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A new dimethylscalarane derivative from the sponge *Cacospongia scalaris*

Salvatore De Rosa*, Antonio Crispino, Alfonso De Giulio, Carmine Iodice, Giuseppina Tommonaro

Istituto per la Chimica di Molecole di Interesse Biologico CNR, Via Toiano, 6 - 80072 Arco Felice (Napoli) Italy (Associated to the National Institute for the Chemistry of Biological Systems, CNR)

Nevenka Zavodnik

Center for Marine Research "R. Boskovic" Institute, Rovinj, Croatia

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Abstract

A new 23,24-bishomoscalarane sesterterpene (**2**) was isolated from the northern Adriatic sponge *Cacospongia scalaris*. The structure was proposed on the basis of spectral data. The absolute stereochemistry was determined using the modified Mosher's method. Compound **2** is the first alkylated scalarane sesterterpenoid with methylation at C-23, to have been identified in nature.

Keywords: Marine metabolites; Sponges; Terpenes and terpenoids.

Introduction

Marine organisms and in particular sponges have provided a large number of sesterterpenoids, most of which incorporate the scalarane skeleton [1]. Metabolite of this class are confined in a small number of related genera of the order Dictyoceratida [2] and the scalarane terpenoids found in nudibranchs are of dietary origin [3-5], in fact these molluscs live in close association with Dictyoceratid sponges. Some of scalarane sesterterpenes exhibit significant antiinflammatory [6-8], cytotoxic [9, 10] and antifeedant [5] activities. Scalarane sesterterpenoids also include alkylated derivatives, sometimes called homoscalaranes, that can be divided into four known skeletal types [11]. Homoscalaranes show methylation at C-20 or C-24, while methylations at C-20 and C-24 are typical in bishomoscalaranes [12,13]. The numbering system which has been used throughout this paper is that proposed by Kazlauskas *et al.* [14].

Continuing our search for marine natural compounds, we have previously isolated 12-deacetoxyscalaradial (**1**) [15], the first scalarane sesterterpenoid without a substituent at C-12,

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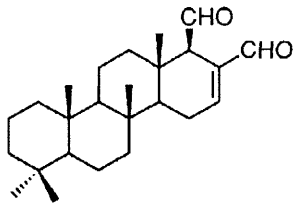
from *Cacospongia mollior*, collected in the Tyrrhenian Sea. We now report the isolation and the structural elucidation of a new scalarane derivatives from *C. scalaris*, collected in the northern Adriatic. From *C. scalaris* collected in the gulf of Naples was isolated scalarin, the first compound possessing a scalarane skeleton to be described [16].

Results and Discussion

The Et_2O-soluble fraction of the Me_2CO extract of *C. scalaris* was chromatographed on Si gel to give compound **2** (0.02% dry weight).

Compound **2**, which did not give crystals suitable for X-ray analysis, had $[\alpha]_D$ +60.4° (c= 0.5, $CHCl_3$) and the molecular formula $C_{27}H_{44}O_3$ from HRMS of the parent ion. The IR spectrum showed the presence of carbonyl (1715 cm^{-1}) and hydroxyl (3390 cm^{-1}) groups. This latter could be acetylated by treatment with pyridine and acetic anhydride, leading to the monoacetyl derivative **3**. The ^{1}H-NMR spectrum of **2** showed the presence of an aldehyde group (δ 9.61, 1H, s), a methyl ketone residue (δ 2.60, 3H, s), four tertiary methyl groups (δ 0.93, 0.84, 0.71 and 0.61, each 3H, s), an ethyl group (δ 0.51, 3H, t, J = 7.7 Hz), and an α-methine of secondary hydroxyl function (δ 3.48, 1H, ddd, J = 11.0, 10.3, 5.5 Hz). The ^{13}C-NMR spectrum contained the expected 27 signals and did not show additional signals due to sp_2 carbon atoms, apart the expected aldehyde (δ 201.9, d) and ketone (δ 211.5, s) groups. The count of unsaturations and the considerations on the functionalities that are present in the molecule indicated a tetracyclic skeleton, and the NMR data suggested that compound **2** has a bishomoscalarane skeleton.



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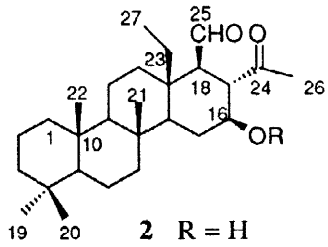

2 R = H
3 R = Ac
4 R = R-MTPA
5 R = S-MTPA

The presence of characteristic fragment ions at *m/z* 205 and 191, in the mass spectrum of **2**, due to fragmentation across the C ring, which are usually found in the scalarane derivatives [15,16] suggested the absence of substituents on A and B rings. Comparison of ^{13}C NMR spectra with those of reported for scalaranes [15-17] fully confirms this hypothesis. The COSY-45 spectrum showed that the methine doublet at δ 2.38 (H-18) was coupled with another methine at δ 2.77 (H-17), which in turn was coupled with an α-hydroxyl proton at δ 3.48 (H-16). This latter was coupled with a non-equivalent methylene at δ 1.40 and 1.05 (H-15), which in turn, was coupled with a methine at δ 0.68 (H-14). From the COSY-45 data it

had been also possible to define the spin system corresponding to the protons H-9/H-11/H-12 and the presence of a tertiary ethyl group. The methyl ketone residue was located at C-17 by presence of HMBC correlation between the methyl at δ 2.60 (H-26) and the carbon at δ 52.5 (d), which was correlated (HETCOR) with the proton at δ 2.77. These data also suggested the location of the hydroxy and the aldehyde groups at C-16 and C-18, respectively. HMBC correlations observed between the H-27 methyl protons (δ 0.51) and the carbons observed at δ 41.4 (C-13), 21.4 (C-23), and the H-18 methine proton (δ 2.38) and the carbons at δ 211.5 (C-24), 201.9 (C-25), 74.8 (C-16), 57.8 (C-14), 41.4 (C-13), and 21.4 (C-23) defined the location of the ethyl group at C-13. Other HMBC correlations reported in Table 1, allowed us to propose the structure **2**, without stereochemical implications.

Table 1. NMR Spectral Data of **2** in C_6D_6 Solution[a].

N.C	^{13}C	^{1}H	HMBC (J_{C-H} =10 Hz)
1	39.8 t	1.58 m, 0.72 m	–
2	18.3 t	1.45 m, 1.25 m	–
3	42.3 t	1.45 m, 1.20 m	0.93 (H-19), 0.84 (H-20)
4	33.5 s	–	0.93 (H-19), 0.84 (H-20)
5	56.2 d	0.72 m	1.48 (H-7), 1.20 (H-3), 0.84 (H-20), 0.71 (H-22)
6	18.8 t	1.20 m	–
7	41.7 t	1.48 m, 0.55 m	0.61 (H-21)
8	37.7 s	–	0.68 (H-14), 0.61 (H-21)
9	60.5 d	0.50 m	1.97 (H-12), 1.48 (H-7), 0.71 (H-22), 0.61 (H-21)
10	37.3 s	–	0.71 (H-22)
11	17.0 t	1.35 m, 1.18 m	–
12	35.7 t	1.97 ddd (10.1, 10.1, 3.3), 0.90m	1.35-1.18 (H-11), 1.30 (H-23)
13	41.4 s	–	2.38 (H-18), 1.40-1.05 (H-15), 0.51 (H-27)
14	57.8 d	0.68 dd (13.2, 2.6)	2.38 (H-18), 1.40-1.05 (H-15), 0.61 (H-21)
15	29.6 t	1.40 m, 1.05 ddd (13.2, 13.2, 11.0)	0.68 (H-14)
16	74.8 d	3.48 ddd (10.3, 11.0, 5.5)	2.77 (H-17), 2.38 (H-18), 1.40-1.05 (H-15), 0.68 (H-14)
17	52.5 d	2.77 dd (11.4, 10.3)	9.61 (H-25), 3.48 (H-16), 2.60 (H-26), 1.40-1.05 (H-15)
18	65.9 d	2.38 d (11.4)	9.61 (H-25), 2.77 (H-17), 1.30 (H-23)
19	33.3 q	0.93 s	0.84 (H-20)
20	21.2 q	0.84 s	0.93 (H-19), 1.45-1.20 (H-3)
21	17.7 q	0.61 s	1.48-0.55 (H-7)
22	16.1 q	0.71 s	–
23	21.4 t	1.30 m	2.38 (H-18), 1.97-0.90 (H-12), 0.51 (H-27)
24	211.5 s	–	3.48 (H-16), 2.77 (H-17), 2.38 (H-18)
25	201.9 d	9.61 brs	2.77 (H-17), 2.38 (H-18)
26	33.7 q	2.60 s	–
27	9.3 q	0.51 t (7.7)	1.30 (H-23)

[a] Chemical shifts are referred to TMS and were assigned by COSY and HETCOR NMR spectra. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses and were determined by double-irradiation experiments.

The NOESY spectrum of **2** exhibited the presence of nOes indicating that the aldehyde group (H-25, δ 9.61), the H-12_{eq} (δ 1.97) and the ethyl group (δ 0.51) are oriented on the same side (β) of the molecule, while H-18_{ax} (δ 2.38) and H-14 (δ 0.68) have the same orientation (α) as the oxymethine (H-16, δ 3.48). The axial position of H-18 (J = 11.4 Hz), H-17 (J = 11.4 and 10.3 Hz), and H-16 (J = 11.0, 10.3 and 5.5 Hz) was deduced from the magnitude of their coupling constants in the ^{1}H-NMR spectra of **2**.

The absolute stereochemistry of compound **2** was determined by application of modified Mosher's method [18,19]. Treatment of **2** with S-(-)- and R-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) yielded the corresponding R- and S-MTPA esters (**4** and **5**, respectively). The ^{1}H-NMR chemical shifts of both diastereomers **4** and **5** were carefully assigned by analysis of their COSY spectra. The proton chemical shift differences ($\Delta\delta=\delta_{S\text{-MTPA ester}} - \delta_{R\text{-MTPA ester}}$) observed for H-21 and H-27 were -22.18 and -25.12 Hz, respectively, while those observed for H-17, H-18 and H-26 were +5.51, +6.60 and +164.98 Hz, respectively [for convenience, δ values were given in Hz (500 MHz)]. From the MTPA determination rule [18,19], the positive and negative Δδ value observed for the signals protons were located on the right and on the left side of the MTPA plane, respectively, showed clearly that the absolute configuration at C-16 is S. According to the relative configuration, the absolute configuration of asymmetric centres C-17 and C-18 were assigned both as S. The absolute stereochemistry of C-5, C-8, C-9, C-10, C-13 and C-14 as shown is compatible with the known absolute stereochemistry of common steroids and terpenoids.

Conclusion

The finding of **2** from *C. scalaris* is the first report of a bishomoscalarane with methylation at C-23. In general scalaranes without a substituent at C-12 are uncommon natural metabolites and up to now only one product (**1**) isolated from two sponges, *C. mollior* [15] and *C. scalaris* [17] collected in the gulf of Naples, Italy, and Southern Coast of Spain, respectively, have been reported. The fact that *Cacospongia* genus is a source of scalaranes without substituent at C-12 may have some taxonomic significance.

Experimental

General experimental procedures.

Melting points were measured on a Kofler apparatus and are uncorrected. Ir spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell. EIMS spectra were recorded on a TRIO VG 2000 spectrometer; HREIMS was recorded on a VG Autospec spectrometer with peak matching technique, using PFK as calibrant. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. The 2D nmr spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder.

Biological material.

Cacospongia scalaris (order Dictyoceratida; family Thorectidae) was collected by dredging (-20 m) at Rovinj (Croatia), and frozen at -20° until extracted and was identified by prof. Roberto Pronzato, Istituto di Zoologia dell'Università di Genova. A voucher specimen is maintained in the Arco Felice institute collection (voucher No. S12R/96).

Extraction and isolation.

The frozen sponge (50 g dry wt after extraction) was extracted with Me_2CO and, after elimination of the solvent *in vacuo*, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The Et_2O extract was evaporated *in vacuo* to obtain a brown oil (2.6 g), which was applied on a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40-70°) to Et_2O. From fractions eluted with petroleum ether-Et_2O (7:3) was recovered compound **2** (10 mg, 0.02% dry weight) that crystallized from *n*-hexane.

Compound **2**: mp 123-125°C; $[\alpha]_D$ +60.4° (c= 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3390 (br), 1715 cm^{-1}; EIMS *m/z* (%) $[M]^+$ 416.3293 ($C_{27}H_{44}O_3$ requires 416.3290) (4), $[M-H_2O]^+$ 398 (8), 387 (12), 370 (20), 273 (20), 205 (35), 191 (100); NMR data see Table 1. Cross peaks were observed in a NOESY spectrum between the following signals: δ 9.61-0.51 (H-25, H_3-27), 9.61-1.97 (H-25, H-12_{eq}), 3.48-2.38 (H-16, H-18), 3.48-0.68 (H-16, H-14), 2.77-2.60 (H-17, H_3-26), 2.77-1.05 (H-17, H-15_{ax}), 2.77-0.51 (H-17, H_3-27), 1.05-0.61 (H-15_{ax}, H_3-21). The ^1H NOESY spectrum was recorded at 500 MHz; only cross peaks not sensitive to strong filtering are reported.

Acetylation of compound **2.**

A solution of compound **2** (2 mg) in pyridine (0.5 mL) and acetic anhydride (0.2 mL) was kept at room temperature over night. The excess reagents were removed *in vacuo*, and the residue was subjected to preparative TLC on Si gel plate (petroleum ether-Et_2O; 4:1) to give acetate **3**.

Acetate **3**: amorphous solid; IR ν_{max} ($CHCl_3$) 1745, 1720, 1230 cm^{-1}; EIMS *m/z* (%) $[M]^+$ 458 (2), $[M-HAc]^+$ 398 (10), 387 (8), 370 (20), 205 (40), 191 (100); ^{1}II-NMR ($CDCl_3$) δ : 9.80 (1H, s, H-25), 4.64 (1H, ddd, *J* = 11.0, 10.3, 5.5 Hz, H-16), 3.07 (1H, dd, *J* = 11.4, 10.3 Hz, H-17), 2.48 (1H, d, *J* = 11.4 Hz, H-18), 2.37 (3H, s, H-26), 2.07 (3H, s, $COC\underline{H}_3$), 0.86 (3H, s, H-21), 0.84 (3H, s, H-19), 0.81 (3H, s, H-22), 0.80 (3H, s, H-20), 0.72 (3H, t, *J* = 7.6 Hz, H-27).

Preparation of R- and S-MTPA esters of compound 2.

S-(-)-MTPA chloride (Aldrich) (20 μL) was added to a solution of compound **2** (1.5 mg) in dry pyridine (0.5 mL) and the resulting mixture was kept at room temperature for 2 h. After the removal of the solvent, *in vacuo*, the residue was subjected to preparative TLC on

Si gel plate (petroleum ether-Et_2O; 4:1) to give R-MTPA ester **4** of compound **2** (1 mg). The S-MTPA ester **5** was obtained in the same manner, starting from R-(+)-MTPA chloride.
R-MTPA ester 4: $[\alpha]_D$ +76.5° (c= 0.1, $CHCl_3$); ^{1}H-NMR (C_6D_6) δ : 9.46 (1H, s, H-25), 7.7-7.0 (5H, m, Ph), 5.21 (1H, ddd, *J* = 11.0, 10.3, 5.5 Hz, H-16), 3.47 (3H, s, OCH_3), 3.00 (1H, dd, *J* = 11.4, 10.3 Hz, H-17), 2.34 (1H, d, *J* = 11.4 Hz, H-18), 1.99 (3H, s, H-26), 0.95 (3H, s, H-19), 0.83 (3H, s, H-20), 0.68 (3H, s, H-22), 0.58 (3H, s, H-21), 0.44 (3H, d, *J* = 7.7 Hz, H-27).
S-MTPA ester 5: $[\alpha]_D$ +7.5° (c= 0.1, $CHCl_3$); ^{1}H-NMR (C_6D_6) δ : 9.47 (1H, s, H-25), 7.6-7.0 (5H, m, Ph), 5.20 (1H, ddd, *J* = 11.0, 10.3, 5.5 Hz, H-16), 3.38 (3H, s, OCH_3), 3.01 (1H, dd, *J* = 11.4, 10.3 Hz, H-17), 2.40 (3H, s, H-26), 2.36(1H, d, *J* = 11.4 Hz, H-18), 0.95 (3H, s, H-19), 0.82 (3H, s, H-20), 0.67 (3H, s, H-22), 0.52 (3H, s, H-21), 0.38 (3H, d, *J* = 7.7 Hz, H-27).

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