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Three New Polyhydroxysteroids from the Tropical Starfish *Asteropsis carinifera*

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Abstract—Thirteen steroidal compounds including three new polyhydroxysteroids, (24*R*,25*S*)-24-methyl-5α-cholestane-3β,6α,8,15β,16β,26-hexaol, (22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,6α,8,15β,16β,26-hexaol, and (22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,16β,26-heptaol, have been isolated along with ten previously known polyhydroxysteroids from the tropical starfish *Asteropsis carinifera* collected near the coast of Vietnam. The structures of the new compounds were elucidated by spectroscopic methods (mainly 2D NMR and ESI mass spectrometry).

Keywords: starfish, Asteropsis carinifera, polyhydroxysteroids, NMR spectra, absolute configuration

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INTRODUCTION

Starfish are characterized by a great diversity of highly oxidized steroid compounds. As a rule, they contain polyhydroxysteroids, mono- and biosides termed asterosaponins [1, 2]. These compounds attract attention due to their diverse biological properties, incuding embryotoxic, antifungal, antiviral, antibacterial, neuritogenic, and other kinds of activities [1, 2]. Continuing our investigations on biologically active steroid metabolites from starfish, we studied the steroid composition of the tropical starfish *Asteropsis carinifera* Lamarck, 1816 (family Asteropseidae, order Valvatida), collected from the coasts of Vietnam. Thirteen steroid compounds were isolated: three new polyhydroxysteroids, (24*R*,25*S*)-24-methyl-5α-cholestan-3β,6α,8,15β,16β, 26-hexaol (**I**), (22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,6α,8,15β,16β,26-hexaol (**II**), and (22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,16β,26-heptaol (**III**), and ten previously known polyhydroxysteroids (**IV**)–(**XIII**). The absolute configuration of the asymmetric centers of the side chains of the new compounds has been determined based on the analysis of the ^{1}H NMR spectra of *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetates of these compounds (*R*-(+)-MTPA derivatives) obtained by the Mosher method.

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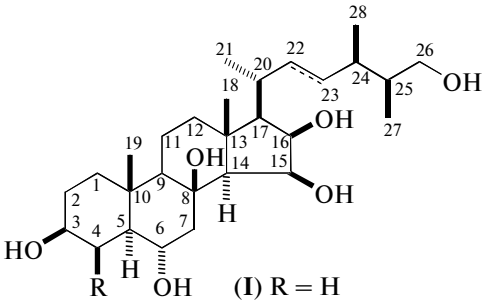
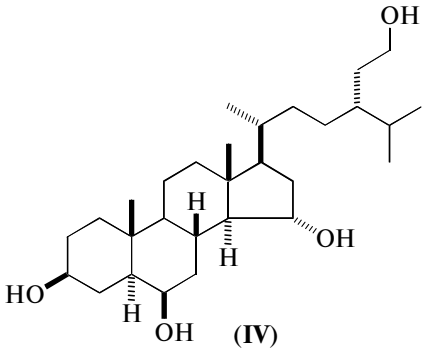
RESULTS AND DISCUSSION

Thirteen polyhydroxylirated steroids (**I**)–(**XIII**) were isolated from an ethanol extract of the starfish *A. carinifera* by means of column chromatography on Amberlite XAD-2, silica gel, florisil, and HPLC in semipreparative Diasfer-110-C18 and Discovery C18 columns. The results of the chromatographic separation of the steroid fractions are shown in Table 1. The structures of new compounds (**I**)–(**III**) were established mainly by means of ^{1}H-^{1}H-COSY-, HSQC-, HMBC-, and DEPT-NMR experiments and of the data of ESI mass spectrometry. Known compounds (**IV**)–(**XIII**) were identified by a comparison of their ^{1}H- and ^{13}C NMR spectra and mass spectra with the corresponding data for these substances available in published data [3–8].

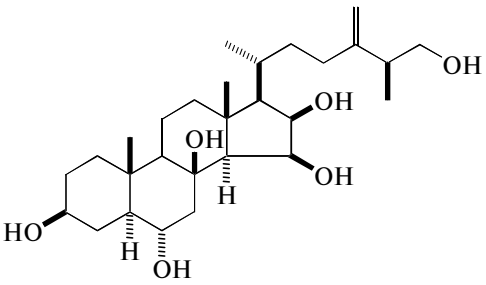
The mass spectrum of the HR-ESI (registration of cations) of compound (**I**) contained the peak of a cationized molecule with *m/z* 505.3466 $[M + Na]^+$, and in the mass spectrum of HR-ESI (registration of anions) there was a peak of a deprotonated molecule with *m/z* 481.3544 $[M - H]^-$. The data of the mass spectra and the NMR spectra indicated that compound (**I**) has a molecular formula $C_{28}H_{50}O_6$. In the ^{13}C- and DEPT-NMR spectra of steroid (**I**), signals of 28 carbon atoms were present, including the signals of 5 methyl, 9 methylene, 11 methine groups, and 3 quaternary carbon atoms; the signals of 6 carbon atoms at δ 66.9, 67.6, 71.2, 72.2, 72.7, and 77.2 ppm were bound with oxygen atoms. The chemical shifts (CS) of the carbon atoms, characteristic protons, and as well as coupling constants of the steroid nucleus of compound (**I**) in



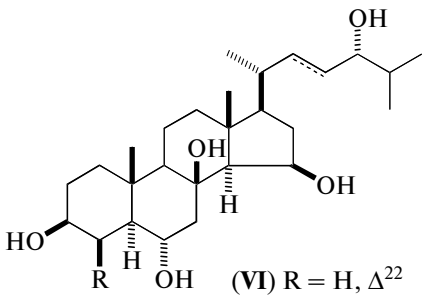
(**I**) R = H
(**II**) R = H, Δ^{22}
(**III**) R = OH, Δ^{22}



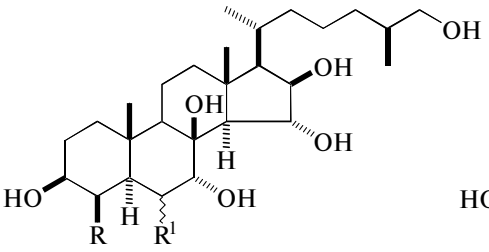
(**IV**)



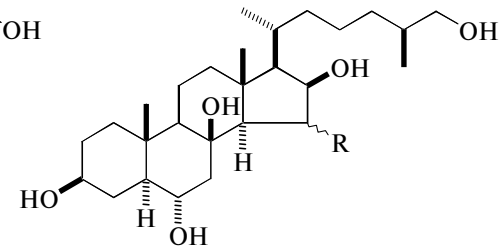
(**V**)



(**VI**) R = H, Δ^{22}
(**VII**) R = OH
(**VIII**) R = OH, Δ^{22}



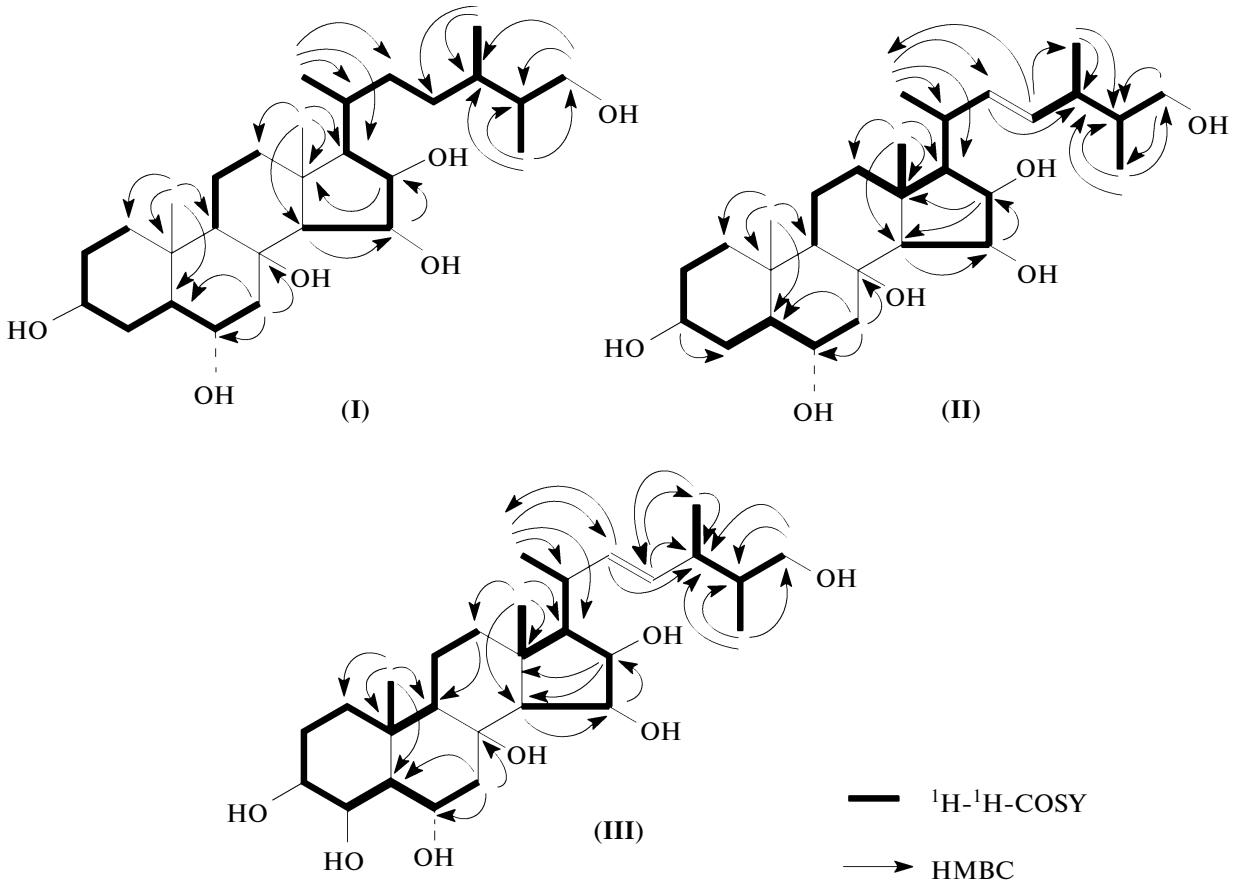
(**IX**) R = H, R^1 = —OH
(**X**) R = H, R^1 = ⁗OH
(**XI**) R = H, R^1 = ⁗OH



(**XII**) R = —OH
(**XIII**) R = ⁗OH

the ^{1}H- and ^{13}C NMR spectra almost coincided with the corresponding signals of 5α-cholest-3β,6α,8,15β,16β,26-hexaol from the starfish *Halityle regularis* [9]. The chemical shifts of carbon atoms of the side chain of steroid (**I**) were close to the analogous values in the spectrum of the ^{13}C NMR of certonardosterol M from starfish *Certonardoa semiregularis* having a 24-methyl-26-hydroxycholestane side chain [10]. ^{1}H-^{1}H-COSY, HSQC, and HMBC-NMR experiments revealed the signals of all protons and carbon atoms in compound (**I**) (Table 2, figure) and confirmed the presence of hydroxygroups at positions 3β,6α,8,15β, and 16β of the steroid nucleus and the 26-hydroxy-24-methylcholestane side chain.

The 20*R* configuration of the asymmetric center was determined based on the chemical shifts of protons of the (Me21) at δ 0.93 ppm (δ 0.90–0.96 ppm for 20*R*-steroids [11]). As in the side chain of steroid (**I**) there are two more asymmetric centers C24 and C25; four stereoisomers by the side chain are possible for it. Two of them belong to a pair of *threo*-isomers and the other two belong to a pair of *erythro*-isomers. According to published data for synthetic 26-hydroxy-24-methylsteroids, these stereoisomers differ from each other in the chemical shifts of carbon atoms C24, C27, and C28 and the protons Me27 and Me28. The values of the chemical shifts of atoms C24 at δ 35.0 ppm, C27 at δ 12.1 ppm, and C28 at δ 14.7 ppm and the chemical shifts of protons Me27 at δ 0.81 ppm and Me28 at



^{1}H-^{1}H COSY and HMBC correlations for compounds (**I**)–(**III**).

δ 0.80 ppm corresponded to the *threo* configuration. At an *erythro* configuration, these signals in the spectra would be shifted to a weaker field [12].

For the determination of the absolute configuration of the asymmetric center C25 and, as a consequence, C24, by treatment of compound (**I**) with chloroanhydride of *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher reagent [13]), an *R*-(+)-MTPA ester of compound (**I**) was obtained. It is known that the signals of two protons of H26 in the spectrum of the ^{1}H NMR of the 25*S* isomer of the *R*-(+)-MTPA ester are close in comparison with those in the spectrum of the 25*R* isomer of the corresponding derivative: at the 25*S* configuration, the signals of protons H26 and H'26 were observed at 4.23 ppm (bsd), and at the 25*R* configuration, at 4.34 and 4.14 ppm (Δ 0.2 ppm) [12]. In the ^{1}H MNR spectrum of *R*-(+)-MTPA ester, the compound (**I**) chemical shifts of protons H26 were 4.24 and 4.19 ppm (Δ 0.05 ppm) indicating the *S* configuration of the symmetric center C24. As a result, the structure (24*R*,25*S*)-24-methyl-5α-cholestane-3β,6α,8,15β,16β,26-hexaol was ascribed to compound (**I**).

In the mass spectrum of the HR-ESI (registration of cations) of compound (**II**), the peak of the cationized molecule was present with *m/z* 503.3340 $[M + Na]^+$, and in the mass spectrum of HR-ESI (registration of anions) the peak of the deprotonized molecule was present with *m/z* 479.3385 $[M - H]^-$. The data of the mass spectra and of the NMR spectra indicated that steroid (**II**) has the molecular formula $C_{28}H_{48}O_6$. A comparison of the chemical shifts of carbon atoms, protons, and the corresponding coupling constants in the spectra of the ^{1}H- and ^{13}C NMR of compounds (**I**) and (**II**) demonstrated that both compounds have an identical 3β,6α,8,15β,16β-tetrahydroxysteroid nucleus. However, in contrast to steroid (**I**), the NMR spectra of compound (**II**) contained the signals of a double bond in the side chain (δ_C 136.6 and 134.4 ppm, δ_H 5.46 and 5.41 ppm), and the molecular weight of compound (**I**) was, correspondingly, lower by two units. The ^{1}H-^{1}H COSY, HSQC, and HMBC NMR experiments gave the possibility of determining the signals of all protons and atoms of the steroid nucleus and of the aglycone chain side (Table 2, figure). With reference to the obtained data, it was found that in steroid (**II**) there is a 26-hydroxy-24-methylcholestane side chain with a

Table 1. Polyhydroxysteroids isolated from the starfish *A. carinifera*

Compound	Quantity, mg	R_f^*	(+)-ESI-mass-spectrum $[M + Na]^+$	Starfish from which the compound was isolated for the first time; reference
(24*R*,25*S*)-24-methyl-5α-cholestane-3β,6α,8,15β,16β,26-hexaol (**I**)	1.5	0.72	505	
(22*E*,24*R*,25*S*)-24-methyl-5α-cholest- 22-ene-3β,6α,8,15β,16β,26-hexaol (**II**)	1.3	0.80	503	
((22*E*,24*R*,25*S*)-24-methyl-5α-cholest- 22-ene-3β,4β,6α,8,15β, 16β,26-heptaol (**III**)	1.2	0.67	519	
(24*R*)-24-ethyl-5α-cholestane-3β,6β,15α,29-tetraol (**IV**)	0.5	0.74	487	*C. semiregularis* [3]
24-methyl-5α-cholest-24 (28)-ene-3β,6α,8,15β,16β,26-hexaol (**V**)	1.2	0.74	503	*Dermasterias imbricata* [4]
22E,24*R*)-5α-holest-22-ene-3β,6α,8,15β,24-pentaol (**VI**)	2.0	0.77	473	*D. imbricata* [4]
(24*S*)-5α-cholestane-3β,4β,6α,8,15β,24-hexaol (**VII**)	2.5	0.69	491	*Gomophia watsoni* [5]
(22*E*,24*R*)-5α-cholest-22-ene-3β,4β,6α,8,15β,24-hexaol (**VIII**)	0.5	0.68	489	family Echinasteridae [6]
(25*S*)-5α-cholestane-3β,6β,7α,8,15α,16b, 26-heptaol (**IX**)	1.5	0.69	507	genus *Rosaster* [7]
(25*S*)-5α-cholestane-3β,6α,7α,15a,16β,26-heptaol (**X**)	0.5	0.56	507	*Protoreaster nodosus* [8]
(25*S*)-5α-cholestane-3β,4b,6α,7α,8,15α,16β,26-octaol (**XI**)	4.0	0.42	523	*P. nodosus* [8]
(25*S*)-5α-cholestane-3β,6α,8,15β,16β,26-hexaol (**XII**)	3.0	0.72	491	*H. regularis* [9]
(25*S*)-5α-cholestane-3β,6α,8,15α,16β,26-hexaol (**XIII**)	1.0	0.53	491	*P. nodosus* [8]

* Determined in the system toluol–EtOH (9 : 5).

22(23) double bond. The value of the corresponding coupling constant of $J_{22,23}$ 15.4 Hz indicated the *trans* configuration of the 22(23) double bond.

The 20*R* configuration of the asymmetric center was determined based on the chemical shifts of protons Me21 at δ 1.03 ppm (δ 1.04 ppm for 20*R*-Δ^{22} steroids [11]. The determination of the stereochemistry of the side chain of steroid (**II**) was made in the same way as for compound (**I**). The values of the chemical shifts of carbon atoms C24 at δ 40.8, C27 at 14.6, and C28 at 17.7 ppm and of the chemical shifts of protons Me27 at δ 0.87 and Me28 at 0.94 ppm corresponded to the 24*R*,25*S*-*threo* configuration of the Δ^{22}-24-methyl-26-hydroxycholestane side chain [12]. In the spectrum of the ^{1}H NMR *R*-(+)-MTPA derivative of compound (**II**), the chemical shifts of two protons H26 were δ 4.29 and 4.20 ppm (Δ 0.09 ppm); at a 25*S* configuration analogous signals were observed at 4.31 and 4.19 ppm (Δ 0.12 ppm) and at a 25*R* configuration at 4.38 and 4.13 ppm (Δ 0.25 ppm) [12]. This confirmed the *S* configuration of the asymmetric center C25 and, respectively, the *R* configuration of the asymmetric center C24. On the basis of the obtained data, the structure of (22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,6α,8,15β,16β,26-hexaol was ascribed to steroid (**II**).

In the mass spectrum of the HR-ESI (registration of cations) of compound (**III**), the peak of the cationized molecule was present with *m/z* 519.3283 $[M + Na]^+$ and in the mass spectrum of HR-ESI (registration of anions) there was the peak of the deprotonized molecule with *m/z* 495.3332 $[M - H]^-$. By the data of the mass spectra and NMR spectra, steroid (**III**) differs from compound (**II**) in the presence in the molecule of an additional hydroxyl group and has the molecular formula $C_{28}H_{48}O_7$. The chemical shifts of carbon atoms, protons, and, respectively, the corresponding coupling constants of the side chain in the NMR spectra of steroids (**III**) and (**II**) were almost identical. This indicated the (22*E*,24*R*,25*S*)-26-hydroxy-24-methylcholestane side chain. An analysis of the NMR data of steroid (**III**) demonstrated that the chemical shifts of the side chain in steroid (**III**) and the corresponding coupling constants of the steroid nucleus are close to the corresponding signals of halityloside A from the starfish *H. regularis* [9] having 3β,4β,6α,8,15β,16β-hydroxyl groups in the steroid nucleus. The signals of all protons and carbon atoms of the steroid nucleus and the side chain of aglycon in compound (**III**) were attributed by means of ^{1}H-^{1}H-COSY, HSQC, and HMBC NMR experiments (Table 2, figure). It was found that compound (**III**) has the structure (22*E*,24*R*,25*S*)-24methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,16β,26-heptaol.

Thus, for the first time the fraction of free polyhydroxysteroids from the tropical starfish *A. carinifera* has been characterized. Seven compounds belong to steroid hexaols and have, principally, hydroxyl groups at positions 3β,6α (or β),8,15β (or α),16β, and 26. In other compounds—tetraol, pentaol, three heptaols, and octaol—there is the hydroxylation by positions 7α,24, or 29 rarer for this group of substances. The

Table 2. Data of the NMR spectra of steroids (**I**), (**II**), and (**III**) (CD_3OD, δ, ppm; *J*, Hz)*

Number of atom	(I)		(II)		(III)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.3	0.96 m	39.3	0.96 m	39.6	1.70 qt (13.1, 3.7)
		1.70 m		1.70 m		0.97 m
2	31.4	1.47 m	31.4	1.47 m	26.2	1.82 qd (13.2, 3.8)
		1.72 m		1.73 m		1.54 m
3	72.2	3.47 m	72.2	3.49 m	73.7	3.42 ddd (11.9, 4.5, 3.7)
4	32.3	1.91 m	32.3	1.20 m	69.1	4.25 brs
		2.18 brd (9.9)		2.17 brd (14.9)		
5	53.8	1.03 m	53.8	1.03 m	57.2	0.93 m
6	67.6	3.71 dt (4.2, 10.9)	67.6	3.71 dt (4.2, 10.9)	64.7	4.18 td (10.9, 4.3)
7	49.6	2.38 dd (4.3, 12.4)	49.5	2.38 dd (3.0, 12.4)	50.0	2.46 dd (12.3, 4.4)
		1.32 dd (10.5, 12.0)		1.31 dd (10.5, 12.5)		1.35 dd (12.1, 11.1)
8	77.2		77.2		77.1	
9	57.4	0.83 m	57.4	0.84 m	58.4	0.82 dd (12.7, 3.1)
10	38.0		38.0		38.2	
11	19.4	1.77 qd (3.0, 12.8)	19.4	1.78 m	18.9	1.75 qd (3.9, 3.2)
		1.46 m		1.47 m		1.41 m
12	43.5	1.95 dt (3.5, 12.7)	43.3	1.93 brd (12.5)	43.3	1.92 kt (12.4, 63.7)
		1.12 td (3.5, 12.5)		1.14 m		1.13 m
13	44.5		44.4		44.5	
14	61.0	1.02 d (5.5)	61.1	1.02 m	61.2	1.01 d (5.6)
15	71.2	4.36 dd (5.5, 6.9)	71.3	4.35 dd (5.7, 6.8)	71.3	4.35 dd (7.0, 5.5)
16	72.7	4.21 t (7.0)	73.2	4.11 t (6.9)	73.2	4.11 t (7.0)
17	63.0	0.99 m	63.5	0.97 m	63.5	0.97 m
18	17.9	1.23 s	17.9	1.26 s	17.9	1.25 s
19	14.0	0.98 s	14.0	0.99 s	17.0	1.16 s
20	31.0	1.92 m	34.7	2.59 m	34.7	2.59 m
21	18.1	0.93 d (7.0)	20.4	1.03 d (7.0)	20.4	1.02 d (6.8)
22	34.6	1.17 m	136.6	5.46 dd (7.4, 15.4)	136.6	5.47 dd (15.4, 7.7)
		1.67 m				
23	32.8	1.28 m	134.4	5.41 dd (7.7, 15.4)	134.4	5.41 dd (15.4, 7.9)
24	35.0	1.57 m	40.8	2.05 dd (7.0, 14.0)	40.9	2.05 m
25	41.2	1.62 m	42.3	1.50 m	42.3	1.50 m
26	66.9	3.47 dd (6.6, 10.6)	66.9	3.51 dd (5.9, 10.8)	67.0	3.54 dd (6.0, 11.0)
		3.35 dd (7.0, 10.7)		3.34 dd (6.3, 11.0)		3.34 m
27	12.1	0.81 d (7.0)	14.6	0.87 d (7.0)	14.6	0.87 d (6.9)
28	14.7	0.80 d (7.0)	17.7	0.94 d (7.0)	17.7	0.95 d (6.9)

* Signals are attributed by methods of two-dimensional NMR spectroscopy ^1H-^1H-COSY, HSQC, and HMBC.

complete structures have been found, including the absolute stereochemistry, for three new steroid polyols.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded in Bruker DPX-300 (^1H, 300, ^{13}C, 75.5 MHz), Bruker DRX-500 (^1H, 500, ^{13}C, 125.7 MHz), and Bruker Avance III 700 (^1H, 700, ^{13}C, 176 MHz) spectrometers with the inner standard being CD_3OD (δ_C 49.0 ppm, δ_H 3.30 ppm). The optical rotation was measured on a Perkin Elmer 343 polarimeter in MeOH. The mass spectra of ESI were obtained on an Agilent 6510 Q-TOF mass spectrometer (United States). The samples were dissolved in MeOH (with 0.01 mg/ml). HPLC was performed in an Agilent chromatograph with a refractometric detector. TLC was performed on Sorbfil plates with a layer of CTX-1A silica gel (5–17 μm, Sorbpolimer, Russia) fixed on foil. The substances were detected by concentrated H_2SO_4 with the subsequent heating of the plates at 110°C over the course of 10 min. Preparative separations were made using column chromatography on Amberlite XAD-2 (20–80 mesh, Sigma Chemical Co.), KSK silica gel (50–160 μm, Sorbpolimer, Russia), and florisil (100–200 mesh, Aldrich, United States).

Animals. Samples of the starfish *A. carinifera* were collected in January 2005 by diving from a depth of 5–10 m in the South China Sea (Van Fong Bay, coast of Vietnam) during the 30th research voyage on board the RV *Akademik Oparin*. The determination of the species of the starfish was made by A.V. Smirnov (Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia).

Isolation of compounds (I)–(III). The fresh starfishes (500 g) were twice extracted with ethanol while heating in a water bath. The ethanol extract was concentrated in a vacuum, dissolved in H_2O (0.5 l), and passed through a column (7 × 26 cm) with Amberlit XAD-2. The column was washed with H_2O until the absence of Cl^- ions in the eluate and then with ethanol. The ethanol eluate was evaporated, and the obtained total fraction of the steroid compounds was consecutively chromatographed in columns with silica gel (6 × 16 cm) in the $CHCl_3$–EtOH system (stepwise gradient, 4 : 1 ⟶ 1 : 4) and with florisil (2 × 14 cm) in the system $CHCl_3$–EtOH (stepwise gradient, 5 : 1 ⟶ 1 : 2). Several fractions were obtained containing by TLC data polyhydroxysteroids (R_f within 0.42–0.80) in the toluol–EtOH system (9 : 5). The obtained fraction were divided by HPLC on a Diasfer column 110-C18 (10 μm, 15 × 250 mm, 2.5 ml/min) in the EtOH–H_2O system (65 : 35); this led to the isolation of compounds (**I**) (1.5 mg, t_R 47.3 min), (**II**) (1.3 mg, t_R 63.2 min), and (**IV**)–(**XIII**) and of the fraction enriched with compound (**III**). This fraction was additionally purified by HPLC on a Discovery C18 column (5 μm, 10 × 250 mm, 2.5 ml/min) in the MeOH–H_2O–1M NH_4OAc system (75 : 24 : 1). Compound (**III**) was obtained (1.2 mg, t_R 45.0 min). The results of the chromatographic separation of the steroid fractions with the isolation of compounds (**I**)–(**XIII**) are shown in Table 1.

(24*R*,25*S*)-24-methyl-5α-cholestane-3β,6α,8,-15β,16β,26-hexaol (I), an amorphous compound, $[\alpha]_D^{20}$ + 23 (*c* 0.1 of MeOH). (+)-HR-ESI mass spectrum, *m/z*: 505.3466 $[M + Na]^+$ (calculated for $C_{28}H_{50}O_6Na$, 505.3499). (–)-HR-ESI mass spectrum, *m/z*: 481.3544 $[M - H]^-$ (calculated for $C_{28}H_{49}O_6$, 481.3535). Spectra of ^1H- and ^{13}C NMR are shown in Table 2.

(22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β, 6α,8,15β,16β,26-hexaol (II), an amorphous compound, $[\alpha]_D^{20}$ + 6 (*c* 0.1 of MeOH). (+)-HR-ESI mass spectrum, *m/z*: 503.3340 $[M + Na]^+$ (calculated for $C_{28}H_{48}O_6Na$, 503.3343). (–)-HR-ESI-mass-spectrum, *m/z*: 479.3385 $[M - H]^-$ (calculated for $C_{28}H_{47}O_6$, 479.3378). Spectra of ^1H- and ^{13}C NMR are shown in Table 2.

(22*E*,24*R*,25*S*)-24-methyl-5α-cholest-22-ene-3β,-4β,6α,8,15β,16β,26-heptaol (III), an amorphous compound, $[\alpha]_D^{20}$ + 7 (*c* 0.1 of MeOH). (+)-HR-ESI mass spectrum, *m/z*: 519.3283 $[M + Na]^+$ (calculated for $C_{28}H_{48}O_7Na$, 519.3292). (–)-HR-ESI mass spectrum, *m/z*: 495.3332 $[M - H]^-$ (calculated for $C_{28}H_{47}O_7$, 495.3327). Spectra of ^1H- and ^{13}C NMR are shown in Table 2.

Preparation of MTPA esters of compounds (I) and (II). The substance in an amount of 0.8 mg was dissolved in 200 μl of dry pyridine, 10 μl of chloroanhydride of (*S*)-(+)-MTPA was added (Sigma-Aldrich, Germany), it was kept for 2 h at room temperature, and then the solution was concentrated under a vacuum. The dry residue was purified by HPLC on a Diasfer-110-C-18 column (5 μm, 4 × 250 mm, 1 ml/min) in the MeOH–H_2O system (97 : 3). The output was 0.7 mg of (*R*)-(+)-MTPA ester of compound (**I**) and 0.7 mg of (*R*)-(+)-MTPA ester of compound (**II**).

3,6,26-Tri-(*R*)-(+)-MTPA ester of compound (I). The spectrum of ^1H NMR (CD_3OD), selected signals: 0.83 (3 H, d, *J* 6.8. Me28); 0.85 (3 H, dd, *J* 6.9, Me27); 0.91 (3 H, d, *J* 6.7, Me21); 1.09 (3 H, s, Me19); 1.24 (3 H, s, Me18); 2.59 (1 H, dd, *J* 4.5, 12.0, H7); 4.18 (1 H, t, *J* 7.0,H16); 4.19 (1 H, dd, *J* 6.6, 10.9, H'26); 4.24 (1 H, dd, *J* 6.6, 10.9,H26); 4.32 (1 H, dd, *J* 6.7, 5.7, H15); 4.83 (1 H, m, H3); 5.29 (1 H, dt, *J* 4.2, 11.1, H6).

3,6,26-Tri-(*R*)-(+)-MTPA-ester of compound (II). The spectrum of ^1H NMR (CD_3OD), selected signals: 0.92 (3 H, d, *J* 6.8, Me28); 0.92 (3 H, d, *J* 6.9, Me27); 1.02 (3 H, d, *J* 6.7, Me21); 1.10 (3 H, s, Me19); 1.27 (3 H, s, Me18); 2.58 (1 H, dd, *J* 4.5, 12.0, H7); 4.05

(1 H, t, *J* 7.1, H16); 4.20 (1 H, dd, *J* 4.4, 10.6, H'26); 4.29 (1 H, dd, *J* 6.4, 10.9, H26); 4.32 (1 H, dd, *J* 6.8, 5.6, H15); 4.83 (1 H, m, H3); 5.29 (1 H, dt, *J* 4.2, 11.1, H6); 5.38 (1 H, dd, *J* 8.2, 15.4, H23); 5.42 (1 H, dd, *J* 7.7, 15.4, H22).

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