A new nortriterpenoid from the deep-sea sponge *Sarcotragus spinulosus*

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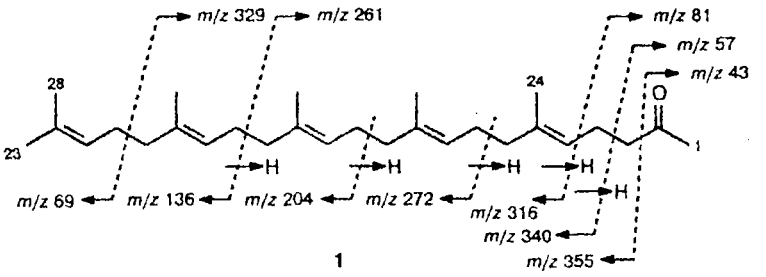
A new nortriterpenoid from the deep-sea sponge *Sarcotragus spinulosus* was isolated and characterized. The structure of geranylfarnesylacetone was assigned to the new compound on the basis of analysis of its chemical transformations and spectral data.

Key words: marine sponge, *Sarcotragus spinulosus*, nortriterpenoid, "biochemical coordination."

In the study of cytotoxic components from marine invertebrates, we found that some toxic species of sponges[1,2] and holothurians[3] contain (instead of cholesterol and related Δ^5-sterols) unusual sterols having a lateral chain "nontypical" of zoosterols or a tetracyclic ring with a different type of unsaturation, for example, with 7(8)- or 9(11)-double bonds. We name the simultaneous and mutually caused presence of two series of secondary metabolites with different biological functions (in this case, toxins and membrane components — sterols) the "biochemical coordination." Continuing our study of this phenomenon, the nonpolar components of the extract of the deep-sea sponge *Sarcotragus spinulosus* were investigated. Cytotoxic sarcohydroquinone sulfates A—C and sarcochromenol sulfates A—C have been isolated previously from this sponge.[4] It has been found that, unlike the majority of other animals, *S. spinulosus* contains no sterols.



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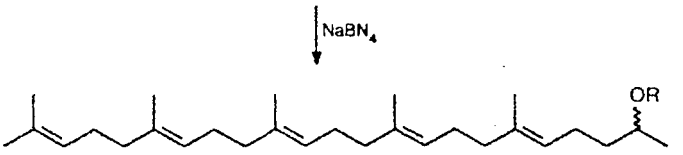
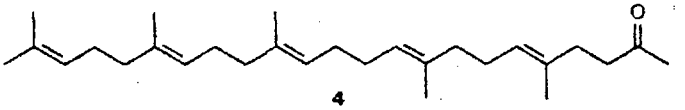


2: R = H; **3**: R = Ac



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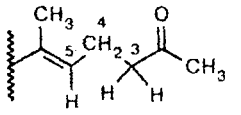
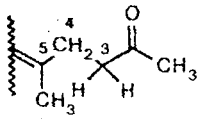


A



B

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Table 1. Data of ^{1}H NMR ($CDCl_3$) spectra for geranylfarnesylacetone (**1**) and its derivatives (**2** and **3**)

Group	δ (*J*/Hz)		
	1	**2**	**3**
CH_3(1)	2.14 (s, 3 H)	1.19 (d, 3 H, J = 6.5)	1.22 (d, 3 H, J = 6.5)
CH(2)	—	3.80 (m, 1 H)	4.88 (m, 1 H)
CH_3(3)	2.46 (t, 2 H, J = 8.0)	1.50 (m, 2 H)	1.50 (m, 2 H)
CH_2(4)	2.26 (q, 2 H, J = 8.0)	2.09 (m, 2 H)	2.09 (m, 2 H)
CH_2(7, 8, 11, 12, 15, 16, 19, 20)	2.02 (m, 16 H)	2.03 (m, 16 H)	2.03 (m, 16 H)
CH(6, 10, 13, 17, 21)	5.10 (m, 5 H)	5.09 (m, 5 H)	5.10 (m, 5 H)
CH_3(25, 26, 27, 28	1.55 (s, 12 H)	1.54 (s, 12 H)	1.60 (s, 12 H)
CH_3(23)	1.62 (s, 3 H)	1.63 (s, 3 H)	1.59 (s, 3 H)
CH_3(24)	1.68 (s, 3 H)	1.68 (s, 3 H)	1.68 (s, 3 H)

Column chromatography of the chloroform—hexane extract on silica gel followed by high-performance liquid chromatography on an Ultrasphere-Si column gave a new nortriterpenoid (**1**) (0.0074% of the dry weight of the animals).

The mass spectral data for compound **1** correspond to the molecular formula $C_{28}H_{46}O$ (m/z 398 $[M]^+$). The mass spectrum contains intense signals at m/z 329 $[M^+-C_5H_9]$; 261 $[M^+-C_5H_9-C_5H_8]$ and 340 $[M^+-C_3H_6O]$; 272 $[M^+-C_3H_6O-C_5H_8]$, 204 $[M^+-C_3H_6O-C_5H_8-C_5H_8]$, 136 $[M^+-C_3H_6O-C_5H_8-C_5H_8-C_5H_8]$, which confirm that this compound contains five prenyl units.

The ^{1}H NMR spectrum of compound **1** (Table 1) exhibits singlet signals of methyl groups at δ 1.55, 1.62, 1.68, and 2.14, a multiplet at δ 5.10 (olefinic protons), and a multiplet at δ 2.02 (allylic protons). The ratio of intensities of the aforementioned signals also indicates that compound **1** contains five isoprene units. The comparison of the ^{1}H NMR spectral data for nortriterpenoid **1** with the corresponding data for sarcohydroquinone sulfates A—C, which have polyprenyl aliphatic chains with the *trans*-configuration of double bonds[4] formed by the "head-to-tail" type connection of prenyl units, shows that the corresponding signals in the spectra coincide. This also confirms that compound **1** contains a linear polyprenyl structural fragment.

Resonances at δ 2.46 and 2.26 indicated the methylene fragments adjacent to the carbonyl and one of the double bonds. A three-proton singlet at δ 2.14 revealed the methyl group adjacent to the carbonyl group.

The presence of the keto group in the compound isolated was confirmed by the reduction of compound **1** by an excess of sodium borohydride to alcohol **2**. The mass spectrum of compound **2** exhibits a peak of the molecular ion at m/z 400 $[M^+]$. The ^{1}H NMR spectrum of compound **2** exhibits a three-proton doublet of the terminal methyl group instead of the three-proton singlet signal, which was observed in the spectrum of the starting compound **1**. In addition, the ^{1}H NMR spectrum of compound **2** contains a multiplet signal at δ 3.80, which was assigned to the carbonyl proton at the C(2) atom.

The acetylation of alcohol **2** gave acetate **3**. In the ^{1}H NMR spectrum of compound **3**, the carbonyl proton for the C(2) atom (4.88 ppm) was shifted to the weak field by 1.08 ppm as compared to the corresponding signal for alcohol **2** (see Table 1).

Based on the data obtained, we can ascribe one of two alternative formulas to the compound isolated: **1** or **4**, which differ by structural fragments **A** and **B**, respectively.

The analysis of the character of splitting of the signal of the methylene group in position 4 in the ^{1}H NMR spectrum of compound **1** made it possible to choose formula **1**. The presence of the two-proton quartet at δ 2.26 with three spin-spin coupling constants equal to 8.0 Hz for the allylic methylene group in position 4 in the ^{1}H NMR spectrum corresponds to fragment **A** only, since this methylene group in fragment **A** has three neighboring H atoms. In fragment **B**, a similar group is present near two protons; therefore, the corresponding signal would be observed as a triplet with two spin-spin coupling constants. The absence of this signal in the spectrum allowed us to rule out the alternative structural formula **4**.

Thus, based on the data obtained, we suggest the structure of geranylfarnesylacetone **1** for the norterpenoid.

Similar linear norterpene ketones with a different number of C atoms have been observed previously in brown algae[5] and crustaceans,[6] in which they perform hormonal functions. It is noteworthy that the sponge studied contains no sterols, which are necessary structural components of biomembranes. Probably, their function can be performed by the new marine secondary metabolite **1** found.

Experimental

^{1}H NMR spectra were recorded on a Bruker WM-250 spectrometer. HPLC was carried out on a Du Pont 8800 chromatograph (a refractometer served as the detector) using an Altex Ultrasphere-Si (10 mm × 25 cm) column.

Thin layer chromatography (TLC) was carried out on glass plates (4.5×6.0 cm) with a fixed layer of silica gel L (Chemapol, Czech Republic).

The sponges were collected by the Sigsby trawl from a depth of 400 m in the Tasman Sea during the trip No. 7 of the Research Ship "Akademik Oparin" in September, 1988. The taxonomic determination of the sponge was carried out by V. M. Koltun (Zoological Institute, RAS, St. Petersburg).

Isolation of geranylfarnesylacetone (1). The lyophilically dried sponge was thoroughly extracted by hexane and then chloroform. The combined extracts were evaporated to dryness, and the residue (3 g) was chromatographed on a column with silica gel in a petroleum ether—chloroform system. The eluates containing, according to the TLC data, compounds less polar than cholesterol were combined and concentrated *in vacuo*. The residue was chromatographed by HPLC in a petroleum ether—ethyl acetate (5 : 1) system to obtain compound **1** (3 mg) as an optically inactive yellowish oil (yield 0.0074%), R_f 0.79 (in a hexane—ethyl acetate (3 : 1) system; R_f of cholesterol = 0.33), $C_{28}H_{46}O$. MS, m/z (I_{rel} (%)): 398 [M^+] (24); 383 (2); 355 (4); 340 (7); 329 (19); 316 (4); 272 (24); 261 (24); 204 (30); 191 (28); 136 (52); 135 (90); 81 (100); 69 (95); 57 (15); 43 (30). The ^1H NMR spectral data are presented in Table 1.

Reduction of geranylfarnesylacetone (1). Compound **1** (1.9 mg) was reduced by sodium borohydride in methanol at room temperature for 10 min. After a standard treatment, compound **2** (1.9 mg, 100%) was obtained as a yellowish oil, R_f 0.50 (in a hexane—ethyl acetate (3 : 1) system; R_f of cholesterol = 0.33), $C_{28}H_{48}O$. MS, m/z (I_{rel} (%)): 400 [M^+] (3.4); 382 (0.8); 332 (12); 317 (2); 289 (3); 279 (15); 269 (10); 264 (10); 263 (10); 204 (31); 191 (20); 163 (20); 161 (23); 137 (100); 136 (93); 135 (68); 81 (100); 69 (95); 68 (100); 43 (95). The ^1H NMR spectral data are presented in Table 1.

Acetylation of alcohol (2). Compound **2** (1.6 mg) was acetylated in a acetic anhydride—pyridine (1 : 1) mixture (1 mL) at room temperature for 16 h. The compound was obtained in a 100% yield (1.8 mg) as a yellow oil. ^1H NMR ($CDCl_3$), δ: 2.03 (s, 3 H, CH_3CO—), and other spectral data are presented in Table 1.

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Preconcentration of poorly volatile impurities in thin-layer chromatography by thermodesorption of a volatile matrix

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A method for preconcentration of poorly volatile impurities by continuous thermodesorption of the major component on a TLC plate is proposed. The method provides multiple (200—500) concentration of the organic impurities present in the initial solution. A simple device for such concentration is suggested.

Key words: preconcentration, poorly volatile impurities, aqueous solutions, thin-layer chromatography, thermodesorption.

Preconcentration is a way of increasing the accuracy of an analytical determination.[1] Development of facile methods and schemes for sample enrichment is of considerable interest from the practical viewpoint.[2—4]

The so-called method of continuous evaporation for thin-layer chromatography (TLC) has been reported; according to this method, some limited evaporation of the mobile phase during separation in the working area

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