Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

METABOLITES OF THE MARINE SPONGE *Suberites* cf. *aurantiacus*

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UDC 547.925:577.115.3:593.433

Fractions of free sterols and sterol esters were observed in the ethylacetate extract of the marine sponge Suberites *cf.* aurantiacus *and their compositions were determined. It was found that cholesterol was the predominant component in all sterol forms. The esters had a high content of unsaturated long-chain fatty acids (C_{24}–C_{26}). Sterol acetates have not been previously observed in marine invertebrates.*

Keywords: marine sponge *Suberites* cf. *aurantiacus*, sterol acetates.

Sponges of the genus *Suberites* contain various hemolytic toxins, proteins, suberitane sesterterpenes, and other natural compounds [1–6]. However, the lipid composition of these sponges is poorly studied. At present sterols and fatty acids of several *Suberites* species have been studied [7–13]. Herein we report results from a study of the principal sterol and fatty-acid components from the EtOAc extract of the Caribbean sponge *Suberites* cf. *aurantiacus*.

The same set of secondary metabolites was observed during preliminary comparison by TLC of the EtOH and EtOAc extracts of *S.* cf. *aurantiacus*. Both extracts showed three spots with R_f 0.48, 0.88, and 0.95 (hexane:EtOAc, 3:1). Further studies used the EtOAc extract because it contained greater amounts of these metabolites. It was separated by normal column chromatography over silica gel. The resulting fractions were homogeneous according to TLC. PMR analysis revealed complicated mixtures of Δ^5 sterols (characteristic resonances at δ 0.68, s, CH_3-18 and 1.01, s, CH_3-19); Δ_5 sterol acetates (characteristic resonances at δ 0.68, s, CH_3-18; 1.02, s, CH_3-19; and 2.02, s, Ac); and fatty-acid esters of Δ^5 sterols [characteristic resonances at δ 0.68, s, CH_3-18; 1.02, s, CH_3-19; and 1.25, s, $(-CH_2-)_n$]. The sterol compositions of these fractions were analyzed further using GC and GC–MS of the sterol acetates. Fatty acids were identified by these same methods using the corresponding methyl esters and pyrrolidide derivatives.

The extract of *S.* cf. *aurantiacus* contained mainly Δ^5 sterols and their derivatives. Fractions of sterols, sterylacetates, and sterol esters of fatty acids had compositions similar to that of the sterols (Table 1). The predominant component in all sterol compounds was cholesterol (cholest-5-en-3β-ol, 42.4–44.6%). Cholestanol (5α-cholestan-3β-ol) occurred in the fractions in insignificant (1.0–2.5%) amounts. Cholestanol was observed as the predominant component in sterol fractions of previously studied sponges *S. domuncula* [7, 8], *S. japonicus* [9], and the Mediterrean *S. carnosus* [10]. However, *S. carnosus* collected in the Indian Ocean [11] contained ergosterol derivatives. The sponge *S. vestigium* produced Δ^5 sterols, similar to the sponge studied by us, in addition to Δ^7 steroidal alcohols, in contrast with it [12].

At least 30 fatty-acid sterol esters were found in *S.* cf. *aurantiacus*:

Fatty acid	*%*	*Fatty acid*	*%*	*Fatty acid*	*%*
14:0	1.0	*i*-19:0 (17-Me-18:0)	0.4	22:1 Δ^9	0.3
4,8,12-Me_3-13:0	2.4	*ai*-19:0 (16-Me-18:0)	0.1	22:0	0.1
15:0	0.2	19:0	0.3	23:1 Δ^9	0.5
16:1Δ^9	0.1	*i*-20:0 (18-Me-19:0)	1.0	23:0	0.1
16:0	3.8	*ai*-20:0 (17-Me-19:0)	0.5	24:1 Δ^{17}	10.9
i-17:0 (15-Me-16:0)	0.2	20:0	0.7	25:2 $\Delta^{5,9}$	3.1
17:0	0.8	*i*-21:0 (19-Me-20:0)	1.3	26:2 $\Delta^{5,9}$	53.3
18:1 Δ^9	0.2	*ai*-21:0 (18-Me-20:0)	0.6	26:1 Δ^{17}	11.6
18:1 Δ^{11}	2.0	21:0	0.2	26:1 Δ^{19}	2.1
18:0	1.1	*i*-22:0 (20-Me-21:0)	0.3	27:2 $\Delta^{5,9}$	0.6.

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0009-3130/10/4603-0335

TABLE 1. Sterol Composition of Various Fractions Isolated from *S.* cf. *aurantiacus*, % of Corresponding Fraction

Sterol	Fraction		
	free sterols	sterol acetates	sterol fatty acid esters
Cholesta-5,22-dien-3β-ol	5.5	3.0	5.3
Cholest-5-en-3β-ol	42.4	44.6	42.8
Cholestan-3β-ol	1.9	1.0	2.5
Unident. $C_{27}\Delta^{5,X}$ sterol	2.1	1.3	2.3
24ξ-Methylcholesta-5,22-dien-3β-ol	11.5	9.6	10.2
24ξ-Methylcholest-5-en-3β-ol	4.8	4.6	4.2
24ξ-Ethylcholesta-5,22-dien-3β-ol	10.7	11.0	8.7
24ξ-Ethylcholest-5-en-3β-ol	21.0	24.8	23.9

Over half of the total mixture of acids was $C_{26}\Delta^{5,9}$-demospongic acid; approximately 15 and 28%, saturated and monoene fatty acids, respectively. The content of polyunsaturated fatty acids reached up to 57%. The fatty-acid composition from the total lipid fraction was identical in principle with that of fatty acids obtained from their sterol esters.

Previously studied fatty-acid fractions from *S. domuncula* [7] and *S. massa* [13] also contained many components and significant quantities of $C_{26}\Delta^{5,9}$, 14.1 and 21.3%, respectively. A sharp difference was observed in the content of C_{28}:3 acids, 23.2% in *S. domuncula* [7] and 11.8% ($C_{28}\Delta^{5,9,19}$ 11.1 and $C_{28}\Delta^{5,9,21}$ 0.7%) in *S. massa* [13], whereas we did not observe such acids. Only C_{19} saturated acid and its methyl ester were found in *S. carnosus* [11]. Fatty acids were present as monoglycerides (batyl ester and its analogs) in *S. vestigium* [12].

Thus, we observed an unusual composition of sterol derivatives in *S.* cf. *aurantiacus* that differed primarily by the presence of sterol acetates. Cholesterol acetate was isolated previously from green algae [14] and mutant yeast cells [15]; sitosterol acetate (24*R*-ethylcholest-5-en-3β-ol), from higher plants [16–18]. The remaining components found in the sterol-acetate fractions should probably be considered to be new natural compounds. This is even more surprising if it is considered that acetates are frequently encountered in marine steroids such as polyhydroxysteroids and glycosides. Among metabolites of marine invertebrates, the closest structural analog to the compounds isolated by us is 5α-pregn-20-en-3α-ol acetate. It was observed as a minor component of the extract from an unidentified soft coral collected near Canton Island [19]. An important difference between it and the compounds identified by us is its shortened side chain that contained only two C atoms and the location of the acetate in the 3α-position.

EXPERIMENTAL

PMR spectra were recorded in $CDCl_3$ on a Bruker DPX-300 spectrometer with TMS internal standard. GC–MS of sterol acetates was carried out on a Hewlett–Packard HP 6890 GS system equipped with an HP-5MS column at 270°C. GC–MS of fatty-acid pyrrolidides used a Shimadzu QP-5050A instrument with an MDN-55 column with temperature gradient 210–270°C at 3°C/min and then 40 min under isothermal conditions at 270°C. The carrier gas was He; ionizing potential, 70 eV. GC of sterol acetates was performed on an Agilent 6850 series GC System with an HP-5MS column at 270°C; GC of fatty-acid methyl esters and pyrrolidides, on a Shimadzu GC-17a chromatograph equipped with an SPB-5 capillary column at 205°C or a Supelcowax-10 at 210°C. The carrier gas was He.

Biological Specimen. The sponge was collected by diving to a depth of 10 m near Pines Island (Cuba) and was identified as *Suberites* cf. *aurantiacus* (Suberitidae, Hadromerida) by V. B. Krasokhin. The sponge was lyophilized immediately after collection and stored at –18°C. The specimen was preserved in the collection of the Pacific Institute of Bioorganic Chemistry (Vladivostok, PIBOCO01-063).

Extraction and Isolation. Lyophilized specimens (160 g) were ground and extracted with EtOAc (3 × 1 L). The combined extract was evaporated in a rotary evaporator to a viscous dark residue (3.4 g) that was separated by normal column chromatography over silica gel (50–100 μm, Sorbfil, Russia) using hexane with an increasing content of EtOAc. This produced three fractions of sterol fatty-acid esters (SFAE, 0.20 g, 5.8 wt% of extract, hexane:EtOAc, 100:1 v/v), sterol acetates (SA, 0.14 g, 4.2 wt% of extract, hexane:EtOAc, 95:1), and free sterols (FS, 1.02 g, 29.6 wt% of extract, hexane:EtOAc, 10:1). The

SFAE fraction was worked up with MeONa in MeOH and then HCl in MeOH as before [20]. Chromatography over silica gel using hexane afforded fatty-acid methyl esters (SFAE-a); hexane:EtOAc (10:1), free steroidal alcohols (SFAE-b). The resulting fractions of FS and SFAE-b were acetylated as usual by a mixture of acetic anhydride and pyridine at room temperature. The resulting sterol acetates were isolated and identified by GC–MS using the appropriate standard sterols [21].

The fraction of total lipids was extracted by $CHCl_3$:MeOH (2:1) [22] from the lyophilized specimen (2 g). Fatty-acid methyl esters were prepared as described above [23] and purified by preparative TLC on Sorbfil (Russia) plates using benzene. Fatty-acid pyrrolidides for MS analysis were prepared as before [24] and purified by preparative TLC using EtOAc. Fatty-acid methyl esters and the corresponding pyrrolidides were identified by comparison of their GC and MS properties with those reported [23].

ACKNOWLEDGMENT

The research was supported by the RAS Program "Molecular and Cellular Biology", a grant of the RFBR No. 08-04-00599-a, and a grant of the RF President for Support of Leading Scientific Schools NSh-2813.2008.4.

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