Additional Cytotoxic Sterols and Saponins from the Starfish *Certonardoa semiregularis*

Weihong Wang,[†] Hyojin Jang,[†] Jongki Hong,[‡] Chong-Ok Lee,[§] Kwang Sik Im,[†] Song-Ja Bae,[⊥] and Jee H. Jung*[,†]

College of Pharmacy, Pusan National University, Busan 609-735, Korea, Basic Science Institute, Seoul, Korea, Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology, Daejon, Korea, and Department of Food and Nutrition, Silla University, Busan, Korea

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Twelve new (**1**−**7**, **9**−**13**) polyhydroxysterols and two new saponins (**14** and **15**) were isolated from the starfish *Certonardoa semiregularis* by activity-guided fractionation. Compounds **1**−**7** are rare examples of 15-keto steroids from starfish. The side chain of compound **11** was also unprecedented in nature. The structures were determined by combined spectroscopic methods and chemical derivatization. These compounds were evaluated for cytotoxicity against a small panel of human solid tumor cell lines, and most of them exhibited considerable activity. One of the 15-keto sterols (**6**) displayed the highest potency, which is comparable to that of doxorubicin.

We recently reported several new cytotoxic steroids from the starfish *Certonardoa semiregularis* Muller & Troschel (family Linckiidae).[1−4] The significant cytotoxic activity of these compounds, especially that of certonardosterol D_2 (ED_{50} 0.01−0.15 μg/mL) against a small panel of human solid tumor cell lines,[4] prompted further isolation of new biologically active steroids from the same starfish. Employing the brine shrimp lethality assay to guide fractionation, we isolated 12 new (**1**−**7**, **9**−**13**) and one known (**8**) polyhydroxysterol and two new saponins (**14** and **15**). Compounds **1**−**7** are rare examples of 15-keto steroids from starfish, although the 23-keto steroids were frequently encountered in asterosaponins of starfish,[5,6] and some steroids with a ketone group at C-6 or C-20 were also isolated from starfish.[5,7,8] The 15-keto steroids were found in a few species of sponges recently.[9,10] The side chain of compound **11** was also unprecedented in nature. The structure elucidation including stereochemistry and cytotoxicity of the compounds is described herein.

Results and Discussion

Certonardosterol Q_1 (**1**) was isolated as colorless needles. The molecular formula of $C_{28}H_{46}O_6$ was established from the observation of a molecular ion peak at *m*/*z* 501.3191 $[M + Na]^+$ (calcd 501.3192, Δ −0.1 mmu) in the HR-FABMS. All 28 carbons and 41 protons attached to carbons were observed in the ^{13}C and ^1H NMR spectra. The presence of a ketone group was indicated by the ^{13}C NMR resonance at δ 216.3 and the IR absorption at 1731 cm^{-1}. The presence of hydroxyl groups was supported by a strong OH stretch (3347 cm^{-1}, br) in the IR spectrum, which also revealed a band attributable to C═C at 1639 cm^{-1}. A double bond, a ketone group, and a sterol nucleus accounted for 6 degrees of unsaturation. Three oxymethine signals at δ 3.42 (H-3), 4.10 (H-6), and 4.24 (H-4), which were associated with ^{13}C signals at δ 73.6 (C-3), 64.2 (C-6), and 69.0 (C-4), respectively, were observed in the HSQC spectrum. The methine proton signal at δ 2.09 (correlated with the ^{13}C signal at δ 70.3) was assigned to H-14, which showed a three-bond HMBC correlation with the H-18 signal (δ 1.04). The relatively deshielded H-14 signal (δ 1.01 in the 15-hydroxy sterol[3]) provided the initial indication that the ketone group is located at C-15. The placement of the ketone group at C-15 was corroborated by the HMBC correlations of the carbonyl carbon (δ 216.3) to H-14 (δ 2.09) and H-16 (δ 2.44 and 1.77). The mutually coupled signals at δ 3.07 and 1.21, which were assigned to H-7, were further coupled to the H-6 oxymethine proton signal (δ 4.10). The extreme downfield shift of H_{eq}-7 to δ 3.07 (δ 2.44 in the 15-hydroxy sterol[3]) can be explained by the significant neighboring group effect of the C-15 ketone group.[9] Most of the known 15-keto polyhydroxysterols from sponges are characterized by the uncommon *cis* C/D ring junction.[9,10] The NMR data of **1** disclosed the upfield shifts of the H-18 and C-18 signals and the downfield shift of the C-14 signal compared to the corresponding signals of H-14β steroids (*cis* C/D ring fusion). Thus, the common *trans* C/D ring fusion of **1** was unequivocally defined.[9,10] In addition to the ^1H NMR signals of the sterol nucleus, two secondary methyl (δ 1.05 and 1.01), an oxymethylene (δ 3.55 and 3.34), and an exomethylene (δ 4.78 and 4.77) group were observed, which indicate the presence of the 26-hydroxy-24-methylcholest-24(24[1])-ene side chain. The stereochemistry at C-25 was assumed as *S* by analogy with the co-occurring sterol certonardosterol A.[3] Thus, the structure of certonardosterol Q_1 (**1**) was defined as (25*S*)-3β,4β,6α,8,-26-pentahydroxy-24-methyl-5α-cholest-24(24[1])-en-15-one.

Certonardosterol Q_2 (**2**) was isolated as colorless needles. The molecular formula of **2** was established as $C_{27}H_{46}O_6$ on the basis of the pseudomolecular ion peak at *m*/*z* 489.3191 $[M + Na]^+$ (calcd for $C_{27}H_{46}O_6Na$, 489.3192). The ^1H NMR data showed that **2** shares the same sterol nucleus with **1**. In addition to the signals attributable to the sterol nucleus, the ^1H NMR spectrum of **2** revealed an oxymethine signal at δ 3.21, which showed long-range correlations with the methyl carbon signals at δ 17.6 and 19.5. This suggested the presence of a 24-hydroxy cholesterol side chain. The stereochemistry of C-24 was defined by analysis of the ^1H NMR data of its (*R*)-MTPA ester. The isopropyl methyl proton signals were observed at δ 0.83 and 0.86, 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 24*R* isomer would appear isochronous and

* To whom correspondence should be addressed. Tel: 82-51-510-2803. Fax: 82-51-510-2803. E-mail: jhjung@pusan.ac.kr.
[†] Pusan National University.
[‡] Korea Basic Science Institute.
[§] Korea Research Institute of Chemical Technology.
[⊥] Silla University.

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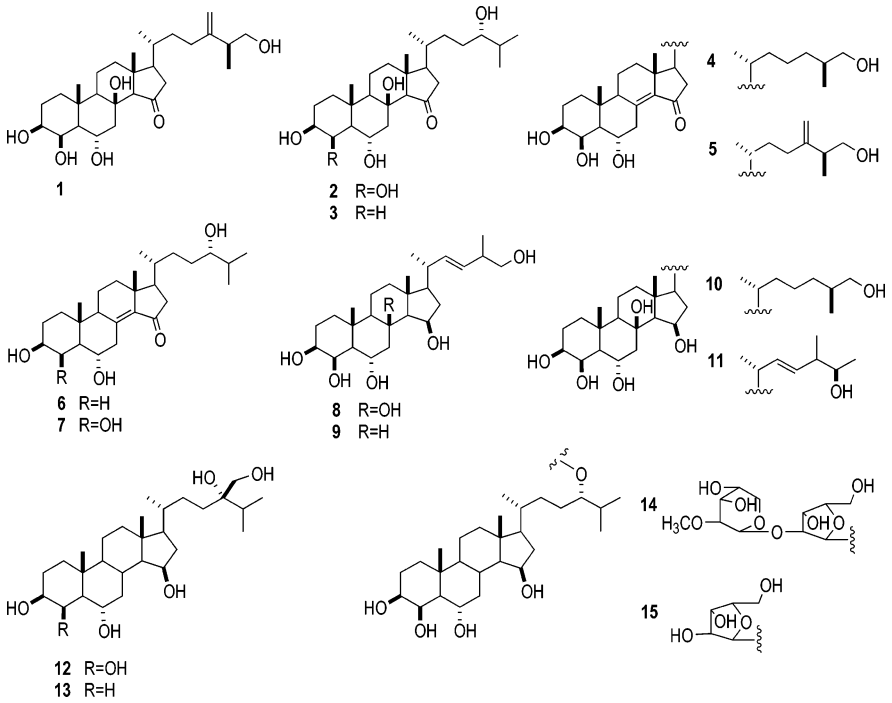
Chart 1



downfield shifted to δ 0.92 (6H, d).[11] Thus, the structure of certonardosterol Q_2 (**2**) was established as (24*S*)-3β,4β,6α,8,24-pentahydroxy-5α-cholestan-15-one.

Certonardosterol Q_3 (**3**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 473.3242 $[M + Na]^+$ (calcd for $C_{27}H_{46}O_5Na$, 473.3243). It was postulated as a 4-deoxy derivative of **2**. Comparison of the ^1H NMR spectrum with that of **2** revealed the lack of the broad signal at δ 4.24, which was assigned to H-4α in **2**, and upfield shifts of the signals of H-6β (δ 4.10→3.64) and H-19 (δ 1.16→1.00). The rest of the signals were almost identical to those of **2**. The stereochemistry at C-24 was proposed as *S* by analogy with the co-occurring compound **2**.

Certonardosterol Q_4 (**4**) was isolated as colorless needles. The molecular formula of **4** was established as $C_{27}H_{44}O_5$ on the basis of HRFABMS and the NMR data. The $[M + Na]^+$ ion was observed at *m*/*z* 471.3085 (calcd for $C_{27}H_{44}O_5Na$, 471.3086). In the HMBC experiment, mutually coupled oxymethylene signals (δ 3.40 and 3.32) showed correlations with the carbon signals at δ 36.9 (C-25), 34.8 (C-24), and 17.2 (C-27), suggesting the presence of the 26-hydroxy cholesterol side chain. Three oxymethine signals were observed at δ 4.23, 3.89, and 3.48, which were assigned to H-4, -6, and -3, respectively. The ketone carbonyl signal at δ 210.0 (C-15), along with the UV absorption at 258 nm and the IR absorption at 1693 cm^{-1}, indicated the presence of an α,β-unsaturated ketone group. The signals of methylene protons α to the carbonyl group were observed at δ 2.34 and 2.10. The significant downfield shift of the H-7 signals (δ 4.46 and 1.56) may be due to anisotropy of the enone function. The H-7 signals showed coupling with the H-6 signal in the COSY spectrum and showed correlations with the carbon signals at δ 149.8 (C-8), 142.1 (C-14), 67.1 (C-6), 55.7 (C-5), and 52.5 (C-9) in the HMBC. The stereochemistry at C-25 was proposed as *S* by analogy with the co-occurring sterol **10** (vide infra). Therefore, the structure of certonardosterol Q_4 (**4**) was defined as (25*S*)-3β,4β,6α,26-tetrahydroxy-5α-cholest-8(14)-en-15-one. Compound **4** may be suspected as a dehydration artifact of the precursors such as **1**−**3**. However, compounds **2** and **3** showed no sign of chemical change after heating at 45 °C for 3 days. Steroids with a conjugated ketone group on the A, B, or C ring have been previously reported from sponges, coelenterates, green algae, brown algae, and red algae,[12] while the $\Delta^{8(14)}$-15-keto steroids were unprecedented in marine organisms.

Certonardosterol Q_5 (**5**) was isolated as colorless needles. In the HRFABMS spectrum, it showed a pseudomolecular ion peak at *m*/*z* 483.3067 $[M + Na]^+$ (calcd for $C_{28}H_{44}O_5Na$, 483.3086). The NMR data indicated that it shares the same sterol nucleus with **4** and shares the same side chain with **1**. Thus, the structure of **5** was defined as (25*S*)-3β,4β,6α,26-tetrahydroxy-24-methyl-5α-cholesta-8(14),24(24^1)-dien-15-one.

Certonardosterol Q_6 (**6**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 455.3130 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_4Na$, 455.3137). The NMR data showed that it shares the same side chain with **2**. The methylene proton signals at δ 2.23 and 1.17 (H-4) showed coupling with the oxymethine signal at δ 3.53 (H-3) and the methine signal at δ 1.30 (H-5) in the COSY spectrum. The oxymethine signal at δ 3.36 was attributed to H-6. The stereochemistry at C-24 was proposed as *S* by analogy with the co-occurring compound **2**. Thus, the structure of **6** was defined as (24*S*)-3β,6α,24-trihydroxy-5α-cholest-8(14)-en-15-one.

Certonardosterol Q_7 (**7**) was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 471.3092 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_5Na$, 471.3086). The NMR data showed that it shares the same sterol nucleus with **5** and shares the same side chain with **6**.

Compound **8** was isolated as colorless needles. The HRFABMS showed a pseudomolecular ion peak at *m*/*z* 475.3030 $[M + Na]^+$ (calcd for $C_{26}H_{44}O_6Na$, 475.3036). The structure was identified by comparison of the NMR data with those reported.[13] Compound **8** was previously isolated from the Antarctic starfish *Acodontaster conspicuus*.[13]

Certonardosterol B_3 (**9**) was isolated as colorless needles. The HRFABMS gave a pseudomolecular ion peak at *m*/*z* 459.3091 $[M + Na]^+$ (calcd for $C_{26}H_{44}O_5Na$, 459.3086). It was an 8-deoxy derivative of **8**, as determined by analysis

of the NMR data. The methylene signals at δ 2.34 and 0.92, which were assigned to H-7, were coupled to the H-6 oxymethine proton signal (δ 3.89) and the H-8 methine proton signal (δ 1.88). The H-8 signal showed additional coupling with the H-9 (δ 0.67) and H-14 (δ 0.89) signals. Thus, the structure of **9** was determined to be (*E*)-26,27-dinor-24ξ-methyl-5α-cholest-22-ene-3β,4β,6α,15β,25-pentol.

Certonardosterol A_3 (**10**) was isolated as colorless needles and displayed a pseudomolecular ion peak at *m*/*z* 491.3358 $[M + Na]^+$ (calcd for $C_{27}H_{48}O_6Na$, 491.3349) in the HR-FABMS. The NMR data indicated that **10** shares the same sterol nucleus with **8** and shares the same side chain with **4**. The stereochemistry at C-25 was determined by analysis of the ^{1}H NMR data of its MTPA esters.[14] It was reported that for the (*R*)-MTPA esters the H-26 signals of the 25*S* isomer are closely spaced, while those of the 25*R* isomer are well-separated. The reverse was observed for the (*S*)-MTPA esters.[14] The ^{1}H NMR spectrum of the (*R*)-MTPA ester of **10** showed the H-26 signals at δ 4.21 and 4.13, and those of the (*S*)-MTPA ester were observed at δ 4.24 and 4.11. Accordingly, the 25*S* configuration was proposed and the structure of **10** was defined as (25*S*)-5α-cholestane-3β,4β,6α,8,15β,26-hexol.

Certonardosterol A_4 (**11**) was isolated as colorless needles. A pseudomolecular ion peak was observed at *m*/*z* 489.3189 $[M + Na]^+$ in the HRFABMS, consistent with a molecular formula of $C_{27}H_{46}O_6$ (calcd for $C_{27}H_{46}O_6Na$, 489.3192). The NMR data showed that the sterol nucleus of **11** is the same as that of **10**. In addition, the ^{1}H NMR spectrum showed a complex olefinic multiplet near δ 5.24, an oxymethine quintet at δ 3.46, and three methyl doublets at δ 1.10, 0.990, and 0.987, which were attributable to the protons of the side chain. The methyl doublet at δ 0.990 was due to H-21. The olefinic proton signals near δ 5.24 were coupled to the carbon signals at δ 138.1 (C-22) and 131.6 (C-23) in the HSQC experiment, indicating the presence of a double bond at C-22. The long-range HMBC correlations of the oxymethine proton signal at δ 3.46 (H-25), the methine proton signal at δ 2.01 (H-24), and the methyl proton signal at δ 0.987 (H-24^1) to the olefinic carbon at δ 131.6 (C-23) and the COSY correlation between the proton signal at δ 3.46 and another methyl proton signal at δ 1.10 (H-26) were observed. These observations suggested the 27-nor-25-hydroxy-24-methylcholest-22-ene side chain. The 22*E* configuration was assigned by the chemical shift of C-20 (δ 41.0), while that of the 22*Z* isomer would be upfield shifted to about δ 35.[15] The absolute configuration at C-25 was determined as *R* by the modified Mosher's method on the basis of the $\Delta\delta$ ($\delta_S - \delta_R$) value of H-26 (+0.08 ppm). Thus, the structure of **11** was determined to be (*E*)-(24ξ,-25*R*)-27-nor-24-methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,-25-hexol. To the best of our knowledge, the 27-nor-25-hydroxy-24-methylcholest-22-ene side chain of **11** was previously undescribed.

Certonardosterol B_4 (**12**) was isolated as colorless needles. The HRFABMS exhibited a pseudomolecular ion peak at *m*/*z* 505.3502 $[M + Na]^+$ (calcd for $C_{28}H_{50}O_6Na$, 505.3505). The NMR data indicated that it shares the same sterol nucleus with **9**. The ^{1}H and ^{13}C NMR data indicated that the side chain of **12** was saturated and also revealed the presence of an oxygenated quaternary carbon (δ 77.1), an oxymethylene, three *sec*-methyl, two methylene, and two methine groups. The location of the hydroxymethyl group was deduced from the splitting pattern of its proton signals (two doublets) and its HMBC correlation with the oxygenated quaternary carbon (C-24) signal. The HMBC correlations of H-23 (δ 1.63 and 1.34), H-25 (δ 1.83), and H-26/27 (δ 0.91 and 0.92) to C-24 were also observed. Further analysis of the 2D NMR data suggested the presence of the 24-hydroxymethyl-24-hydroxycholestane side chain. The NMR data of the synthesized 24*R* and 24*S* epimers of the 24-hydroxymethyl-24-hydroxycholestanes showed small but still significant differences in the H-24^1 and C-24^1 signal (24*R* epimer: δ_H 3.52, 3.48, $\Delta\delta_H = 0.04$ ppm, δ_C 66.0; 24*S* epimer: δ_H 3.53, 3.47, $\Delta\delta_H = 0.06$ ppm, δ_C 66.3).[16] The NMR data ($\Delta\delta_H = 0.07$ ppm; δ_C 66.3) of **12** were close to those of the 24*S* epimer. The 24*S* configuration was further corroborated by comparison of the ^{1}H NMR data of its MTPA esters with those of the synthesized (24*R*)- and (24*S*)-24-hydroxymethyl-24-hydroxycholesterols. It was reported that the H-24^1 signals of the (*R*)-MTPA ester of the 24*R* isomer appear as two well-separated doublets (δ 4.37 and 4.17), while those of the 24*S* isomer appear as closely spaced doublets (δ 4.33 and 4.21). The reverse was apparent for those of the (*S*)-MTPA esters.[16] The H-24^1 signals were observed at δ 4.30 and 4.16 for the (*R*)-MTPA esters of **12** and at δ 4.50 and 4.06 for the (*S*)-MTPA ester, which are in close agreement with the 24*S* configuration. Thus, the structure of **12** was defined as (24*S*)-24-methyl-5α-cholestane-3β,4β,6α,15β,24,24^1-hexol.

Certonardosterol D_5 (**13**) was isolated as colorless needles. The FABMS exhibited a pseudomolecular ion peak at *m*/*z* 489 $[M + Na]^+$. Analysis of the NMR data showed that it shares the same 3β,6α,15β-trihydroxy sterol nucleus with certonardosterol D[3] and the same side chain with **12**.

Certonardoside H_3 (**14**) was isolated as colorless needles. The FABMS gave a pseudomolecular ion peak at *m*/*z* 753 $[M + Na]^+$. The NMR data showed that **14** shares the same sterol nucleus with **12** and shares the same side chain with **2**. In addition to the signals attributable to the aglycon, a total of 15 oxymethine, oxymethylene, and oxymethyl protons were observed at δ 2.83−5.10 in the ^{1}H NMR spectrum, and a total of 11 oxygenated carbons were observed at δ 61.2−108.8 in the ^{13}C NMR spectrum. By careful examination of these signals and by the aid of COSY and HMBC data, the sugar moiety 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl unit was established. Comparison of the sugar moiety of **14** with that of certonardoside H revealed that the difference is the lack of a methoxyl group on C-4″ in **14**.[1] The location of the sugar residue was determined on the basis of the long-range correlation between C-24 and H-1′. The 24*S* configuration was proposed by analogy with the co-occurring saponin **15** (vide infra). Therefore, the structure of certonardoside H_3 (**14**) was assigned as the 4″-*O*-demethyl derivative of certonardoside H,[1] that is, (24*S*)-24-*O*-[2-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl]-5α-cholestane-3β,4β,6α,-15β,24-pentol. To the best of our knowledge, the sugar moiety 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylofuranosyl unit was previously undescribed.

Certonardoside H_4 (**15**) was isolated as colorless needles. The HRFABMS exhibited a pseudomolecular ion peak at *m*/*z* 607.3813 $[M + Na]^+$ (calcd for $C_{32}H_{56}O_9Na$, 607.3822). The NMR data showed that **15** shares the same aglycon with **14**. In addition to the aglycon signals, four oxymethine proton signals at δ 4.02, 4.03, 4.18, and 4.94 and two oxymethylene proton signals at δ 3.75 and 3.86, reminiscent of the β-D-xylofuranosyl sugar unit of certonardoside N, were observed.[2] Methanolysis (4.5% HCl in MeOH) of **15** gave the 5α-cholestane-3β,4β,6α,15,24-pentol (**15a**), 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

Table 1. ^{1}H NMR Data of **1**−**4** and **6** (CD_3OD, 500 MHz)[a]

position	1	2	3	4	6
1	1.71 (dt, 13.0, 3.5)	1.71 (dt, 13.5, 3.5)	1.73 (dt, 13.0, 3.3)	1.70 (dt, 13.0, 3.3)	1.73 (dt, 13.0, 3.3)
	1.00 (m)	1.00 (m)	0.99 (m)	1.26 (m)	1.26 (m)
2	1.82 (m)	1.81 (m)	1.72 (m)	1.80 (qd, 12.5, 2.5)	1.81 (m)
	1.55 (m)	1.54 (m)	1.48 (m)	1.59 (m)	1.40 (m)
3	3.42 (ddd, 11.0, 5.0, 3.5)	3.42 (ddd, 12.0, 5.0, 3.5)	3.47 (m)	3.48 (ddd, 11.5, 4.0, 3.0)	3.53 (m)
4	4.24 (br s)	4.24 (br s)	2.18 (m)	4.23 (br s)	2.23 (dt, 12.5, 2.5)
			1.22 (m)		1.17 (m)
5	0.93 (dd, 11.0, 2.5)	0.93 (dd, 11.0, 2.3)	1.03 (m)	1.20 (dd, 10.5, 3.0)	1.30 (m)
6	4.10 (td, 11.0, 4.5)	4.10 (td, 11.0, 4.5)	3.64 (td, 10.5, 4.0)	3.89 (td, 11.0, 5.3)	3.36 (m)
7	3.07 (dd, 13.0, 4.5)	3.07 (dd, 13.0, 4.0)	3.00 (dd, 13.0, 3.5)	4.46 (dd, 13.5, 5.0)	4.37 (dd, 13.5, 5.0)
	1.21 (dd, 13.0, 11.0)	1.21 (dd, 13.0, 11.0)	1.19 (dd, 13.0, 11.0)	1.56 (m)	1.54 (m)
9	0.82 (dd, 13.0, 3.0)	0.82 (dd, 12.0, 2.3)	0.84 (td, 12.0, 2.5)	1.92 (dd, 10.0, 8.0)	1.95 (dd, 10.0, 7.5)
11	1.71 (m)	1.71 (m)	1.75 (m)	1.59 (m)	1.68 (m)
	1.47 (m)	1.47 (m)	1.52 (m)	1.55 (m)	1.57 (m)
12	2.15 (dt, 13.0, 2.8)	2.14 (dt, 12.5, 3.3)	2.15 (dt, 12.3, 3.5)	2.14 (dt, 12.5, 3.5)	2.15 (dt, 13.0, 3.5)
	1.44 (m)	1.43 (m)	1.43 (m)	1.29 (m)	1.31 (m)
14	2.09 (s)	2.08 (s)	2.10 (s)		
16	2.44 (dd, 18.5, 7.5)	2.42 (dd, 19.0, 8.0)	2.43 (dd, 18.8, 8.8)	2.34 (dd, 18.5, 8.0)	2.36 (dd, 19.0, 7.5)
	1.77 (dd, 18.5, 9.0)	1.80 (dd, 19.0, 9.5)	1.80 (dd, 18.8, 9.8)	2.10 (dd, 18.5, 12.0)	2.12 (dd, 19.0, 12.3)
17	1.59 (m)	1.58 (m)	1.56 (m)	1.49 (m)	1.51 (m)
18	1.04 (s)	1.04 (s)	1.05 (s)	0.99 (s)	1.00 (s)
19	1.16 (s)	1.16 (s)	1.00 (s)	0.95 (s)	0.72 (s)
20	1.56 (m)	1.52 (m)	1.52 (m)	1.61 (m)	1.62 (m)
21	1.01 (d, 6.0)	1.00 (d, 6.0)	1.00 (d, 6.5)	1.03 (d, 6.5)	1.04 (d, 7.0)
22	1.53 (m)	1.61 (m)	1.60 (m)	1.42 (m)	1.65 (m)
	1.21 (m)	1.01 (m)	1.01 (m)	1.10 (m)	1.05 (m)
23	2.14 (m)	1.57 (m)	1.58 (m)	1.44	1.55 (m)
	1.98 (m)	1.23 (m)	1.23 (m)	1.20	1.24 (m)
24		3.21 (m)	3.21 (m)	1.41 (m)	3.22 (m)
				1.02 (m)	
25	2.25 (sextet, 7.0)	1.62 (m)	1.62 (m)	1.57 (m)	1.62 (m)
26	3.55 (dd, 11.0, 6.0)	0.89 (d, 7.0)	0.89 (d, 6.5)	3.40 (dd, 10.5, 6.0)	0.89 (d, 7.0)
	3.34 (m)[b]			3.32 (m)[b]	
27	1.05 (d, 7.0)	0.90 (d, 7.0)	0.90 (d, 7.5)	0.90 (d, 6.5)	0.90 (d, 7.0)
24^1	4.78 (br s)				
	4.77 (br s)				

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

doublets of the isopropyl methyl protons at δ 0.83 and 0.86, which matched well with those of the (*R*)-MTPA ester of the (24*S*)-24-hydroxy steroid (δ 0.84 and 0.86).[11] Thus, the structure of **15** was defined as (24*S*)-24-*O*-β-D-xylofuranosyl-5α-cholestane-3β,4β,6α,15β,24-pentol.

The compounds were evaluated for cytotoxicity against a small panel of human solid tumor cell lines (Table 6). Compounds **1**−**4**, **6**, **7**, **9**−**11**, and **14** showed moderate to significant cytotoxicity. Compound **6** displayed the highest potency, which is comparable to that of doxorubicin. The potency of the compounds might be partly governed by the polarity of the compounds, since the number of sugar units (**14**, **15**) or the degree of oxygenation of the nucleus (**6**−**9**) makes a difference.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH, using a Shimadzu mini 1240 UV−vis spectrophotometer. IR spectra were measured by a JASCO FT/IR-410 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 500 and Bruker AC200 instruments. Chemical shifts were reported with reference to the respective solvent peaks [δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 8.74 (H-2) and δ_C 150.4 (C-2) for pyridine-d_5]. 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 × 20 mm, 4 μm, 80 Å), a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å), a Vydac column (250 × 10 mm, 5 μm, 90 Å), a YMC-Pack NH_2 column (250 × 10 mm, 5 μm, 120 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 5 (0.8 g) was very active in the brine shrimp assay (LD_{50} 15 μg/mL) and was further separated by normal-phase MPLC (silica gel 60, 400/230 mesh), eluting with a solvent system of 0 to 70% MeOH/CH_2Cl_2, to afford 11 fractions. Fraction 5-4 was subjected to reversed-phase HPLC (Vydac column) eluting with 75% MeOH to give compound **6**. Fraction 5-5 was subjected to a YMC-Pack ODS column (250 × 20 mm) eluting with 75% MeOH, followed by purification on a Vydac column eluting with the same mobile phase, to yield compounds **1** (0.7 mg), **2** (1.7 mg), **3** (0.9 mg), **4** (0.7 mg), **5** (0.6 mg), and **7** (1.2 mg). Fraction 5-7 was chromatographed on a C18-5E Shodex packed column eluting with 75% MeOH, followed by purification on a YMC-Pack NH_2 column eluting with 90% MeCN, to give compounds **8** (3.7 mg), **9** (2.0 mg), **10** (4.2 mg), **11** (1.2 mg), and **13** (0.4 mg). Fraction 5-9 was subjected to a YMC-Pack preparative ODS column eluting with 75% MeOH, followed by purification on a YMC-Pack C8

Table 2. ^{1}H NMR Data of **8**−**12** and the Aglycon of **14** (CD_3OD, 500 MHz)[a]

position	**8**[b]	**9**	**10**[b]	**11**	**12**	**14**
1	1.91 (dt, 13.0, 3.5) 1.17 (m)	1.68 (dt, 13.5, 3.5) 1.04 (m)	1.92 (dt, 13.0, 3.5) 1.16 (m)	1.70 (dt, 13.0, 3.5) 0.98 (m)	1.64 (dt, 13.2, 3.3) 1.02 (m)	1.68 (dt, 13.0, 3.3) 1.04 (m)
2	2.39 (m) 1.98 (m)	1.78 (m) 1.56 (m)	2.40 (qd, 12.5, 2.0) 1.98 (m)	1.83 (m) 1.55 (m)	1.80 (m) 1.56 (m)	1.81 (qd, 12.5, 3.0) 1.55 (m)
3	3.96 (ddd, 12.0, 5.0, 3.5)	3.42 (ddd, 12.0, 4.8, 3.5)	3.96 (m)	3.42 (ddd, 11.5, 4.8, 3.3)	3.43 (m)	3.44 (m)
4	5.25 (br s)	4.21 (br s)	5.25 (br s)	4.25 (br s)	4.21 (br s)	4.21 (br s)
5	1.46 (dd, 11.0, 2.5)	0.91 (m)	1.47 (dd, 11.0, 2.5)	0.94 (m)	0.90 (m)	0.91 (m)
6	5.07 (td, 10.8, 4.5)	3.89 (td, 11.0, 4.5)	5.06 (td, 10.8, 3.8)	4.15 (td, 11.0, 4.0)	3.90 (td, 11.0, 4.5)	3.90 (td, 10.0, 5.0)
7	3.16 (dd, 12.0, 4.0) 1.86 (m)	2.34 (dt, 11.5, 4.3) 0.92 (m)	3.16 (dd, 12.3, 4.3) 1.86 (m)	2.43 (dd, 12.0, 4.5) 1.29 (m)	2.35 (dt, 11.5, 4.3) 0.92 (m)	2.36 (dt, 12.0, 4.0) 0.92 (m)
8		1.88 (m)			1.89 (m)	1.88 (m)
9	1.04 (dd, 12.5, 2.5)	0.67 (td, 11.8, 3.5)	1.03 (dd, 12.5, 2.3)	0.82 (dd, 12.0, 3.0)	0.67 (td, 11.5, 3.5)	0.68 (td, 12.0, 3.5)
11	2.13 (qd, 13.0, 3.0) 1.62 (dd, 13.0, 3.0)	1.44 (m) 1.31 (m)	2.13 (m) 1.62 (m)	1.77 (m) 1.43 (m)	1.42 (m) 1.32 (m)	1.43 (m) 1.30 (m)
12	2.08 (dt, 12.0, 3.3) 1.25 (m)	1.92 (m) 1.11 (m)	2.10 (m) 1.23 (m)	1.96 (dt, 12.5, 3.3) 1.16 (m)	1.94 (dt, 11.0, 2.5) 1.10 (m)	1.94 (dt, 12.5, 3.0) 1.07 (m)
14	1.16 (m)	0.89 (m)	1.13 (d, 5.5)	1.01 (d, 5.5)	0.88 (m)	0.87 (m)
15	4.71 (br t, 6.2)	4.13 (td, 6.5, 2.5)	4.75 (br t, 6.0)	4.38 (td, 6.5, 2.5)	4.16 (td, 6.8, 1.5)	4.16 (td, 7.0, 2.0)
16	2.36 (m) 1.74 (ddd, 14.3, 11.0, 2.3)	2.20 (m) 1.33 (m)	2.44 (dt, 14.5, 8.0) 1.68 (m)	2.18 (dt, 14.5, 8.3) 1.34 (m)	2.44 (dt, 15.0, 8.0) 1.36 (m)	2.42 (dt, 15.0, 8.5) 1.34 (m)
17	1.06 (m)	1.11 (m)	1.06 (m)	1.03 (m)	1.09 (m)	1.07 (m)
18	1.66 (s)	0.94 (s)	1.65 (s)	1.27 (s)	0.93 (s)	0.93 (s)
19	1.86 (s)	1.06 (s)	1.86 (s)	1.15 (s)	1.06 (s)	1.06 (s)
20	2.31 (m)	2.14 (m)	1.66 (m)	2.14 (m)	1.50 (m)	1.50 (m)
21	1.14 (d, 6.5)	1.01 (d, 6.5)	1.03 (d, 6.5)	0.990 (d, 7.0)	0.96 (d, 6.5)	0.95 (d, 6.5)
22	5.48 (dd, 15.5, 8.0)	5.30 (dd, 15.0, 8.0)[c]	1.48 (m) 1.12 (m)	5.26 (dd, 15.0, 8.0)[c]	1.47 (m) 1.11 (m)	1.60 (m) 0.99 (m)
23	5.54 (dd, 15.5, 6.5)	5.27 (dd, 15.0, 7.0)[c]	1.59 (m) 1.31 (m)	5.23 (dd, 15.0, 7.0)[c]	1.63 (m) 1.34 (m)	1.58 (m) 1.37 (m)
24	2.56 (m, 6.5)	2.22 (m)	1.69 (m) 1.21 (m)	2.01 (q, 6.5)		3.34 (m)
25	3.84 (dd, 10.0, 5.5) 3.70 (dd, 10.0, 7.0)	3.42 (dd, 11.0, 6.5) 3.30 (m)	1.86 (m)	3.46 (quint, 6.8)	1.83 (m)	1.85 (m)
26			3.81 (dd, 10.5, 5.5) 3.71 (dd, 10.5, 6.5)	1.10 (d, 6.5)	0.92 (d, 6.5)	0.89 (d, 7.0)
27			1.13 (d, 7.0)		0.91 (d, 7.0)	0.90 (d, 7.0)
24[1]	1.23 (d, 7.0)	0.97 (d, 7.0)		0.987 (d, 7.0)	3.50 (d, 11.3) 3.43 (d, 11.3)	

[a] Multiplicities and coupling constants are in parentheses. [b] Spectra were recorded in pyridine-d_5 with a few drops of CD_3OD. [c] The δ and J values were analyzed by spectrum simulation.

column eluting with 75% MeOH, to afford compounds **12** (1.4 mg), **14** (2.5 mg), and **15** (2.6 mg).

Certonardosterol Q_1 (1): colorless needles; IR (film) ν_{max} 3347, 2935, 1731, 1639, 1554, 1457, 1384 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 501 [M + Na]$^+$; HRFABMS *m/z* 501.3191 (calcd for $C_{28}H_{46}O_6Na$, 501.3192).

Certonardosterol Q_2 (2): colorless needles; $[\alpha]^{21}_D$ 60.0° (*c* 0.07, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 489 [M + Na]$^+$; HRFABMS *m/z* 489.3191 (calcd for $C_{27}H_{46}O_6Na$, 489.3192).

***(R)*-MTPA ester of certonardosterol Q_2:** selected ^{1}H NMR (CD_3OD) δ 1.27 (3H, s, H-19), 1.05 (3H, s, H-18), 1.00 (3H, d, J = 6.5 Hz, H-21), 0.86 (3H, d, J = 6.5 Hz, H-26/27), 0.83 (3H, d, J = 7.0 Hz, H-26/27).

Certonardosterol Q_3 (3): colorless needles; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 473 [M + Na]$^+$; HRFABMS *m/z* 473.3242 (calcd for $C_{27}H_{46}O_5Na$, 473.3243).

Certonardosterol Q_4 (4): colorless needles; UV (MeOH) λ_{max} (log ϵ) 258 (3.78) nm; IR (film) ν_{max} 3341, 2938, 1739, 1693, 1619, 1550, 1373 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 471 [M + Na]$^+$; HRFABMS *m/z* 471.3085 (calcd for $C_{27}H_{44}O_5Na$, 471.3086).

Certonardosterol Q_5 (5): colorless needles; ^{1}H NMR δ 4.79 (1H, br s, H-24[1]), 4.78 (1H, br s, H-24[1]), 4.46 (1H, dd, J = 13.5, 5.0 Hz, H_e-7), 4.23 (1H, br s, H-4), 3.90 (1H, td, J = 11.0, 5.0 Hz, H-6), 3.55 (1H, dd, J = 11.0, 6.5 Hz, H-26), 3.49 (1H, ddd, J = 11.5, 4.8, 3.3 Hz, H-3), 3.36 (1H, overlapped with solvent signal, H-26), 2.37 (1H, dd, J = 19.0, 7.5 Hz, H-16), 2.26 (1H, sextet, J = 6.5 Hz, H-25), 2.14 (1H, dt, J = 13.0, 3.5 Hz, H_e-12), 2.11 (1H, dd, J = 19.0, 12.0 Hz, H-16), 1.92 (1H, dd, J = 9.5, 8.0 Hz, H-9), 1.06 (6H, d, J = 7.0 Hz, H-21 and H-27), 0.99 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR data, see Table 3; FABMS *m/z* 483 [M + Na]$^+$; HRFABMS *m/z* 483.3067 (calcd for $C_{28}H_{44}O_5Na$, 483.3086).

Certonardosterol Q_6 (6): colorless needles; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 455 [M + Na]$^+$; HRFABMS *m/z* 455.3130 (calcd for $C_{27}H_{44}O_4Na$, 455.3137).

Certonardosterol Q_7 (7): colorless needles; ^{1}H NMR δ 4.46 (1H, dd, J = 13.8, 5.3 Hz, H_e-7), 4.23 (1H, br s, H-4), 3.90 (1H, td, J = 11.0, 5.0 Hz, H-6), 3.49 (1H, ddd, J = 11.5, 4.5, 3.0 Hz, H-3), 3.22 (1H, m, H-24), 2.36 (1H, dd, J = 19.0, 8.0 Hz, H-16), 2.26 (1H, sextet, J = 6.5 Hz, H-25), 2.14 (1H, dt, J = 13.0, 3.8 Hz, H_e-12), 2.13 (1H, dd, J = 19.0, 12.5 Hz, H-16), 1.92 (1H, dd, J = 10.0, 7.5 Hz, H-9), 1.04 (3H, d, J = 7.0 Hz, H-21), 0.99 (3H, s, H-18), 0.95 (3H, s, H-19), 0.91 (3H, d, J = 7.0 Hz, H-26/27), 0.89 (3H, d, J = 6.5 Hz, H-26/27); ^{13}C NMR data, see Table 3; FABMS *m/z* 471 [M + Na]$^+$; HRFABMS *m/z* 471.3092 (calcd for $C_{27}H_{44}O_5Na$, 471.3086).

Compound 8: colorless needles; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 475 [M + Na]$^+$; HRFABMS *m/z* 475.3030 (calcd for $C_{26}H_{44}O_6Na$, 475.3036).

Certonardosterol B_3 (9): colorless needles; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 459 [M + Na]$^+$; HRFABMS *m/z* 459.3091 (calcd for $C_{26}H_{44}O_5Na$, 459.3086).

Certonardosterol A_3 (10): colorless needles; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS (+ve) *m/z* 491 [M + Na]$^+$; HRFABMS (+ve) *m/z* 491.3358 (calcd for $C_{27}H_{48}O_6Na$, 491.3349).

Table 3. ^{13}C NMR Data of **1**−**12** and the Aglycon of **14** (CD_3OD, 50 MHz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a]	**9**	**10**[a]	**11**	**12**	**14**
1	39.8	39.8	39.5	38.22[b]	38.22[b]	37.7	38.22[b]	39.5	38.8	39.5	39.7	38.8	38.8
2	26.1	26.1	31.4	26.2	26.2	31.8	26.2	26.9	26.3	26.9	26.2	26.3	26.3
3	73.6	73.6	72.1	73.5	73.5	71.6	73.5	73.1	73.7	73.1	73.7	73.7	73.7
4	69.0	69.0	32.3	69.0	69.0	33.4	69.0	68.9	69.1	68.9	69.1	69.1	69.1
5	57.0	57.0	53.6	55.7	55.6	52.0	55.6	57.4	56.6	57.4	57.3	56.6	56.6
6	64.2	64.2	67.1	67.1	67.1	70.5	67.1	63.8	66.6	63.8	64.7	66.6	66.6
7	48.5	48.5	48.2	38.19[b]	38.19[b]	38.0	38.19[b]	50.6	41.9	50.6	49.7	41.9	41.9
8	74.6	74.6	74.7	149.8	149.8	149.6	149.8	76.6	31.5	76.6	77.4	31.5	31.4
9	57.7	57.7	56.7	52.5	52.4	51.6	52.5	57.8	56.5	57.7	58.4	56.5	56.5
10	38.0	38.0	38.0	39.0	39.0	39.0	39.0	37.7	37.5	37.7	38.2	37.5	37.5
11	18.8	18.8	19.3	19.8	19.6	20.2	19.8	18.8	21.3	19.3	19.2	21.5	21.5
12	42.2	42.3	42.4	38.16[b]	38.16[b]	38.1	38.16[b]	42.4	42.5	42.5	43.2	42.6	42.6
13	44.6	44.6	44.6	43.9	43.9	43.9	43.9	43.5	43.3	43.7	44.2	43.4	43.4
14	70.3	70.2	70.2	142.1	142.1	142.1	142.1	62.1	62.4	61.9	62.8	62.3	62.3
15	216.3	216.5	216.5	210.0	210.0	210.1	210.0	70.1	70.6	70.1	71.1	70.7	70.6
16	43.0	43.0	43.1	43.2	43.2	43.0	43.2	43.1	42.7	42.3	43.4	42.2	42.3
17	53.3	53.4	53.3	52.4	52.2	52.4	52.4	56.7	57.5	57.1	57.8	57.6	57.8
18	15.1	15.1	15.0	19.0	19.0	18.8	19.0	16.7	15.3	16.6	16.6	15.1	15.2
19	17.1	17.1	14.2	16.2	16.1	13.7	16.1	17.4	16.1	17.4	17.0	16.1	16.1
20	35.9	36.5	36.5	35.9	35.6	35.8	36.1	40.0	41.4	35.3	41.0	37.4	37.1
21	19.1	19.3	19.3	19.7	19.3	19.5	19.8	20.7	21.4	18.7	21.0	19.3	19.3
22	35.3	33.3	33.4	37.1	35.3	33.3	33.2	136.6	137.9	36.6	138.1	30.0	33.0
23	32.7	31.7	31.7	24.5	32.5	31.5	31.6	131.3	131.6	24.0	131.6	31.6	28.8
24	153.5	78.0	78.1	34.8	153.5	77.9	78.0	40.2	40.6	34.4	46.0	77.1	85.8
25	43.3	34.7	34.6	36.9	43.3	34.5	34.6	67.8	68.4	36.7	72.7	33.7	31.5
26	67.5	17.6	17.6	68.4	67.5	17.2	17.6			67.5	21.3	17.4	18.5
27	17.3	19.5	19.5	17.2	17.2	19.3	19.5			17.5		17.3	18.1
24^1	109.6				109.4			17.5	17.4		17.4	66.3	

[a] Spectra were recorded in pyridine-d_5 with a few drops of CD_3OD. [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 of **14** and **15** (CD_3OD)[a]

	14		**15**	
position	δ_H	δ_C	δ_H	δ_C
1′	5.10 (br s)	108.8	4.94 s	109.6
2′	4.12 (br s)	89.7	4.02 m	82.1
3′	4.20 (dd, 5.0, 2.0)	75.7	4.03 m	77.2
4′	4.15 (q, 5.5)	83.9	4.18 m	83.7
5′	3.87 (dd, 11.8, 5.3)	62.3	3.86 dd (11.8, 5.3)	62.3
	3.76 (dd, 11.8, 6.0)	105.0	3.75 dd (11.8, 6.3)	
1″	4.41 (d, 7.5)	84.8		
2″	2.83 (dd, 9.3, 7.8)	77.4		
3″	3.31 (m)	71.2		
4″	3.48 (m)	67.0		
5″	3.82 (dd, 11.3, 5.3)	61.2		
	3.15 (dd, 11.3, 10.3)			
2″-OMe	3.54 (s)			

[a] Multiplicities and coupling constants are in parentheses.

(*S*)-MTPA ester of certonardosterol A_3: selected ^1H NMR (CD_3OD) δ 4.24 (1H, dd, J = 11.0, 6.0 Hz, H-26), 4.11 (1H, dd, J = 11.0, 6.0 Hz, H-26), 1.26 (3H, s, H-18), 1.26 (3H, s, H-19), 0.92 (3H, d, J = 7.0 Hz, H-21), 0.89 (3H, d, J = 6.5 Hz, H-27).

(*R*)-MTPA ester of certonardosterol A_3: selected ^1H NMR (CD_3OD) δ 4.21 (1H, dd, J = 10.5, 6.0 Hz, H-26), 4.13 (1H, dd, J = 10.5, 6.0 Hz, H-26), 1.27 (3H, s, H-18), 1.26 (3H, s, H-19), 0.93 (3H, d, J = 6.5 Hz, H-21), 0.89 (3H, d, J = 6.5 Hz, H-27).

Certonardosterol A_4 (11): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS m/z 489 [M + Na]$^+$; HRFABMS m/z 489.3189 (calcd for $C_{27}H_{46}O_6Na$, 489.3192).

(*S*)-MTPA ester of certonardosterol A_4: selected ^1H NMR (CD_3OD) δ 1.28 (3H, d, J = 6.0 Hz, H-26), 1.25 (3H, s, H-18), 1.24 (3H, s, H-19), 1.00 (3H, d, J = 7.0 Hz, H-21), 0.99 (3H, d, J = 6.5 Hz, H-24[1]).

(*R*)-MTPA ester of certonardosterol A_4: selected ^1H NMR (CD_3OD) δ 1.28 (3H, s, H-18), 1.26 (3H, s, H-19), 1.20 (3H, d, J = 6.5 Hz, H-26), 1.00 (3H, d, J = 6.5 Hz, H-21), 0.98 (3H, d, J = 7.0 Hz, H-24[1]).

Table 5. Selected ^1H NMR Data of the MTPA Esters of **2**, **10**, **11**, **12**, and **15a** (CD_3OD, 500 MHz)[a]

MTPA ester	H-26	H-26, H-27	H-24[1]
(*R*)-MTPA ester of **2**		0.86 (d, 6.5)	
		0.83 (d, 7.0)	
(*S*)-MTPA ester of **10**	4.24 (dd, 11.0, 6.0)		
	4.11 (dd, 11.0, 6.0)		
(*R*)-MTPA ester of **10**	4.21 (dd, 10.5, 6.0)		
	4.13 (dd, 10.5, 6.0)		
(*S*)-MTPA ester of **11**	1.28 (d, 6.8)		
(*R*)-MTPA ester of **11**	1.20 (d, 6.5)		
(*S*)-MTPA ester of **12**			4.50 (d, 11.0)
			4.06 (d, 11.0)
(*R*)-MTPA ester of **12**			4.30 (d, 11.5)
			4.16 (d, 11.5)
(*R*)-MTPA ester of **15a**		0.86 (d, 6.5)	
		0.83 (d, 7.0)	

[a] Multiplicities and coupling constants are in parentheses.

Certonardosterol B_4 (12): colorless needles; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS m/z 505 [M + Na]$^+$; HRFABMS m/z 505.3502 (calcd for $C_{28}H_{50}O_6Na$, 505.3505).

(*S*)-MTPA ester of certonardosterol B_4: selected ^1H NMR (CD_3OD) δ 4.50 (1H, d, J = 11.0 Hz, H-24[1]), 4.06 (1H, d, J = 11.0 Hz, H-24[1]), 1.17 (3H, s, H-19), 0.95 (3H, d, J = 6.5 Hz, H-21), 0.93 (3H, s, H-18), 0.90 (3H, d, J = 6.5 Hz, H-26/27), 0.89 (3H, d, J = 7.0 Hz, H-26/27).

(*R*)-MTPA ester of certonardosterol B_4: selected ^1H NMR (CD_3OD) δ 4.30 (1H, d, J = 11.5 Hz, H-24[1]), 4.16 (1H, d, J = 11.5 Hz, H-24[1]), 1.17 (3H, s, H-19), 0.95 (3H, d, J = 6.5 Hz, H-21), 0.94 (3H, s, H-18), 0.90 (3H, d, J = 7.0 Hz, H-26/27), 0.89 (3H, d, J = 7.0 Hz, H-26/27).

Table 6. Cytotoxicity Data of **1**−**12**, **14**, and **15** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	12.0	10.9	5.57	4.95	11.6
2	6.94	6.85	4.20	4.10	5.82
3	5.43	12.3	7.30	12.6	13.6
4	3.80	4.10	3.40	2.90	4.20
5	>30	>30	>30	>30	>30
6	0.43	0.22	0.17	0.12	0.48
7	4.58	6.65	4.66	3.80	7.10
8	>30	>30	>30	>30	>30
9	12.5	12.1	7.13	14.7	10.4
10	11.7	16.2	5.24	18.6	20.3
11	17.0	16.4	5.70	28.4	19.3
12	>30	36.6	7.50	>30	>30
14	3.65	2.80	0.82	0.52	7.20
15	>30	>30	8.3	>30	>30
doxorubicin	0.04	0.12	0.05	0.12	0.18

[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.

Certonardosterol D_5 (13): colorless needles; the ^{1}H and ^{13}C NMR data of the sterol nucleus were identical with those of certonardosterol D,[3] the ^{1}H and ^{13}C NMR data of the side chain were identical with those of certonardosterol B_4 (**12**); FABMS *m*/*z* 489 $[M + Na]^+$; ESIMS *m*/*z* 489 $[M + Na]^+$.

Certonardoside H_3 (14): colorless needles; $[\alpha]^{21}_D$ −12.5° (*c* 0.14, MeOH); ^{1}H NMR data, see Tables 2 and 4; ^{13}C NMR data, see Tables 3 and 4; FABMS (+ve) *m*/*z* 753 $[M + Na]^+$; ESIMS (+ve) *m*/*z* 753 $[M + Na]^+$; ESIMS (−ve) *m*/*z* 729 $[M - H]^-$.

Certonardoside H_4 (15): colorless needles; $[\alpha]^{21}_D$ −27.1° (*c* 0.12, MeOH); the ^{1}H NMR data of the aglycon moiety were identical with those of certonardoside H_3 (**14**); ^{1}H NMR data of the sugar moiety, see Table 4; ^{13}C NMR data, see Tables 3 and 4; FABMS *m*/*z* 607 $[M + Na]^+$; HRFABMS *m*/*z* 607.3813 (calcd for $C_{32}H_{56}O_9Na$, 607.3822).

Compound 15a: white amorphous powder; ^{1}H NMR (CD_3OD) δ 4.21 (1H, br s, H-4), 4.16 (1H, td, J = 6.5, 2.0 Hz, H-15), 3.90 (1H, td, J = 10.8, 4.3 Hz, H-6), 3.45 (1H, m, H-3), 2.39 (1H, dt, J = 15.5, 8.5 Hz, H-16), 2.35 (1H, dt, J = 11.5, 4.0 Hz, H_{eq}-7), 1.94 (1H, dt, J = 12.0, 2.5 Hz, H_{eq}-12), 1.79 (1H, qd, J = 13.5, 3.0 Hz, H_{eq}-2), 1.68 (1H, dt, J = 13.5, 3.5 Hz, H_{eq}-1), 1.06 (3H, s, H-19), 0.95 (3H, d, J = 7.0 Hz, H-21), 0.93 (3H, s, H-18), 0.91 (3H, d, J = 7.0 Hz, H-26/27), 0.89 (3H, d, J = 6.5 Hz, H-26/27), 0.67 (1H, td, J = 11.3, 3.0 Hz, H-9).

(*R*)-MTPA ester of compound 15a: selected ^{1}H NMR (CD_3OD) δ 1.17 (3H, s, H-19), 0.95 (3H, d, J = 6.5 Hz, H-21), 0.94 (3H, s, H-18), 0.86 (3H, d, J = 6.5 Hz, H-26/27), 0.83 (3H, d, J = 7.0 Hz, H-26/27).

Preparation of MTPA Esters. The (*S*)-MTPA and (*R*)-MTPA esters of compounds **10** (1 μmol), **11** (1 μmol), and **12** (1 μmol) were prepared as described previously.[1] To the solutions of compounds in dry pyridine (25 μL) were added (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μmol) to afford (*S*)- and (*R*)-MTPA esters, 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 and analyzed by ^{1}H NMR spectroscopy. Compounds **2** (1 μmol) and **15a** (1 μmol) were treated with only (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (6 μmol) in dry pyridine (25 μL) for 24 h at room temperature, respectively. The following procedure was the same as that for compounds **10**, **11**, and **12**.

Methanolysis of Saponins. A solution of compound **15** (1.5 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 supernatant was taken to dryness under N_2. The residue was purified by HPLC (YMC-Pack ODS column, 250 × 10 mm, 5 μm, 120 Å, $MeOH/H_2O$ (9:1)] to give **15a**.

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