0.69	0.40	0.33	0.73
8	1.75	1.51	0.48	1.22	1.25
9	0.15	0.16	<0.10	0.08	0.25
10	2.33	2.64	2.23	2.21	2.71
11	1.75	1.13	0.67	0.89	2.54
12	1.25	2.00	0.68	0.74	2.28
13	0.87	0.89	0.26	0.35	2.17
14	3.41	3.29	2.36	3.53	8.20
15	0.83	0.74	0.30	0.45	2.76
16	4.26	3.06	2.39	3.69	8.32
17	6.61	3.71	2.10	3.00	5.04
18	4.44	4.32	2.93	3.82	8.13
19	4.13	4.92	3.85	4.58	6.14
doxorubicin	0.01	0.08	0.08	0.10	0.17

[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 CNS cancer; HCT 15: human colon cancer. [b] Compounds were assayed in two separate batches.

487 [M + Na]$^+$ (100), 469 (2.0), 427 (1.5), 345 (1.0), 289 (0.8); HRFABMS (+ve) *m/z* 487.3388 (calcd for $C_{28}H_{48}O_5Na$, 487.3399).

Certonardosterol B_2 (10): colorless needles; ^{1}H NMR (CD_3OD, 500 MHz), δ 5.26 (1H, dd, J = 15.0, 8.0 Hz, H-23), 5.19 (1H, dd, J = 15.0, 8.3 Hz, H-22), 4.21 (1H, br s, H-4), 4.13 (1H, td, J = 6.8, 1.8 Hz, H-15), 3.89 (1H, td, J = 11.0, 4.5 Hz, H-6), 3.57 (1H, dd, J = 11.0, 5.5 Hz, H-26), 3.42 (1H, ddd, J = 12.0, 5.0, 3.5 Hz, H-3), 3.27 (1H, dd, J = 11.0, 7.5 Hz, H-26), 2.34 (1H, dt, J = 12.5, 4.3 Hz, H_{eq}-7), 2.22 (1H, dt, J = 15.5, 7.8 Hz, H-16), 1.80 (1H, qd, J = 12.8, 2.5 Hz, H_{eq}-2), 1.69 (1H, dt, J = 13.0, 3.8 Hz, H_{eq}-1), 1.06 (3H, s, H-19), 1.00 (3H, d, J = 6.5 Hz, H-21), 0.94 (3H, s, H-18), 0.92 (3H, d, J = 6.5 Hz, H-24[1]), 0.88 (3H, d, J = 6.5 Hz, H-27), 0.67 (1H, td, J = 11.5, 3.0 Hz, H-9); ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 487 [M + Na]$^+$ (100), 469 (1.5), 345 (1.3), 289 (2.0); HRFABMS (+ve) *m/z* 487.3389 (calcd for $C_{28}H_{48}O_5Na$, 487.3399).

Certonardosterol A_2 (11): colorless needles; $[\alpha]^{21}_D$ +23.8° (*c* 0.15, MeOH); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 503 [M + Na]$^+$ (100), 485 (2.0), 443 (1.7), 361 (0.5), 305 (2.0); HRFABMS (+ve) *m/z* 503.3337 (calcd for $C_{28}H_{48}O_6Na$, 503.3349).

(*S*)-MTPA ester of 11: ^{1}H NMR (CD_3OD, 500 MHz), δ 4.37 (1H, dd, J = 11.0, 4.5 Hz, H-26), 4.10 (1H, dd, J = 11.0, 6.5 Hz, H-26), 1.27 (3H, s, H-18), 1.25 (3H, s, H-19), 0.98 (3H, d, J = 6.5 Hz, H-21), 0.94 (3H, d, J = 7.0 Hz, H-24[1]), 0.90 (3H, d, J = 7.0 Hz, H-27).

(*R*)-MTPA ester of 11: ^{1}H NMR (CD_3OD, 500 MHz), δ 4.26 (1H, dd, J = 10.8, 4.5 Hz, H-26), 4.18 (1H, dd, J = 10.8, 6.8 Hz, H-26), 1.28 (3H, s, H-18), 1.26 (3H, s, H-19), 0.97 (3H, d, J = 6.5 Hz, H-21), 0.93 (3H, d, J = 7.0 Hz, H-24[1]), 0.90 (3H, d, J = 7.0 Hz, H-27).

Certonardoside O_1 (12): colorless needles; ^{1}H and ^{13}C NMR data of the sterol nucleus are identical with those of certonardosterol N_1 (**3**); ^{1}H and ^{13}C NMR data of sugar moiety, see Table 4; ^{1}H NMR (CD_3OD, 500 MHz), δ (side chain) 3.85 (1H, td, J = 8.8, 6.0 Hz, H-24[2]), 3.53 (1H, q, J = 8.2 Hz, H-24[2]), 1.68 (1H, m, H-24[1]), 1.44 (1H, m, H-24[1]), 0.95 (3H, d, J = 7.0 Hz, H-21), 0.87 (3H, d, J = 7.0 Hz, H-27), 0.84 (3H, d, J = 7.0 Hz, H-26); ^{13}C NMR (CD_3OD, 50 MHz), δ (side chain) 70.1 (CH_2, C-24[2]), 42.3 (CH, C-24), 34.9 (CH_2, C-22), 31.9 (CH, C-20), 31.7 (CH_2, C-24[1]), 30.6 (CH, C-25), 28.7 (CH_2, C-23), 20.1 (CH_3, C-27), 19.1 (CH_3, C-21), 19.0 (CH_3, C-26); FABMS (+ve) *m/z* 649 [M + Na]$^+$ (100), 617 [M + Na − OMe − H]$^+$ (1.0), 531 (2.0), 501 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (1.1), 348 (0.8), 273 (0.3); HRFABMS (+ve) *m/z* 649.4289 (calcd for $C_{35}H_{62}O_9$-Na, 649.4292).

Certonardoside P_1 (13): colorless needles; ^{1}H and ^{13}C NMR data of the sterol nucleus were identical with those of certonardosterol O_1 (**4**); ^{1}H and ^{13}C NMR data of the side chain were identical with those of certonardoside O_1 (**12**); ^{1}H and ^{13}C NMR data of sugar moiety, see Table 4; FABMS (+ve) *m/z* 665 [M + Na]$^+$ (100), 633 [M + Na − OMe − H]$^+$ (1.8), 547 (2.5), 517 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (1.7), 361 (0.8), 289 (0.4); HRFABMS (+ve) *m/z* 665.4246 (calcd for $C_{35}H_{62}O_{10}Na$, 665.4241).

Certonardoside J_2 (14): colorless needles; $[\alpha]^{21}_D$ −6.5° (*c* 0.16, MeOH); ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 4; FABMS (+ve) *m/z* 665 [M + Na]$^+$ (100), 633 [M + Na − OMe − H]$^+$ (1.5), 547 (2.2), 517 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (2.5), 361 (0.8), 289 (2.1); HRFABMS (+ve) *m/z* 665.4245 (calcd for $C_{35}H_{62}O_{10}Na$, 665.4241).

Certonardoside J_3 (15): colorless needles; ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 4; FABMS (+ve) *m/z* 663 [M + Na]$^+$ (100), 631 [M + Na − OMe − H]$^+$ (1.2), 619 (1.5), 545 (2.2), 515 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (1.1), 361 (0.4), 289 (1.1); HRFABMS (+ve) *m/z* 663.4092 (calcd for $C_{35}H_{60}O_{10}Na$, 663.4084).

Certonardoside I_2 (16): colorless needles; ^{1}H and ^{13}C NMR data of the sterol nucleus were identical with those of certonardosterol P_1 (**5**); ^{1}H and ^{13}C NMR data of the side chain were identical with those of certonardoside O_1 (**12**); ^{1}H and ^{13}C NMR data of sugar moiety, see Table 4; FABMS (+ve) *m/z* 681 [M + Na]$^+$ (100), 649 [M + Na − OMe − H]$^+$ (1.7), 563 (2.5), 533 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (1.5), 378 (0.7), 305 (1.8); HRFABMS (+ve) *m/z* 681.4185 (calcd for $C_{35}H_{62}O_{11}Na$, 681.4190).

Certonardoside H_2 (17): colorless needles; ^{1}H NMR data of the sugar moiety, see Table 4, δ (aglycon) 4.21 (1H, br s, H-4), 4.16 (1H, td, J = 6.8, 1.5 Hz, H-15), 3.90 (1H, td, J = 11.0, 4.0 Hz, H-6), 3.75 (1H, dd, J = 9.5, 5.5 Hz, H-24[1]), 3.43 (1H, dd, J = 9.5, 5.5 Hz, H-24[1]), 3.43 (1H, m, H-3), 2.41 (1H, dt, J = 14.5, 8.0 Hz, H-16), 2.35 (1H, dt, J = 11.5, 4.0 Hz, H_{eq}-7), 1.94 (1H, dt, J = 12.5, 3.5 Hz, H_{eq}-12), 1.68 (1H, dt, J = 13.5, 3.3 Hz, H_{eq}-1), 1.06 (3H, s, H-19), 0.93 (3H, s, H-18), 0.93 (3H, d, J = 6.5 Hz, H-21), 0.89 (3H, d, J = 7.0 Hz, H-27), 0.87 (3H, d, J = 7.0 Hz, H-26), 0.67 (1H, td, J = 11.5, 3.5 Hz, H-9); ^{13}C NMR data, see Tables 3 and 4; FABMS (+ve) *m/z* 635 [M + Na]$^+$ (100), 603 [M + Na − OMe − H]$^+$ (1.0), 517 (1.6), 487 [M + Na − $C_6H_{11}O_4$ − H]$^+$ (1.0), 346 (0.5), 289 (2.5); HRFABMS (+ve) *m/z* 635.4130 (calcd for $C_{34}H_{60}O_9Na$, 635.4135).

Compound 17a: amorphous powder; ^{1}H NMR (CD_3OD, 500 MHz), δ 4.21 (1H, br s, H-4), 4.16 (1H, td, J = 6.8, 1.3 Hz,

H-15), 3.90 (1H, td, J = 11.0, 4.5 Hz, H-6), 3.48 (2H, d, J = 5.5 Hz, H-24[1]), 3.43 (1H, m, H-3), 2.41 (1H, dt, J = 14.5, 7.8 Hz, H-16), 2.33 (1H, dt, J = 11.0, 4.0 Hz, H_{eq}-7), 1.92 (1H, dt, J = 12.0, 3.5 Hz, H_{eq}-12), 1.70 (1H, dt, J = 13.3, 3.5 Hz, H_{eq}-1), 1.06 (3H, s, H-19), 0.94 (3H, d, J = 7.0 Hz, H-21), 0.93 (3H, s, H-18), 0.90 (3H, d, J = 7.0 Hz, H-27), 0.87 (3H, d, J = 6.5 Hz, H-26), 0.67 (1H, td, J = 11.5, 3.0 Hz, H-9).

Certonardoside B_2 (18): colorless needles; ^{1}H and ^{13}C NMR data of the sterol nucleus were identical with those of certonardosterol D_3 (**2**); ^{1}H and ^{13}C NMR data of sugar moiety, see Table 4; ^{1}H NMR (CD_3OD, 500 MHz), δ (side chain) 3.72 (1H, dd, J = 9.0, 6.0 Hz, H-24[1]), 3.25 (1H, m, H-24[1]), 0.96 (3H, d, J = 6.5 Hz, H-21), 0.91 (3H, d, J = 7.0 Hz, H-27), 0.89 (3H, d, J = 7.0 Hz, H-26); ^{13}C NMR (CD_3OD, 50 MHz), δ (side chain) 70.0 (CH_2, C-24[1]), 45.9 (CH, C-24), 37.1 (CH, C-20), 34.8 (CH_2, C-22), 29.5 (CH, C-25), 25.6 (CH_2, C-23), 19.7 (CH_3, C-27), 19.4 (CH_3, C-21), 19.0 (CH_3, C-26); FABMS (+ve) m/z 765 $[M + Na]^+$ (100), 747 (1.9), 721 (0.6), 633 (1.3), 605 $[M + Na - C_7H_{13}O_4 + H]^+$ (0.4), 501 (1.0), 471 $[M + Na - C_7H_{13}O_4 - C_5H_8O_4 - H]^+$ (0.4); HRFABMS (+ve) m/z 765.4766 (calcd for $C_{40}H_{70}O_{12}$-Na, 765.4765).

Compound 18a: amorphous powder; ^{1}H NMR (CD_3OD, 500 MHz), δ (side chain) 3.55 (1H, dd, J = 10.5, 5.5 Hz, H-24[1]), 3.46 (1H, dd, J = 10.5, 5.5 Hz, H-24[1]), 0.95 (3H, d, J = 7.0 Hz, H-21), 0.89 (3H, d, J = 6.5 Hz, H-27), 0.88 (3H, d, J = 7.0 Hz, H-26).

Certonardoside B_3 (19): colorless needles; ^{1}H and ^{13}C NMR data of the sterol nucleus were identical with those of certonardosterol D_2 (**1**); ^{1}H and ^{13}C NMR data of sugar moiety, see Table 4; ^{1}H NMR (CD_3OD, 500 MHz), δ (side chain) 5.26 (1H, dd, J = 15.0, 8.0 Hz, H-22), 5.23 (1H, dd, J = 15.0, 7.0 Hz, H-23), 3.71 (1H, dd, J = 9.5, 6.5 Hz, H-24[1]), 3.33 (1H, m, H-24[1]), 1.03 (3H, d, J = 6.5 Hz, H-21), 0.90 (3H, d, J = 7.0 Hz, H-27), 0.84 (3H, d, J = 7.0 Hz, H-26); ^{13}C NMR (CD_3OD, 50 MHz), δ (side chain) 140.3 (CH, C-22), 128.0 (CH, C-23), 70.8 (CH_2, C-24[1]), 50.0 (CH, C-24), 41.7 (CH, C-20), 29.6 (CH, C-25), 21.5 (CH_3, C-21), 21.4 (CH_3, C-27), 19.0 (CH_3, C-26); FABMS (+ve) m/z 763 $[M + Na]^+$ (100), 745 (2.0), 719 (1.0), 631 (1.5), 603 $[M + Na - C_7H_{13}O_4 + H]^+$ (0.4), 499 (0.9), 469 $[M + Na - C_7H_{13}O_4 - C_5H_8O_4 - H]^+$ (0.5), 331 (0.3); HRFABMS (+ve) m/z 763.4598 (calcd for $C_{40}H_{68}O_{12}Na$, 763.4608).

Compound 19a: amorphous powder; ^{1}H NMR data are identical with those of certonardosterol D_2 (**1**).

***(R)*-MTPA ester of 19a:** ^{1}H NMR (CD_3OD, 500 MHz), δ 5.35 (1H, dd, J = 15.5, 8.0 Hz, H-22), 5.21 (1H, dd, J = 15.5, 9.0 Hz, H-23), 4.30 (2H, br d, J = 6.0 Hz, H-24[1]), 1.00 (3H, d, J = 7.0 Hz, H-21), 0.96 (3H, s, H-19), 0.95 (3H, s, H-18), 0.87 (3H, d, J = 6.5 Hz, H-27), 0.83 (3H, d, J = 7.0 Hz, H-26).

Preparation of MTPA Esters. The (*S*)-MTPA and (*R*)-MTPA esters of compound **11** (3 μmol) were prepared as described previously.[1] To the solutions of **11** in dry pyridine (25 μL) were added (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (12 μmol), respectively. Each mixture was allowed to stand at room temperature for 24 h. The reaction was monitored by TLC (ODS, MeOH) and stopped when the original spot had disappeared. After removal of solvent, the product was purified by reversed-phase HPLC on a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) and analyzed by ^{1}H NMR. Compounds **1** (2 μmol) and **19a** (1.5 μmol) were treated with only (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (12 μmol) in dry pyridine (25 μL) at room temperature for 24 h. The following procedure was the same as that for compound **11**.

Methanolysis of Saponins. Each solution of compounds **17** (1.0 μmol), **18** (0.5 μmol), and **19** (2.0 μmol) in anhydrous 4.5% HCl in MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial. After 50 min, TLC analysis [ODS with 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, 250 × 10 mm, 5 μm, 120 Å, MeOH/H_2O (9:1)] to give **17a**, **18a**, and **19a**, respectively.

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