New Sesterterpene Metabolites from the Mediterranean Sponge *Cacospongia scalaris*

Maria Tsoukatou, Helen Siapi, Constantinos Vagias, and Vassilios Roussis*

School of Pharmacy, Department of Pharmacognosy and Chemistry of Natural Products, University of Athens, Panepistimioupolis Zografou, Athens 157 71, Greece

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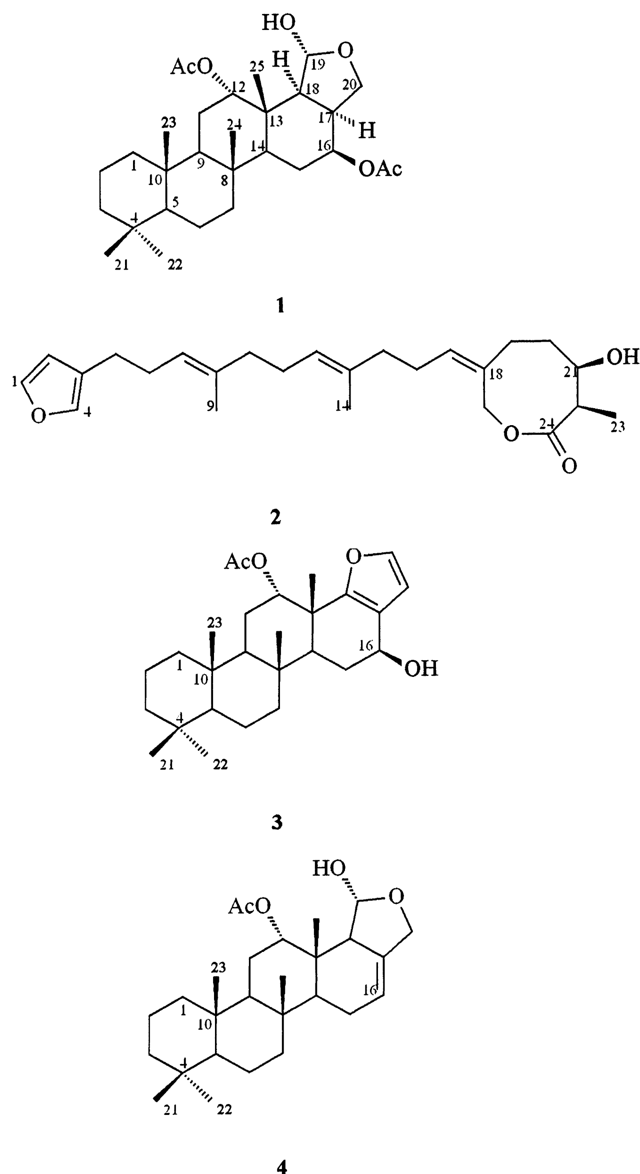
Two new sesterterpene metabolites, 16-acetoxy-dihydrodeoxoscalarin (**1**) and astakolactin (**2**), were isolated from the sponge *Cacospongia scalaris,* collected from the gulf of Astakos, Greece, along with furoscalarol (**3**) and deoxoscalarin (**4**), which have not been reported from *C. scalaris* in the past. The unpalatability of the sponge to fish was traced by field feeding assays to the fractions containing dihydrofurospongin-2. The structures of the new metabolites were elucidated by interpretation of their NMR data and high-resolution mass spectral measurements.

Sponges are prolific producers of novel terpenoids with a wide spectrum of biological activities.[1] The order Dictyoceratida is a rich source of sesterterpenoids with the scalarane skeleton, acyclic furanosesterterpenes, and degraded C-21 furanoterpenes.[2]

In the genus *Cacospongia* are important producers of polycyclic sesterterpenoids, many of which have been reported to possess pharmacological[3−7] and antipredatory[8] activities. It is noteworthy that members of this genus collected from different locations appear to produce chemically distinct metabolites. Mediterranean *Cacospongia* species collected from the Bay of Naples and the bay of Taranto yielded mainly tetracarbocyclic scalarane sesterterpenoids and related pyrroloterpenes,[9−11] whereas specimens collected at the Cap de Nice afforded linear C_{21} difuran terpenoids.[12] Moreover the same species collected from the northern Adriatic has been reported to produce 23,24-bishomoscalaranes.[13] Specimens of *C. cf. linteiformis* from the Caribbean yielded a series of bi-, tetra-, and pentacyclic sesterterpenes,[14,15] while Australian collections have been reported to yield a number of brominated meroterpenoids.[16,17] Finally, a Philippine *Cacospongia* species produced furanolabdanes and furanoditerpenes as well as diterpene-benzenoids which have not been detected in this genus before.[18]

In the course of our investigations on bioactive metabolites from marine organisms and the elucidation of their ecological roles[19,20] we were intrigued by the geographic variability in the chemistry of *Cacospongia.* Therefore, we examined the chemical composition of specimens of *C. scalaris* collected from the Gulf of Astakos in the Ionian Sea. The CH_2Cl_2/MeOH extract of the freeze-dried sponge was subjected to a combination of open column and HPLC separations to afford new metabolites **1** and **2** along with furoscalarol[23,24] (**3**) and deoxoscalarin[21,22] (**4**), which have not been reported from this species in the past. Along with the above-mentioned compounds the previously reported *C. scalaris* metabolites, scalarin, acetylfuroscalarol, and dihydrofurospongin-2, were isolated.



Compound **1**, 16-acetoxy-dihydrodeoxoscalarin, was isolated as a colorless oil with a molecular formula of $C_{29}H_{46}O_6$, as established by HREIMS and ^{13}C NMR data. The degree of unsaturation in combination with the presence of two acetoxy groups and the absence of multiple bonds suggested a pentacyclic sesterterpenoid. The ^1H NMR revealed resonances for five methyl groups (δ 0.78 $\times$ 2, 0.83, 0.84, and 1.00), two acetoxy methyls (δ 2.00 and 2.10), three protons on oxygenated methines (δ 5.26, 5.12,

* To whom correspondence should be addressed. Tel: +30 210 7274 592. Fax: +30 210 7274 592. E-mail: roussis@pharm.uoa.gr.

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Table 1. ^{13}C NMR, ^{1}H NMR, and HMBC Data for Metabolites **1** and **2**

	1			2		
position[a]	δ_C	δ_H (J in Hz)	HMBC (H→C)	δ_C	δ_H (J in Hz)	HMBC (H→C)
1	39.64	0.58/1.52 m	C-23, C-11	142.5	7.32 brs	C-2, C-3, C-4
2	18.49	1.37/1.56 m		111.1	6.26 brs	C-1, C-3, C-4
3	41.98	1.33/1.12 m	C-21, C-22	124.8		C-1, C-2, C-4, C-5,
4	33.21			138.8	7.19 brs	C-1, C-2, C-3, C-5
5	56.52	0.82 m	C-21, C-22	25.04	2.43 t (7.3)	C-3, C-4, C-6
6	18.09	1.36/1.56 m		32.4	2.23 m	C-5
7	41.67	1.69/0.96 m		123.8	5.14 t (6.87)	C-9
8	36.87			135.7		C-9
9	52.04	1.23 m	C-23	16.0	1.55 s	C-7, C-8, C-10
10	37.62		C-9, C-23	39.6	1.99 m	C-9, C-11
11	21.90	1.66 m	C-23, C-1	26.5	2.05 m	C-10
12	74.23	4.92 m	C-25	124.8	5.07 t (6.25)	C-14
13	37.62		C-25	134.2		C-14
14	51.07	1.12 m	C-24, C-25	15.9	1.55 s	C-12, C-13, C-15
15	21.89	1.46/1.72 m	C-20	39.6	1.95 m	C-14
16	72.61	5.12 m		25.7	2.05 m	
17	37.62	3.17 m	C-18	125.6	5.21 t (6.87)	C-25
18	51.21	2.19 d (7.2)	C-20, C-25	131.9		C-25
19	99.73	5.26 d (7.2)	C-20	28.4	2.26 m	
20	69.45	3.82 dd (8.5, 8.8) 4.01 dd (8.8, 11.6)	C-18, C-19	29.1	1.73/1.48 m	
21	33.21	0.84 s	C-5	78.0	3.68 m	C-22, C-24
22	21.30	0.78 s	C-5	43.8	2.70 m	C-21, C24
23	16.06	0.78 s	C-1, C-10, C-9	12.1	1.18 d (6.5)	C-24
24	14.19	0.83 s	C-9, C-14	175		C-21, C-22, C-23
25	16.86	1.00 s	C-12, C-13, C-18, C-14	67.3	4.67/3.83 d (12.5)	C-17, C-18
*CH₃*CO(12)	21.07	2.00 s				
*CH₃*CO(16)	21.30	2.10 s				
CH_3*CO*	171					

[a] Assignments were made from the HSQC spectrum.

and 4.92), and a deshielded methylene (δ 4.01/3.82). The ^{13}C NMR spectrum showed resonances for 29 carbons including seven methyls, eight methylenes, eight methines, and six quaternary carbons. Four oxygenated carbons (δ 69.45, 72.61, 74.23, and 99.73) were observed, with one belonging to a hemiacetal moiety.

A comparison with metabolites in the literature revealed structural similarities with the scalarane type sesterterpenoids. The spectroscopic data for rings A−C showed close resemblance to those of scalarin and deoxoscalarin. Moreover, the characteristic fragment ions at m/z 205 and 191 in the mass spectrum of **1**, due to fragmentation across the C ring usually found in the scalarane derivatives, supported the absence of substituents on the A and B rings. The absence of any double bonds and the presence of a second acetoxy group suggested that structural alterations existed on the D and E rings. Connectivities about the D and E ring system were deduced by COSY and HMBC and are listed in Table 1. The S^* stereochemistry of C-12 was proposed on the basis of NOE correlations between H-12 and the methyl group at C-13. In the same NOE experiment, the spatial proximity of H-16 with H-14, H-15α (δ 1.72), and H-17 was obvious, suggesting S^* stereochemistry at C-16. The *cis* D/E ring fusion was proposed on the basis of a lack of NOE between H-18 and H-25 and the observed close proximity of H-20β with H_3-25 and H-15β as well as of H-20α with H-17. The R^* stereochemistry at C-19 was suggested on the basis of the correlations between H-19 and H_3-25 and the absence of coupling between H-19 and H-18 (90° dihedral angle).

Compound **2**, astakolactin, was obtained as a colorless oil with a molecular formula of $C_{25}H_{36}O_4$, as established by HREIMS and ^{13}C NMR data. Evident in the ^{1}H NMR spectrum of **2** were a monosubstituted furan ring (δ 7.32, 7.19, and 6.26), two singlets corresponding to vinyl methyls (δ 1.55 × 2), a doublet corresponding to a third methyl group (δ 1.18), and three triplets resulting from olefinic protons (δ 5.21, 5.14, and 5.07). The resonances of a deshielded methylene appeared as doublets at δ 4.67 and 3.83, and the presence of a proton on an oxygenated methine was located at δ 3.68.

The ^{13}C NMR spectrum of compound **2** showed resonances for 25 carbon atoms including three methyls, nine methylenes, eight methines, and five quaternary carbons. The chemical shifts in the ^{13}C NMR spectrum confirmed the presence of the furan ring and the three trisubstituted double bonds. Additionally, these data showed the presence of an oxygenated methine, an oxygenated methylene, and an ester/lactone carbonyl group. The inherent degrees of unsaturation, in combination with the presence of an ester carbonyl and five double bonds, suggested that compound **2** was a bicyclic sesterterpenoid. By comparison with metabolites in the literature sharing relevant structural features, astakolactin (**2**) showed a resemblance to the variabilin skeleton.[25]

Two-dimensional NMR experiments (HMBC, COSY) assisted in confirming the connectivity of the terpenoid side chain on C-3 of the furan ring. The presence of a deshielded oxygenated methylene in **2**, and the absence of the characteristic aliphatic methyl of sesterterpene tetronic acids, suggested a cyclization involving this methyl group. The downfield shift of the oxygenated methylene is influenced by the adjacent double bond, and this was confirmed by HMBC correlations of H-25 with C-17 and C-18. The COSY spectrum revealed the connectivity of the H-22 methine, the H_3-23 aliphatic methyl group, the H-21 oxygenated methine, and the H_2-20 methylene. Furthermore, the HMBC correlation between the carbonyl carbon C-24 and H_3-23, H-22, and H-21 supported the proposed lactone structure.

The NOESY correlations between H-22 and H-21 supported the 21R^*, 22R^* relative stereochemistry at these two chiral centers. The spatial proximity between H-17 and H-19 was also shown by NOE and suggests Z geometry for the corresponding double bond. The absence of NOE between H-7 and H-12 with H_3-9 and H_3-14 (δ 16.0, 15.9),

respectively, as well as the chemical shifts of the vinyl methyls, support *E* geometry for the two double bonds at C-7 and C-12.

Furoscalarol (**3**), deoxoscalarin (**4**), scalarin, acetylfuroscalarol, and dihydrofurospongin-2 were also isolated as major constituents of the sponge extract, and their identification was based on comparison with literature spectroscopic data.

Field experiments verified the antifeedant activity of the *C. scalaris* extract and suggested that the activity results mainly from dihydrofurospongin-2.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer model 341 polarimeter and a 10 cm cell. UV spectra were determined in spectroscopic grade C_6H_{14} on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained using a Paragon 500 Perkin-Elmer spectrophotometer.

^{1}H and ^{13}C NMR spectra were recorded using Bruker AC 200 and DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard. High-resolution FAB mass spectral data were recorded on a JEOL AX505HA mass selective detector and were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN. Low-resolution EI mass spectral data were recorded on a HP 5973 mass selective detector. Column chromatography was performed with Kieselgel 60 (Merck), and HPLC was conducted using a Hewlett-Packard Agilent 1100 Pharmacia LKB 2248 model and an EBC LC-1240 equipped with a refractive index detector, with Spherisorb S10W and Supelcosil SPLC-Si CC1593 25 cm × 10 mm, 5 um, columns. TLCs were performed with Kieselgel 60 F_{254} (Merck aluminum support plates).

Collection, Extraction, and Isolation. The black massive sponge, *Cacospongia scalaris*, was hand collected by scuba diving from a depth of 30 m in May 2000 at Astakos Gulf, Etoloakarnania, Greece. A voucher specimen has been deposited at the Herbarium of the Pharmacognosy Laboratory, University of Athens (ATPH/MO/133). The collected organism was kept frozen until processed. The freeze-dried diced sponge material (41 g) was exhaustively extracted at room temperature in a 2:1 mixture of CH_2Cl_2/MeOH. The combined extracts were concentrated under vacuum to yield a dark brown oily residue (7.22 g), which was fractionated by Si gel column chromatography with a step gradient 10% hexanes/EtOAc and finally MeOH. The medium-polarity fractions following repetitive column fractionations yielded metabolites **1**−**4**, which were further purified by normal-phase HPLC.

The preliminary palatability of the sponge extract was evaluated in laboratory aquaria using the generalist predator fish *Thalassoma pavo* and following the methods described by Pawlik et al.[26] Groups of fish, randomly chosen, were offered either a treated or control food pellet, followed by the other choice. Control and treated pellets were given one at a time to 10 different fish, kept separately. Tank assays were repeated at least seven times in order to establish a statistically significant number of experiments. Under these conditions, the extract was found to exhibit significant deterrence.

Bioassay-guided fractionation showed activity to reside in the fractions containing dihydrofurospongin-2. The crude extract and the fractions of the initial chromatographic separation were tested in the field in areas of Astakos Gulf. Control and treated food strips were prepared according to the same protocol and were suspended, in pairs, on plastic ropes at the depth of approximately 20−30 m. The ropes were left in the sea until at least 50% of the control strips were consumed. The eaten percentage of the strips was determined volumetrically. During the experiment several fishes, common in the Mediterranean sea, were observed feeding on test strips (e.g., *Sargus annularis, Chrysophrys aurata, Coris julis*). The Wilcoxon paired-sample test (one tailed[27]) was used to analyze the results of these assays, which were found to follow the pattern of the tank experiments.

16-Acetoxy-dihydrodeoxoscalarin (1): colorless oil (3.2 mg); $[\alpha]_D$ −10° (*c* 0.07, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2974, 1726, 1440, 1281 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HREIMS *m/z* 473.3282 (calcd for $C_{29}H_{45}O_5$, $[M - OH]^+$, 473.3267).

Astakolactin (2): colorless oil (3.9 mg); $[\alpha]_D$ −36° (*c* 0.05, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3060, 1758, 1419, 1270; ^{1}H and ^{13}C NMR, see Table 1; HREIMS *m/z* 400.2610 (calcd for $C_{25}H_{36}O_4$, $[M]^+$, 400.2613).

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