Bioactive Sterols from the Starfish *Certonardoa semiregularis*

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Received October 30, 2002

Thirteen new polyhydroxysterols (**1**−**5**, **7**−**12**, **14**, **15**) and two known polyhydroxysterols (**6**, **13**) 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. These compounds displayed considerable cytotoxicity against a small panel of human solid tumor cell lines. These compounds were also evaluated for antibacterial activity against 20 clinically isolated strains. Most of the compounds showed weak antibacterial activity against *Streptococcus pyogenes* 308A, *Pseudomonas aeruginosa* 1771, and *Pseudomonas aeruginosa* 1771M.

Starfish appear to be the richest source of polyhydroxysterols. Although polyhydroxysterols have been isolated from only a limited number of species in other marine phyla, they are widespread in almost all species of starfish (phylum Echinodermata) examined.[1]

In the course of the search for bioactive metabolites from the starfish *Certonardoa semiregularis*, we have isolated 11 saponins.[2] In our continuing study on the same starfish, 13 new polyhydroxysterols (**1**−**5**, **7**−**12**, **14**, **15**, Chart 1), along with two known ones (**6**, **13**), were isolated. Compound **6** was previously isolated from the starfish *Gomophia watsoni*,[3] *Dermasterias imbricata*,[4] *Culcita novaeguineae*,[5] and *Nardoa tuberculata*.[6] Compound **13** has been isolated from two Antarctic starfish.[7,8] It is known that most of the polyhydroxysterols so far isolated from starfish possess the 8-hydroxy group,[1] but compounds **2**, **4**, **5**, **7**−**9**, **11**, and **14** lack this distinctive feature. The side chains of **10** and **11** were first encountered in naturally occurring sterols.

Results and Discussion

Certonardosterol A (**1**) was isolated as colorless needles. The molecular formula of **1** was established as $C_{28}H_{48}O_6$ on the basis of the pseudomolecular ion peak at *m*/*z* 503.3362 $[M + Na]^+$ (calcd for $C_{28}H_{48}NaO_6$, 503.3349) and the NMR data (Tables 1 and 4). Examination of its ^1H and ^{13}C NMR spectra indicated the presence of the 3*β*,4*β*,6α,8,15*β*-pentahydroxy steroidal nucleus as observed in certonardoside A.[2] In the ^1H NMR spectrum, the signals at *δ* 1.05 and 0.94 (methyl doublets), *δ* 3.55 and 3.34 (oxymethylene), and *δ* 4.78 and 4.76 (olefinic methylene) were observed, which indicate the presence of the 26-hydroxy-24-methylcholest-24(24[1])-ene side chain. The stereochemistry at C-25 was determined by analysis of the MTPA esters. It was reported that C-26 methylene protons appear as a doublet at *δ* 4.16 for the (*R*)-MTPA esters of (25*S*)-26-hydroxysteroids, while those of the 25*R* isomers appear as two well-separated doublets of doublets at *δ* 4.24 and 4.08. The reversal of the pattern was observed in those of the (*S*)-MTPA esters.[9] The ^1H NMR spectrum of the (*R*)-MTPA ester of **1** showed the H-26 methylene proton signal as two close doublets of doublets at *δ* 4.33 and 4.20, while that of the (*S*)-MTPA ester showed two well-separated doublets of doublets at *δ* 4.38 and 4.15. Accordingly, the 25*S* configuration was proposed for compound **1**. 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] Thus, the structure of **1** was defined as (25*S*)-24-methyl-5α-cholest-24(24[1])-ene-3*β*,4*β*,6α,8,15*β*,26-hexol, which corresponds to the aglycone of certonardoside A, although the stereochemistry at C-25 of certonardoside A remains unassigned.[2]

Comparison of the ^1H NMR spectra of **2**−**5** with those of **1** revealed that they share the same (25*S*)-26-hydroxy-24-methylcholest-24(24[1])-ene side chain. Certonardosterol B (**2**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 487.3397 $[M + Na]^+$ (calcd for $C_{28}H_{48}NaO_5$, 487.3399). It was an 8-deoxy derivative of **1**, as determined by comparison of the ^1H NMR data with those of **1** (Table 1). The two coupled signals at *δ* 2.36 and 0.93, which were assigned to H-7, were coupled to the H-6 oxymethine proton signal. The H-7 signal showed additional coupling with the H-8 signal (*δ* 1.89). The signals of H-18 and -19 methyl protons, H-15 oxymethine proton, H_{eq}-11, and H_{ax}-11 protons were shifted upfield by 0.31, 0.08, 0.25, 0.35, and 0.12 ppm, respectively, relative to those of **1**. The signals of C-6, C-11, and C-15 were shifted by +1.8, +2.3, and −0.5 ppm, respectively, relative to those of **1**. These shifts agree with the elimination of the 1,3-diaxial interaction between the angular methyl groups and the 8-hydroxy group. The same pattern of shifts was observed in amurensosides A and C[11] and moniloside C.[12] Thus, the structure of **2** was established as (25*S*)-24-methyl-5α-cholest-24(24[1])-ene-3*β*,4*β*,6α,15*β*,26-pentol.

Certonardosterol C (**3**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 487.3389 $[M + Na]^+$ (calcd for $C_{28}H_{48}NaO_5$, 487.3399). It was postulated as a 4-deoxy derivative of **1**. Comparison of the ^1H NMR spectrum with that for **1** revealed the lack of the broad signal at *δ* 4.25 assigned to H-4α in **1**, and upfield shifts of the signals of H-6*β* (*δ* 4.16→3.70) and H-19 (*δ* 1.15→0.99). The remaining signals were almost identical to those of **1**. Compound **3** corresponds to the aglycone of certonardoside C, although the stereochemistry at C-25 of certonardoside C remains unassigned.[2]

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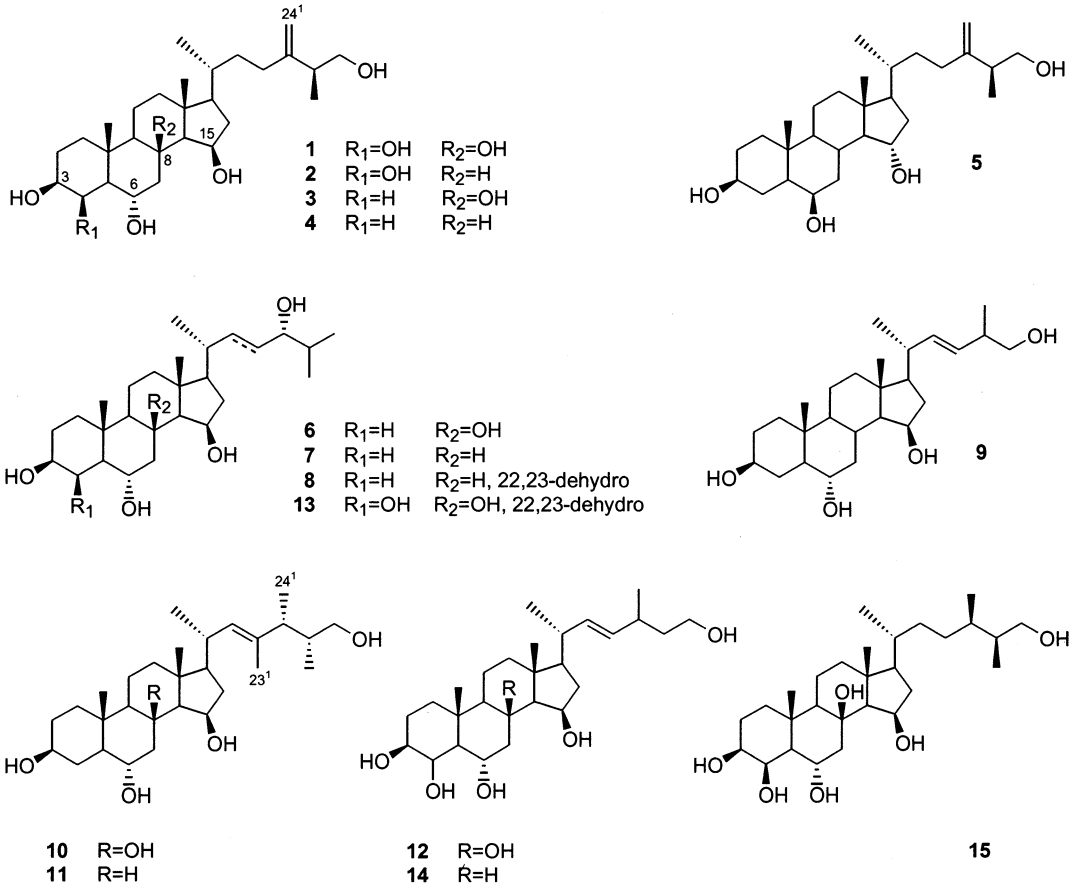
10.1021/np020507i CCC: $25.00
Published on Web 03/07/2003

Chart 1



Certonardosterol D (**4**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 471.3448 $[M + Na]^+$ (calcd for $C_{28}H_{48}NaO_4$, 471.3450). Comparison of the ^1H and ^{13}C NMR data with those of **3** indicated that **4** is the 8-deoxy derivative of **3** and corresponds to the aglycone of certonardoside B, although the stereochemistry at C-25 of certonardoside B remains unassigned.[2]

Certonardosterol E (**5**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 471.3449 $[M + Na]^+$ (calcd for $C_{28}H_{48}NaO_4$, 471.3450). It was a diastereomer of **4** with opposite stereochemistry at C-6 and 15. The H-6 proton was observed as a quartet at δ 3.75 (J = 2.5 Hz), which was typical for an equatorial proton coupled with three other equatorial or axial protons. The coupling constant between H-14 and -15 (9.3 Hz) was close to that of a 15α-hydroxy steroid (10 Hz) and distinct from that of a 15β-hydroxy steroid (5 Hz).[11] In the ^{13}C NMR spectrum, due to the alteration of the 1,3-diaxial interaction with the corresponding hydroxy group, the signals of C-18 and -19 were shifted by −1.5 and +2.5 ppm, respectively, relative to those of **4**. The 3β,6β,15α-trihydroxy steroidal nucleus has been encountered in granulatoside B,[13] solasteroside S_1,[14] and the synthesized compound.[15]

Compound **6** was isolated as light yellow needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 475.3408 $[M + Na]^+$ (calcd for $C_{27}H_{48}NaO_5$, 475.3399). The structure was identified by comparison of the NMR data with those reported.[3] The stereochemical assignment at C-24 was now confirmed by analysis of the ^1H NMR data for its (*R*)-MTPA ester. The isopropyl methyl proton signals were observed at δ 0.82 and 0.85, which matched well with those of the (*R*)-MTPA ester of the 24*S* model compound (δ 0.84 and 0.86), while those of the (*R*)-MTPA ester of the 24*R* isomer would appear downfield shifted to δ 0.92 (6H, d).[16]

Certonardosterol F (**7**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 459.3438 $[M + Na]^+$ (calcd for $C_{27}H_{48}NaO_4$, 459.3450). The NMR data showed that **7** shares the same steroidal nucleus as that of **4** and shares the same side chain as that of **6**. Thus, the structure of **7** was defined as (24*S*)-5α-cholestane-3β,6α,15β,24-tetrol.

Certonardosterol G (**8**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 457.3303 $[M + Na]^+$ (calcd for $C_{27}H_{46}NaO_4$, 457.3294). Comparison of its NMR data with those for **7** indicated that they share the same steroidal nucleus. The ^1H NMR spectrum of **8** showed two doublets of doublets at δ 5.46 and 5.38, which could be assigned to the Δ^{22E} protons (J = 15 Hz). The 22*E* configuration was also supported by the chemical shift of C-20 at δ 41.1, while that of the 22*Z* isomer would be upfield shifted to about δ 35.[8] The coupling of the oxymethine signals (δ 3.69) to the olefinic signal at δ 5.38 suggested the 24-hydroxycholest-22-ene side chain. The configuration of C-24 was proposed on the basis of the analysis of the ^1H NMR data of the MTPA esters. It was known that the isopropyl methyl signals appear at δ 0.87−0.90 for the (*R*)-MTPA ester of the (22*E*, 24*R*) model compounds, while they are shifted to δ 0.95−0.98 for that of the (22*E*, 24*S*) isomer.[7] The isopropyl methyl signals were shifted upfield to δ 0.84 and 0.83 in the spectrum of the (*R*)-MTPA ester of **8** and shifted downfield to δ 0.94 and 0.92 in that of the (*S*)-MTPA ester. Thus, compound **8** can be defined as (*E*)-(24*R*)-5α-cholest-22-ene-3β,6α,15β,24-tetrol.

Table 1. ^{1}H NMR Data of Compounds **1**−**5** (CD_3OD, 600 MHz)[a]

position	**1**[b]	**2**	**3**	**4**	**5**
1	1.70 (dt, 13.0, 3.8)	1.69 (dt, 13.2, 3.3)	1.72 (m)	1.72 (dt, 13.2, 3.2)	1.65 (dt, 13.0, 3.3)
	0.99 (m)	1.03 (m)	0.98 (m)	1.03 (m)	0.98 (m)
2	1.82 (m)	1.82 (m)	1.73 (m)	1.77 (m)	1.75 (m)
	1.56 (m)	1.57 (m)	1.49 (m)	1.43 (m)	1.43 (m)
3	3.43 (ddd, 12.5, 4.8, 3.5)	3.43 (ddd, 11.9, 4.5, 3.5)	3.49 (m)	3.48 (m)	3.53 (m)
4	4.25 (br s)	4.22 (br s)	2.19 (dt, 12.4, 2.1)	2.19 (dt, 12.5, 2.2)	1.75 (m)
			1.22 (m)	1.18 (m)	1.55 (m)
5	0.93 (m)	0.91 (m)	1.03 (m)	1.02 (m)	1.14 (m)
6	4.16 (td, 11.0, 4.3)	3.91 (td, 10.9, 4.6)	3.70 (td, 10.8, 4.2)	3.39 (td, 10.8, 4.4)	3.75 (q, 2.5)
7	2.44 (dd, 12.3, 4.3)	2.36 (dt, 11.8, 4.1)	2.38 (m)	2.27 (m)	2.13 (dt, 13.5, 3.1)
	1.30 (dd, 12.3, 11.0)	0.93 (m)	1.28 (m)	0.92 (m)	1.31 (td, 13.5, 3.2)
8		1.89 (m)		1.88 (m)	1.95 (m)
9	0.81 (dd, 12.3, 2.8)	0.68 (td, 11.5, 3.5)	0.84 (dd, 12.7, 2.9)	0.73 (td, 11.5, 3.4)	0.72 (td, 10.7, 4.3)
11	1.79 (m)	1.44 (m)	1.83 (m)	1.53 (m)	1.54 (m)
	1.44 (m)	1.32 (m)	1.49 (m)	1.33 (m)	1.41 (m)
12	1.98 (dt, 13.0, 3.3)	1.96 (m)	2.00 (dt, 12.6, 3.4)	1.96 (dt, 12.2, 3.6)	1.97 (m)
	1.17 (m)	1.11 (m)	1.18 (m)	1.13 (m)	1.24 (m)
14	1.01 (m)	0.90 (m)	1.02 (m)	0.92 (m)	1.08 (d, 9.3)
15	4.42 (td, 6.5, 1.8)	4.17 (td, 6.6, 1.9)	4.42 (td, 6.6, 2.0)	4.17 (td, 6.5, 2.1)	3.88 (td, 9.3, 3.2)
16	2.36 (dt, 15.0, 7.0)	2.41 (dt, 14.8, 8.2)	2.37 (m)	2.41 (dt, 14.6, 8.2)	1.90 (m)
	1.39 (m)	1.35 (m)	1.39 (m)	1.35 (m)	1.73 (m)
17	1.03 (m)	1.09 (m)	1.01 (m)	1.11 (m)	1.43 (m)
18	1.25 (s)	0.94 (s)	1.27 (s)	0.95 (s)	0.75 (s)
19	1.15 (s)	1.07 (s)	0.99 (s)	0.87 (s)	1.04 (s)
20	1.57 (m)	1.57 (m)	1.57 (m)	1.57 (m)	1.42 (m)
21	0.94 (d, 6.0)	0.97 (d, 6.5)	0.95 (d, 6.5)	0.97 (d, 6.5)	0.96 (d, 5.8)
22	1.57 (m)	1.58 (m)	1.57 (m)	1.59 (m)	1.56 (m)
	1.18 (m)	1.19 (m)	1.18 (m)	1.20 (m)	1.17 (m)
23	2.10 (m)	2.10 (m)	2.11 (m)	2.12 (m)	2.11 (m)
	1.95 (m)	1.95 (m)	1.95 (m)	1.95 (m)	1.95 (m)
25	2.26 (sextet, 6.7)	2.27 (sextet, 6.7)	2.27 (sextet, 6.8)	2.26 (m)	2.26 (sextet, 6.7)
26	3.55 (dd, 11.0, 6.0)	3.57 (dd, 10.7, 5.8)	3.56 (dd, 10.7, 5.8)	3.57 (dd, 10.7, 5.8)	3.56 (dd, 10.7, 5.8)
	3.34 (dd, 11.0, 7.5)	3.35 (dd, 10.7, 7.5)	3.35 (dd, 10.7, 7.5)	3.34 (dd, 10.7, 7.5)	3.35 (dd, 10.7, 7.6)
27	1.05 (d, 7.0)	1.07 (d, 6.6)	1.06 (d, 6.9)	1.07 (d, 6.5)	1.06 (d, 6.9)
24[1]	4.78 (br s)	4.79 (br s)	4.79 (br s)	4.80 (br s)	4.79 (br s)
	4.76 (br s)	4.76 (br s)	4.77 (br s)	4.77 (br s)	4.77 (br s)

[a] Multiplicities and coupling constants are in parentheses. [b] Spectrum of **1** was recorded at 500 MHz.

Certonardosterol H (**9**) was isolated as light yellow needles. The HRFABMS showed a pseudomolecular ion peak at *m/z* 443.3134 $[M + Na]^+$ (calcd for $C_{26}H_{44}NaO_4$, 443.3137). Comparison of its NMR data with those for **8** revealed the presence of a 3*β*,6α,15*β*-trihydroxylation pattern. In addition to the signals attributable to the steroidal nucleus, the ^{13}C NMR spectrum showed seven more signals, including two olefinic carbons, one oxymethylene, and two methyl carbons. In the HMBC experiment, the olefinic carbon at *δ* 131.6 showed correlations with methyl protons (*δ* 0.97) and oxymethylene protons (*δ* 3.42 and 3.30), and the cross-peak between the other olefinic carbon (*δ* 137.9) and methyl protons (*δ* 1.01) was observed. Thus, the structure of **9** was established as (*E*)-26,27-dinor-24*ξ*-methyl-5α-cholest-22-ene-3*β*,6α,15*β*,25-tetrol. Similar oxidized and shortened side chains have been found in polyhydroxysterols from the starfish *Hacelia attenuata*, *Myxoderma platyacanthum*, and *Acodontaster conspicuus*.[8] Compound **9** may be of a dietary origin since it has been suggested that the marine C_{26} sterols (i.e., 26,27-dinor-24-methylcholestane) originate from phytoplankton.[17]

Certonardosterol I (**10**) was isolated as light yellow needles and showed a pseudomolecular ion peak at *m/z* 501.3545 $[M + Na]^+$ (calcd for $C_{29}H_{50}NaO_5$, 501.3556) in the HRFABMS spectrum. Compound **10** possessed the same steroidal nucleus as that of **6** as implied by the NMR data (Tables 2 and 4). The NMR data of the side chain were characterized by a trisubstituted olefin, four methyl groups, and an oxymethylene group. The long-range coupling of the olefinic proton at *δ* 4.91 with the methyl protons at *δ* 1.59 established the location of the latter at C-23.[1] The H-21 and H-24[1] methyl protons were assigned on the basis of the HMBC data, which showed cross-peaks between C-22 (*δ* 132.9) and H-21 (*δ* 0.94) and between C-23 (*δ* 136.2) and H-24[1] (*δ* 0.95). The doublet at *δ* 0.88 was attributed to H-27 methyl protons. The geometry of the double bond was assumed to be *E* by analogy with similar NMR data of dinosterol,[18] acerosterol,[19] and other related compounds from starfish.[20] The NMR data of H-26 (*δ* 3.49, 3.15), H-27 (*δ* 0.88), and H-24[1] (*δ* 0.95) were indicative of the *threo* configuration at C-24 and -25, while the *erythro* analogue would show the corresponding signals at *δ* 3.53, 3.34, 0.87, and 1.02, respectively.[21] Because of the structural similarity between the 26-hydroxy-23,24-dimethylcholest-22-ene side chain and the 26-hydroxy-24-methylcholest-22-ene side chain, the configuration at C-24 and -25 in **10** could be assigned on the basis of the ^{1}H NMR analysis of the MTPA esters in the same manner as that for the 26-hydroxy-24-methylcholest-22-ene side chain.[21] It was reported that the ^{1}H NMR spectrum of the (*R*)-MTPA ester of the 25*S* isomer displays a smaller Δ*δ* of the H-26 geminal proton signals than that of the 25*R* isomer. Such behavior is reversed in the (*S*)-MTPA ester; the H-26 signal of the 25*R* isomer displays a smaller Δ*δ*.[21] The ^{1}H NMR spectrum of the (*R*)-MTPA ester of **10** showed the H-26 methylene proton signals as two well-separated doublets of doublets at *δ* 4.32 and 3.97, while that of the (*S*)-MTPA ester showed two close doublets of doublets at *δ* 4.20 and 4.06. Thus, the (24*S*,25*R*) configuration was assigned and the structure of **10** was defined as (*E*)-(24*S*,25*R*)-23,24-dimethyl-5α-cholest-22-ene-3*β*,6α,8,15*β*,26-pentol.

Certonardosterol J (**11**) was isolated as colorless needles. In the HRFABMS spectrum, it showed a pseudomolecular ion peak at *m/z* 485.3603 $[M + Na]^+$ (calcd for $C_{29}H_{50}NaO_4$,

Table 2. ^{1}H NMR Data of Compounds **6**−**10** (CD_3OD, 500 MHz)[a]

position	6	7	8[b]	9	10
1	1.71 (m)	1.71 (dt, 13.0, 3.5)	1.72 (dt, 13.1, 3.4)	1.71 (dt, 13.0, 3.5)	1.72 (dt, 13.0, 3.5)
	0.98 (m)	1.03 (m)	1.04 (m)	1.02 (m)	0.98 (m)
2	1.73 (m)	1.76 (m)	1.77 (m)	1.75 (m)	1.74 (m)
	1.48 (m)	1.43 (m)	1.43 (m)	1.42 (m)	1.49 (m)
3	3.48 (m)	3.47 (m)	3.48 (m)	3.47 (m)	3.47 (m)
4	2.18 (dt, 12.5, 2.0)	2.18 (dt, 12.5, 2.3)	2.19 (m)	2.18 (m)	2.19 (m)
	1.19 (t, 12.5)	1.16 (m)	1.18 (m)	1.17 (m)	1.19 (t, 11.8)
5	1.03 (m)	1.02 (m)	1.02 (m)	1.02 (m)	1.03 (m)
6	3.69 (td, 10.8, 4.5)	3.38 (td, 10.8, 4.5)	3.39 (td, 10.7, 4.4)	3.37 (td, 10.8, 4.5)	3.69 (td, 10.5, 4.0)
7	2.37 (m)	2.27 (dt, 12.0, 4.0)	2.28 (m)	2.26 (dt, 11.5, 4.3)	2.36 (dd, 12.5, 4.5) 1.27 (m)
	1.27 (m)	0.92 (m)	0.92 (m)	0.90 (m)	
8		1.88 (m)	1.88 (qd, 11.3, 2.9)	1.87 (m)	
9	0.83 (dd, 13.0, 3.0)	0.71 (td, 11.5, 3.3)	0.73 (td, 11.6, 3.4)	0.71 (td, 11.8, 4.0)	0.84 (dd, 12.5, 2.5)
11	1.80 (qd, 13.0, 3.0)	1.52 (m)	1.53 (m)	1.53 (m)	1.81 (qd, 13.0, 3.0)
	1.49 (m)	1.33 (m)	1.34 (m)	1.33 (m)	1.50 (m)
12	1.99 (dt, 13.0, 3.3)	1.95 (dt, 12.0, 3.0)	1.94 (dt, 12.5, 3.2)	1.92 (dt, 12.0, 3.0)	1.97 (dt, 13.0, 3.3)
	1.16 (m)	1.10 (m)	1.15 (m)	1.13 (m)	1.18 (m)
14	1.01 (m)	0.91 (m)	0.92 (m)	0.91 (m)	1.01 (d, 5.5)
15	4.41 (td, 6.8, 2.8)	4.16 (td, 6.8, 2.3)	4.14 (td, 6.6, 2.1)	4.12 (td, 6.5, 2.0)	4.36 (td, 6.5, 2.0)
16	2.36 (m)	2.39 (dt, 15.0, 8.0)	2.28 (m)	2.21 (m)	2.18 (m)
	1.39 (m)	1.36 (m)	1.36 (m)	1.33 (m)	1.27 (m)
17	0.99 (m)	1.07 (m)	1.13 (m)	1.09 (m)	1.03 (m)
18	1.26 (s)	0.94 (s)	0.97 (s)	0.94 (s)	1.31 (s)
19	0.98 (s)	0.86 (s)	0.88 (s)	0.86 (s)	0.98 (s)
20	1.53 (m)	1.51 (m)	2.22 (m)	2.14 (m)	2.52 (m)
21	0.93 (d, 6.5)	0.95 (d, 6.5)	1.05 (d, 6.6)	1.01 (d, 6.5)	0.94 (d, 6.5)
22	1.62 (m)	1.59 (m)	5.46 (dd, 15.4, 8.5)	5.29 (dd, 15.0, 8.0)[c]	4.91 (d, 9.5)
	0.98 (m)	1.00 (m)			
23	1.54 (m)	1.54 (m)	5.38 (dd, 15.4, 6.8)	5.26 (dd, 15.0, 7.0)[c]	
	1.23 (m)	1.23 (m)			
24	3.20 (m)	3.21 (m)	3.69 (t, 6.3)	2.22 (m)	1.92 (m)
25	1.61 (m)	1.61 (m)	1.65 (sextet, 6.6)	3.42 (dd, 10.8, 6.3)	1.64 (m)
				3.30 (m)[d]	
26	0.90 (d, 7.0)	0.91 (d, 7.0)	0.92 (d, 6.8)		3.49 (dd, 10.5, 5.0)
					3.15 (dd, 10.5, 7.8)
27	0.88 (d, 7.0)	0.89 (d, 7.0)	0.87 (d, 6.8)	0.87 (d, 6.8)	0.88 (d, 6.5)
23[1]					1.59 (d, 1.0)
24[1]				0.97 (d, 7.0)	0.95 (d, 7.0)

[a] Multiplicities and coupling constants are in parentheses. [b] Spectrum of **8** was recorded at 600 MHz. [c] The δ and J values were analyzed by spectrum simulation. [d] Overlapped with the solvent signal.

485.3607). The NMR data indicated that it shares the same sterol nucleus with **9** and shares the same side chain with **10** (Tables 2, 3, and 4). Thus, the structure of **11** was defined as (*E*)-(24*S*,25*R*)-23,24-dimethyl-5α-cholest-22-ene-3β,6α,15β,26-tetrol. To the best of our knowledge, compounds **10** and **11** are the first example of sterols of natural origin with the 26-hydroxy-23,24-dimethylcholest-22-ene side chain. Two sterols with further hydroxylation of the same side chain had been isolated from the starfish *Styracaster caroli*.[20] The 23,24-dimethylcholest-22-ene side chain is typical of dinosterol, which was reported from dinoflagellates and diatoms.[22,23] Since dinoflagellates along with diatoms constitute the very basis of the marine food chain, the occurrence of **10**, **11**, and the above-mentioned sterols from starfish may be of some interest as an indication of the capability of starfish to oxidize dietary sterols.

Certonardosterol K (**12**) was obtained as an inseparable mixture with the known compound **13**, previously isolated from two Antarctic starfish.[7,8] The NMR data implied that the two compounds shared the same steroidal nucleus with **1**. In addition, the ^{1}H NMR spectrum showed two isolated olefinic proton signals in an integral ratio of 3:1. The olefinic proton signal of the major component was observed as a complex multiplet at δ 5.18 (δ_C134.6 and 136.7). In the HMBC experiment, the cross-peaks between the signals at δ_C134.6 and δ 0.97 and between the signals at δ_C136.7 and δ 0.98 were observed, and the oxymethylene protons (δ 3.55) showed correlations with signals at δ 34.7 and 40.9. These suggested the presence of a 27-nor-26-hydroxy-24-methylcholest-22-ene side chain for the major compound **12**. The olefinic proton signal of the minor component was observed as two doublets of doublets at δ 5.43 and 5.36, and the latter was coupled with a triplet at δ 3.68, which is ascribable to an oxymethine proton. Thus, the 24-hydroxycholest-22-ene side chain was assigned to the minor compound **13**. The 24*R* configuration was proposed for **13** by analogy with co-occurring sterol **8**.

Certonardosterol L (**14**) was isolated as colorless needles. In the HRFABMS spectrum, it gave a pseudomolecular ion peak at *m*/*z* 473.3242 $[M + Na]^+$ (calcd for $C_{27}H_{46}NaO_5$, 473.3243). The NMR data (Tables 3 and 4) showed that it is the 8-deoxy derivative of **12**. The sterol with the 27-nor-26-hydroxy-24-methylcholest-22-ene side chain of natural origin was not described before, while the steroid saponins with this side chain have been found in the starfish *Coscinasterias tenuispina*,[24] *Sphaerodiscus placenta*,[25] and *Henricia downeyae*.[26]

Certonardosterol M (**15**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 505.3497 $[M + Na]^+$ (calcd for $C_{28}H_{50}NaO_6$, 505.3505). The NMR data indicated that it shares the same steroidal nucleus with **1**. In the methyl region of the ^{1}H NMR spectrum, three doublets were observed. The doublet at δ 0.92 was assigned to H-21. The other two doublets at δ 0.80 and 0.77 showed correlations with the carbon signals at δ 41.0 and 34.8, respectively, and the former showed correlation with the oxymethine carbon at δ 66.9. These implied the presence of the 26-hydroxy-24-methylcholestane side chain. The *threo* configuration at C-24 and -25

Table 3. ^{1}H NMR Data of Compounds **11**−**15** (CD_3OD, 500 MHz)[a]

position	**11**[b]	**12**	**13**	**14**	**15**
1	1.72 (m)	1.71 (dt, 13.5 3.5)	1.71 (dt, 13.5 3.5)	1.68 (dt, 13.5, 3.5)	1.71 (dt, 13.0, 3.5)
	1.02 (m)	0.98 (m)	0.98 (m)	1.04 (m)	0.99 (m)
2	1.76 (m)	1.82 (m)	1.82 (m)	1.80 (m)	1.83 (m)
	1.43 (m)	1.55 (m)	1.55 (m)	1.56 (m)	1.56 (m)
3	3.48 (m)	3.43 (ddd, 12.0, 5.0, 3.5)	3.43 (ddd, 12.0, 5.0, 3.5)	3.42 (m)	3.43 (m)
4	2.19 (dt, 12.5, 2.1)	4.25 (br s)	4.25 (br s)	4.21 (br s)	4.25 (br s)
	1.16 (m)				
5	1.01 (m)	0.94 (m)	0.94 (m)	0.91 (m)	0.93 (m)
6	3.39 (td, 10.7, 4.4)	4.15 (td, 11.0, 4.2)	4.15 (td, 11.0, 4.2)	3.89 (td, 11.5, 5.0)	4.16 (td, 11.0, 4.4)
7	2.27 (dt, 12.0, 4.0)	2.43 (dd, 12.5, 4.3)	2.43 (dd, 12.5, 4.3)	2.34 (dt, 11.5, 4.3)	2.44 (dd, 12.5, 4.3)
	0.91 (m)	1.29 (m)	1.29 (m)	0.92 (m)	1.29 (m)
8	1.88 (m)			1.88 (m)	
9	0.73 (td, 11.5, 3.4)	0.82 (dd, 12.5, 3.0)	0.82 (dd, 12.5, 3.0)	0.67 (td, 11.5, 3.7)	0.82 (m)
11	1.54 (m)	1.78 (m)	1.78 (m)	1.43 (m)	1.75 (m)
	1.33 (m)	1.44 (m)	1.44 (m)	1.30 (m)	1.43 (m)
12	1.93 (m)	1.96 (dt, 12.5, 3.3)	1.96 (dt, 12.5, 3.3)	1.91 (dt, 12.5, 2.5)	1.98 (dt, 13.0, 3.3)
	1.12 (m)	1.17 (m)	1.17 (m)	1.11 (m)	1.15 (m)
14	0.91 (m)	1.00 (d, 5.5)	1.00 (d, 5.5)	0.88 (m)	1.00 (d, 5.5)
15	4.12 (td, 6.6, 2.0)	4.38 (td, 6.5, 2.3)	4.38 (td, 6.5, 2.3)	4.13 (td, 6.5, 2.0)	4.41 (td, 6.5, 2.3)
16	2.23 (dt, 15.1, 8.6)	2.20 (m)	2.20 (m)	2.21 (m)	2.35 (dt, 14.5, 8.0)
	1.26 (m)	1.36 (m)	1.36 (m)	1.32 (m)	1.38 (m)
17	1.03 (m)	1.02 (m)	1.02 (m)	1.09 (m)	0.98 (m)
18	0.98 (s)	1.27 (s)	1.28 (s)	0.94 (s)	1.25 (s)
19	0.88 (s)	1.15 (s)	1.15 (s)	1.06 (s)	1.15 (s)
20	2.52 (m)	2.11 (m)	2.21 (m)	2.12 (m)	1.53 (m)
21	0.96 (d, 6.5)	0.98 (d, 7.0)	1.02 (d, 6.5)	1.00 (d, 7.0)	0.92 (d, 6.5)
22	4.94 (d, 9.8)	5.19 (dd, 15.0, 8.0)[c]	5.43 (dd, 15.5, 8.0)	5.19 (dd, 15.0, 8.0)[c]	1.39 (m)
					1.08 (m)
23		5.16 (dd, 15.0, 7.0)[c]	5.36 (dd, 15.5, 7.0)	5.16 (dd, 15.0, 7.0)[c]	1.23 (m)
24	1.92 (m)	2.18 (m)	3.68 (t, 6.3)	2.18 (m)	1.55 (m)
25	1.64 (m)	1.47 (m)	1.64 (sextet, 6.6)	1.47 (m)	1.60 (m)
26	3.51 (dd, 10.7, 4.7)	3.55 (m)	0.90 (d, 7.0)	3.56 (m)	3.46 (dd, 11.0, 6.5)
	3.17 (dd, 10.7, 8.0)	3.17 (dd, 10.7, 8.0)			3.34 (dd, 11.0, 7.3)
27	0.89 (d, 6.7)	0.89 (d, 6.7)	0.86 (d, 6.5)	0.86 (d, 6.5)	0.80 (d, 7.0)
23^1	1.60 (d, 1.1)	1.60 (d, 1.1)			
24^1	0.97 (d, 7.0)	0.97 (d, 7.0)		0.97 (d, 6.5)	0.77 (d, 6.5)

[a] Multiplicities and coupling constants are in parentheses. [b] Spectrum of **11** was recorded at 600 MHz. [c] The δ and J values were analyzed by spectrum simulation.

Table 4. ^{13}C NMR Data of Compounds **1**−**15** (CD_3OD, 50 MHz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	39.7	38.8	39.4	38.6	39.9	39.4	38.6	38.6	38.6	39.4	38.6	39.7	39.7	38.8	39.7
2	26.2	26.3	31.5	32.0	32.2	31.5	32.0	32.0	32.0	31.5	32.0	26.2	26.2	26.3	26.2
3	73.7	73.7	72.2	72.0	72.4	72.2	72.0	72.0	72.0	72.2	72.0	73.7	73.7	73.7	73.7
4	69.1	69.1	32.4	33.0	36.3	32.3	33.0	33.0	33.0	32.4	33.0	69.1	69.1	69.1	69.1
5	57.3	56.6	53.9	53.1	48.9	53.9	53.1	53.1	53.1	53.9	53.1	57.3	57.3	56.6	57.3
6	64.8	66.6	67.7	70.0	72.5	67.7	70.0	70.0	70.0	67.6	70.0	64.7	64.7	66.6	64.8
7	49.7	41.9	49.4	41.8	40.8	49.6	41.8	41.8	41.8	49.5	41.8	49.6	49.6	41.9	49.8
8	77.4	31.5	77.5	31.6	31.4	77.5	31.6[a]	31.6	31.6	77.5	31.6	77.4	77.4	31.5	77.4
9	58.4	56.5	57.4	55.7	55.8	57.4	55.7	55.7	55.7	57.4	55.8	58.4	58.4	56.6	58.4
10	38.1	37.5	38.0	37.5	36.6	38.0	37.5	37.5	37.5	38.0	37.5	38.2	38.2	37.5	38.1
11	19.2	21.5	19.7	22.2	22.1	19.7	22.2	22.2	22.2	19.7	22.2	19.2	19.2	21.7	19.2
12	43.3	42.6	43.4	42.6	41.6	43.4	42.7	42.5	42.8	43.3	42.6	43.2	43.2	42.5	43.3
13	44.4	43.5	44.4	43.5	45.0	44.4	43.4	43.4	43.3	44.2	43.3	44.2	44.2	43.3	44.4
14	62.7	62.3	62.6	62.1	63.8	62.6	62.2	62.2	62.2	62.7	62.2	62.9	62.9	62.4	62.7
15	71.1	70.6	71.1	70.6	74.2	71.1	70.6	70.6	70.6	71.2	70.7	71.1	71.1	70.6	71.1
16	42.5	42.2	42.5	42.3	41.8	42.5	42.3	42.8	42.5	42.8	42.3	43.4	43.4	43.0	42.5
17	57.8	57.7	57.9	57.7	54.9	58.0	57.8	57.5	57.5	58.5	58.4	57.6	57.6	57.5	57.9
18	16.5	15.1	16.5	15.2	13.7	16.5	15.2	15.3	15.3	16.8	15.5	16.6	16.6	15.3	16.5
19	17.0	16.1	14.1	13.8	16.3	14.1	13.8	13.8	13.8	14.1	13.8	17.0	17.0	16.1	17.0
20	36.0	36.6	36.0	36.6	36.3	36.5	37.1	41.1	41.3	35.3	35.8	41.0[a]	40.6	41.4	36.3
21	18.9	19.2	18.9	19.2	19.0	19.5	19.4	21.2	21.3	20.7	21.0	21.1	20.8	21.4	19.0
22	35.5	35.7	35.5	35.7	35.7	33.3	33.5	139.2	137.9	132.9	132.9	136.7	139.1	136.8	34.7[a]
23	32.7	32.7	32.7	32.7	32.6	31.7	31.7[a]	130.1	131.6	136.2	136.2	134.6	130.1	134.6	32.4
24	153.8	153.7	153.8	153.7	153.7	78.1	78.1	78.9	40.5	45.9	45.9	34.7	78.9	34.7	34.8[a]
25	43.3	43.3	43.3	43.3	43.3	34.5	34.5	35.3	68.4	39.2	39.2	40.9[a]	35.3	41.0	41.0
26	67.5	67.5	67.5	67.5	67.5	19.0	19.5	18.8		67.2	67.2	61.3	18.8	61.3	66.9
27	17.2	17.2	17.2	17.2	17.2	17.5	17.5	18.7		14.3	14.3		18.7		11.9
23^1										14.0	14.0				
24^1	109.4	109.4	109.4	109.4	109.4				17.3	16.4	16.4	21.7		21.5	14.6

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

was assigned by comparison of the ^{1}H and ^{13}C NMR data with those for model compounds.[21] In particular, the chemical shifts of H-27, -24^1 and C-27, -24^1 (Tables 3 and 4) were very close to those of the *threo* model compounds

Table 5. Selected ^{1}H NMR Data of the MTPA Esters of **1**, **6**, **8**, **10**, and **15** (CD_3OD, 500 MHz)[a]

MTPA ester	H-26	H-26, H-27
(*S*)-MTPA ester of **1**	4.38 (dd, 10.8, 6.5)	
	4.15 (dd, 10.8, 6.5)	
(*R*)-MTPA ester of **1**	4.33 (dd, 11.3, 6.8)	
	4.20 (dd, 11.3, 6.8)	
(*R*)-MTPA ester of **6**		0.85 (d, 6.5)
		0.82 (d, 7.0)
(*S*)-MTPA ester of **8**		0.94 (d, 7.0)
		0.92 (d, 6.5)
(*R*)-MTPA ester of **8**		0.84 (d, 7.0)
		0.83 (d, 7.0)
(*S*)-MTPA ester of **10**	4.20 (dd, 10.5, 3.8)	
	4.06 (dd, 10.5, 5.5)	
(*R*)-MTPA ester of **10**	4.32 (dd, 11.0, 4.0)	
	3.97 (dd, 11.0, 6.3)	
(*R*)-MTPA ester of **15**	4.24 (br d)	

[a] Multiplicities and coupling constants are in parentheses.

Table 6. Cytotoxicity Data of Compounds **1**−**11**, **14**, and **15** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	3.1	7.3	4.1	4.4	4.5
2	3.6	6.9	4.9	4.7	6.4
3	14.1	20.2	15.1	13.3	23.9
4	5.3	8.5	5.5	4.8	9.6
5	3.9	4.9	4.1	4.1	4.2
6	11.3	15.6	12.4	11.4	19.4
7	3.8	4.4	3.8	4.3	5.5
8	3.6	3.6	3.4	3.7	4.0
9	7.1	9.4	6.4	8.6	10.6
10	5.1	8.8	5.3	7.3	6.3
11	8.0	10.3	7.1	11.4	12.5
14	4.3	6.0	4.3	4.5	4.2
15	>30	>30	>30	>30	>30
doxorubicin	0.02	0.17	0.02	0.06	0.03

[a] Data as expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer.

[(24*R*,25*S*) and (24*S*,25*R*)] and quite distinct from those of the *erythro* model compounds (24*R*,25*R* isomer: δ 0.91, 0.91 and δ_C 17.4, 14.1; 24*S*,25*S* isomer: δ 0.93, 0.92 and δ_C 17.6, 14.4).[21] In the ^{1}H NMR spectrum of the (*R*)-MTPA ester of **15**, the H-26 signal appeared as a broad doublet at δ 4.24, which is very close to that of the 24*R*,25*S* isomer (δ 4.23, br d) and distinct from that of the 24*S*,25*R* isomer (δ 4.34, dd and 4.14, dd). Thus, the 24*R*,25*S* configuration was assigned, and the structure was established as (24*R*,25*S*)-24-methyl-5α-cholestane-3β,4β,6α,8,15β,26-hexol.

The isolated compounds have been tested for cytotoxicity against a small panel of human solid tumor cell lines (Table 6), and most of them showed moderate to significant cytotoxicity. In general, the cytotoxicity is related to both the hydroxylation pattern of the steroidal nucleus and the functionalization of the side chain. Among the sterols with 26-hydroxy-24-methylcholest-24(24^1)-ene side chains or 24-hydroxylated side chains, compounds **3** and **6**, with the 3β,6α,8,15β-tetrahydroxylation pattern, displayed less potent cytotoxicity than the others. Compound **15** was inactive, although it shares the same steroidal nucleus with the active compound **1**.

Certain sterols of structure similar to compounds **1**−**11** were reported to show antimicrobial activity.[8,27] Therefore, compounds **1**−**11** were assayed for antibacterial activity against 20 clinically isolated strains. Most of the compounds displayed only weak antibacterial activity against *Streptococcus pyogenes* 308A, *Pseudomonas aeruginosa* 1771, and *Pseudomonas aeruginosa* 1771M.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a JASCO DIP-370 digital polarimeter. IR spectra were measured by a JASCO FT/IR-410 spectrophotometer. ^{1}H 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 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 i.d., 5 μm, 120 Å), a C18-5E Shodex packed column (250 × 10 mm i.d., 5 μm, 100 Å), and a Vydac column (250 × 10 mm i.d., 5 μm, 90 Å) using a Shodex RI-71 detector.

Animal Material. The starfish was collected in July 2000, off the coast of Komun Island, Korea.[2] The specimen was identified by Prof. Sook Shin, Sahmyook University, Seoul, Korea. The voucher specimen (J00K-4) of the starfish was deposited at the Marine Natural Product 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 6 (0.84 g) was very active in the brine shrimp assay (LD_{50} 38 ppm) and was further separated by normal-phase MPLC (Silica gel 60, 400/230 mesh), eluting with a solvent system of 20 to100% MeOH/$CHCl_3$, to afford 20 fractions. Compounds **1** (6.9 mg) and **2** (7.0 mg) were obtained by separation of subfraction 6-9 on a reversed-phase HPLC (Vydac, 250 × 10 mm i.d., 5 μm, 90 Å) column eluting with 80% MeOH, followed by purification on the same column eluting with 75% MeOH. Compounds **3**−**11** (**3**, 1.9 mg; **4**, 2.6 mg; **5**, 2.2 mg; **6**, 3.5 mg; **7**, 9.4 mg; **8**, 10.8 mg; **9**, 3.1 mg; **10**, 3.3 mg; **11**, 1.4 mg) were obtained by separation of subfraction 6-8 on a reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm i.d., 5 μm, 120 Å) column eluting with 80% MeOH, followed by purification on a C18-5E Shodex packed column (250 × 10 mm i.d., 5 μm, 100 Å) eluting with the same mobile phase. Compounds **12** and **13** were obtained as a mixture (6.8 mg) by separation of subfraction 6-11 on a reversed-phase HPLC (Vydac, 250 × 10 mm i.d., 5 μm, 90 Å) column eluting with 80% MeOH, followed by purification on a C18-5E Shodex packed column (250 × 10 mm i.d., 5 μm, 100 Å) eluting with the same mobile phase. Compounds **14** (1.1 mg) and **15** (2.2 mg) were obtained by separation of subfraction 6-10 on a reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm i.d., 5 μm, 120 Å) column eluting with 80% MeOH, followed by purification on the same column eluting with the same mobile phase.

Preparation of MTPA Esters. Compounds **1** (4 μmol), **8** (3 μmol), and **10** (3 μmol) were treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (12 μmol) 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 original spot had disappeared. After removal of solvent, the product was purified by reversed-phase HPLC on a YMC-Pack ODS column (250 × 10 mm i.d., 5 μm, 120 Å) and analyzed by ^{1}H NMR. Compounds **6** (3 μmol) and **15** (3 μmol) were treated with only (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (12 μmol) in dry pyridine (25 μL) for 24 h at room temperature, respectively. The following procedure was the same as that for compound **1**.

Certonardosterol A (1): colorless needles; $[\alpha]^{21}_D$ +16° (*c* 0.23, MeOH); IR (KBr disk) ν_{max} 3367, 2943, 1639, 1434, 1161, 1044, 1023, 964 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR

data, see Table 4; FABMS (+ve) *m/z* 503 $[M + Na]^+$ (9.0), 473 (1.0), 445 (6.0); HRFABMS (+ve) *m/z* 503.3362 (calcd for $C_{28}H_{48}NaO_6$, 503.3349).

(*S*)-MTPA ester of certonardosterol A: ^1H NMR (CD_3OD), δ 4.38 (1H, dd, 10.8, 6.5, H-26), 4.15 (1H, dd, 10.8, 6.5, H-26), 1.27 (3H, s, H-19), 1.25 (3H, s, H-18), 1.05 (3H, d, 7.0, H-27), 0.92 (3H, d, 6.5, H-21).

(*R*)-MTPA ester of certonardosterol A: ^1H NMR (CD_3OD), δ 4.33 (1H, dd, 11.3, 6.8, H-26), 4.20 (1H, dd, 11.3, 6.8, H-26), 1.28 (3H, s, H-19), 1.26 (3H, s, H-18), 1.05 (3H, d, 7.0, H-27), 0.92 (3H, d, 6.5, H-21).

Certonardosterol B (2): colorless needles; $[\alpha]^{21}_D$ −9.1° (*c* 0.15, MeOH); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 487 $[M + Na]^+$ (31.5), 457 (2.6), 429 (14.0); HRFABMS (+ve) *m/z* 487.3397 (calcd for $C_{28}H_{48}NaO_5$, 487.3399).

Certonardosterol C (3): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 487 $[M + Na]^+$ (6.2), 457 (2.4), 429 (2.0), 403 (5.5), 349 (16.0); HRFABMS (+ve) *m/z* 487.3389 (calcd for $C_{28}H_{48}NaO_5$, 487.3399).

Certonardosterol D (4): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 471 $[M + Na]^+$ (8.5), 441 (2.0), 413 (11.0), 329 (16.0); HRFABMS (+ve) *m/z* 471.3448 (calcd for $C_{28}H_{48}NaO_4$, 471.3450).

Certonardosterol E (5): colorless needles; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 471 $[M + Na]^+$ (15.5), 449 $[M + H]^+$ (2.2), 413 (17.5), 329 (18.0); HRFABMS (+ve) *m/z* 471.3449 (calcd for $C_{28}H_{48}NaO_4$, 471.3450).

Compound 6: light yellow needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 475 $[M + Na]^+$ (12.5), 435 (8.0), 417 (12.0), 399 (15.5); HRFABMS (+ve) *m/z* 475.3408 (calcd for $C_{27}H_{48}NaO_5$, 475.3399).

(*R*)-MTPA ester of compound 6: ^1H NMR (CD_3OD), δ 1.27 (3H, s, H-18), 1.08 (3H, s, H-19), 0.93 (3H, d, 6.0, H-21), 0.85 (3H, d, 6.5, H-26 or H-27), 0.82 (3H, d, 7.0, H-26 or H-27).

Certonardosterol F (7): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 459 $[M + Na]^+$ (17.0), 419 (15.0), 401 (16.5), 383 (20.0); HRFABMS (+ve) *m/z* 459.3438 (calcd for $C_{27}H_{48}NaO_4$, 459.3450).

Certonardosterol G (8): colorless needles; IR (KBr disk) ν_{max} 3364, 2930, 1666, 1591, 1451, 1042, 959 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 457 $[M + Na]^+$ (26.5), 417 (10.0), 399 (28.0), 381 (19.0), 329 (16.0); HRFABMS (+ve) *m/z* 457.3303 (calcd for $C_{27}H_{46}NaO_4$, 457.3294).

(*S*)-MTPA ester of certonardosterol G: ^1H NMR (CD_3OD), δ 1.00 (3H, d, 6.0, H-21), 0.97 (3H, s, H-18), 0.95 (3H, s, H-19), 0.94 (3H, d, 7.0, H-26 or H-27), 0.92 (3H, d, 6.5, H-26 or H-27).

(*R*)-MTPA ester of certonardosterol G: ^1H NMR (CD_3OD), δ 1.05 (3H, d, 6.5, H-21), 0.97 (3H, s, H-18), 0.96 (3H, s, H-19), 0.84 (3H, d, 7.0, H-26 or H-27), 0.83 (3H, d, 7.0, H-26 or H-27).

Certonardosterol H (9): light yellow needles; $[\alpha]^{21}_D$ −7.5° (*c* 0.11, MeOH); ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 443 $[M + Na]^+$ (31.0), 413 (13.6), 385 (10.3), 360 (9.5), 329 (26.5); HRFABMS (+ve) *m/z* 443.3134 (calcd for $C_{26}H_{44}NaO_4$, 443.3137).

Certonardosterol I (10): light yellow needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 501 $[M + Na]^+$ (25.0), 441 (2.0), 413 (4.5), 360 (4.0), 349 (14.0); HRFABMS (+ve) *m/z* 501.3545 (calcd for $C_{29}H_{50}NaO_5$, 501.3556).

(*S*)-MTPA ester of certonardosterol I: ^1H NMR (CD_3OD), δ 4.20 (1H, dd, 10.5, 3.8, H-26), 4.06 (1H, dd, 10.5, 5.5, H-26), 1.55 (3H, d, 1.0, H-23[1]), 1.31 (3H, s, H-18), 1.10 (3H, s, H-19), 0.97 (3H, d, 7.0, H-24[1]), 0.93 (3H, d, 6.5, H-21), 0.91 (3H, d, 6.5, H-27).

(*R*)-MTPA ester of certonardosterol I: ^1H NMR (CD_3OD), δ 4.32 (1H, dd, 11.0, 4.0, H-26), 3.97 (1H, dd, 11.0, 6.3, H-26), 1.57 (3H, d, 1.0, H-23[1]), 1.31 (3H, s, H-18), 1.09 (3H, s, H-19), 0.97 (3H, d, 6.5, H-24[1]), 0.93 (3H, d, 6.5, H-21), 0.88 (3H, d, 6.5, H-27).

Certonardosterol J (11): colorless needles; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 485 $[M + Na]^+$ (12.5), 427 (4.0), 345 (2.0), 329 (19.5); HRFABMS (+ve) *m/z* 485.3603 (calcd for $C_{29}H_{50}NaO_4$, 485.3607).

Certonardosterol K (12) and compound 13: white needles; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 489 $[M + Na]^+$; FABMS (−ve) *m/z* 465 $[M - H]^-$; HRFABMS (+ve) *m/z* 489.3191 (calcd for $C_{27}H_{46}NaO_6$, 489.3192).

Certonardosterol L (14): colorless needles; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 473 $[M + Na]^+$; FABMS (−ve) *m/z* 449 $[M - H]^-$ (100), 429 (19); HRFABMS (+ve) *m/z* 473.3242 (calcd for $C_{27}H_{46}NaO_5$, 473.3243).

Certonardosterol M (15): colorless needles; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS (+ve) *m/z* 505 $[M + Na]^+$; FABMS (−ve) *m/z* 481 $[M - H]^-$ (100), 463 (6), 443 (4.5); HRFABMS (+ve) *m/z* 505.3497 (calcd for $C_{28}H_{50}NaO_6$, 505.3505).

(*R*)-MTPA ester of certonardosterol M: ^1H NMR (CD_3OD), δ 4.24 (2H, br d, H-26), 1.28 (3H, s, H-19), 1.25 (3H, s, H-18), 0.88 (3H, d, 7.0, H-21), 0.84 (3H, d, 7.0, H-27), 0.79 (3H, d, 7.0, H-24[1]).

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

Acknowledgment. A research grant by the Korea Research Foundation (2001-041-F00279) is gratefully acknowledged. High-resolution (600 MHz) NMR spectra were provided by the Korea Basic Science Institute.

Supporting Information Available: Evaluation of antimicrobial activity of compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP020507I