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Spatane diterpenoids from the brown alga *Stoechospermum marginatum* (Dictyotaceae)

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Abstract

Two spatane diterpenes, the new 5(*R*),16(*S*)-diacetoxyspata-13,17-diene and the known 5(*R*),16(*S*)-dihydroxyspata-13,17-diene have been isolated from the brown alga *Stoechospermum marginatum*. The structures were elucidated from chemical and spectroscopic evidence. The absolute stereochemistry was determined using the modified Mosher's method. The ^{13}C chemical shifts were totally assigned by extensive NMR experiments

Keywords: *Stoechospermum marginatum*; Dictyotaceae; Spatane diterpenoids; ^{13}C NMR

1. Introduction

The Phaeophyta, are a rich source of unique diterpenoids with a very complex cyclic systems, produced by mechanisms of cyclization not previously encountered in nature (De Rosa, 1991). Spatane diterpenoids are characterized by unique tricyclic [5.3.0.$0^{2,6}$] decane ring and the first compound to be reported was spatol (**1**) (Gerwick, Fenical, Engen, & Clardy, 1980), isolated from *Spatoglossum schmitii*. Subsequently, many spatane diterpenoids were isolated from *S. howleii*, *Stoechospermum marginatum*, *Dilophus marginatus* and *D. okamurai* (Kurata, Tanaguchi, Shiraishi, & Suzuki, 1990). Algae of the family Dictyotaceae are frequently encountered as the major vegetation in shallow water in tropical and subtropical habitats. *S. marginatum* (C. Agardh) Kuetzing, a representative of the family Dictyotaceae is found in abundance in shallow water along the coast of Iran in the gulf of Oman. This paper describes the isolation and the structural elucidation, including the absolute stereochemistry, of spatane diterpenes from *S. marginatum*, and the complete assignment of ^{13}C chemical shifts never reported for this class of compounds.

2. Results and discussion

The lipid-soluble extract of *S. marginatum* was chromatographed on silica gel, using increasing concentration of diethyl ether in petrol as the eluent. Two diterpenoids were isolated. In order of increasing polarity, these were 5(*R*),16(*S*)-diacetoxyspata-13,17-diene (**2**, 0.01% dry weight) and 5(*R*),16(*S*)-dihydroxyspata-13,17-diene (**3**, 0.017% dry weight). An initial examination of the spectral data revealed that the two compounds were very similar.

Compound **2** had $[\alpha]_D$ +10.5° and a molecular formula $C_{24}H_{36}O_4$ (MS and NMR data). The IR absorption at 1720 cm^{-1}, the ^1H NMR signal at δ 2.01 (6H, s) and the ^{13}C NMR signals at δ 170.6 (s), 170.4 (s), 21.3 (q) and 21.2 (q) indicated the presence of two acetate groups, and this was further substantiated by the mass fragments at *m*/*z* 328 and 268, corresponding to the subsequent loss of two molecules of AcOH. In addition, the ^1H NMR spectrum showed two α-acetoxy proton signals at δ 5.61 (ddd, J=8.4, 7.6 and 6.9 Hz) and 4.88 (d, J=4.1 Hz), two vinyl methyl groups at δ 1.68 and 1.71, a tertiary methyl group at δ 0.89 and a methyl doublet at δ 0.85 (J=6.6 Hz) coupled with a methine at δ 1.80 (H-1) which was in turn linked to a nonequivalent methylene at δ 1.72 and 1.30 (H-2), and to a proton (H-8) resonating at δ 2.00 (from COSY). Further signals at δ 4.84 (1H, br s), 4.76 (1H, br s) and 5.06 (1H, br d, J=8.4 Hz) indicated the presence of an exomethylene group and a trisubstituted double bond, confirmed by the presence in the low-field region of ^{13}C NMR spectrum of signals at δ 143.6 (s), 137.3 (s), 123.5 (d) and 112.0 (t). The count of unsaturations and the considerations on the functionalities that are present in the molecule indicated a

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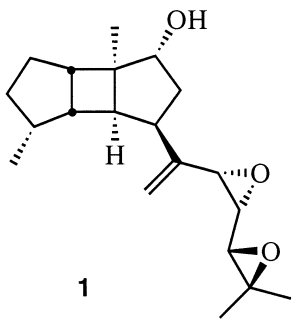

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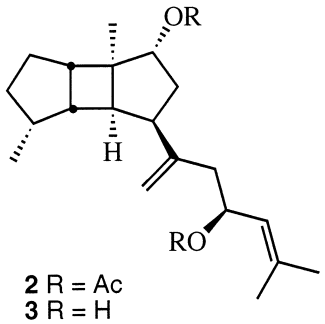

2 R = Ac
3 R = H
4 R = R-MTPA
5 R = S-MTPA

tricyclic skeleton, and the NMR data (Table 1) suggested that compound **2** has a spatane skeleton.

The COSY-45 spectrum showed that the α-acetoxy proton at δ 4.88 (H-5) was coupled with a nonequivalent methylene at δ 2.25 and 1.70 (H-6), which in turn was coupled with a methine at δ 2.86 (H-7). This latter was coupled with a methine at δ 2.07 (H-8) and long-range with the exomethylene protons (H-14). From the COSY-

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **2** in $CDCl_3$ solution[a]

NC	^{13}C	^{1}H	Long-range connectivities[b]
1	36.5 d	1.80 m	H-3
2	34.9 t	1.72 m, 1.30 m	–
3	27.8 t	1.70 m, 1.45 m	H-9, H-10
4	46.1 s	–	H-5, H-6, H-9, H-12
5	82.2 d	4.88 brd (4.1)	H-12, H-10, H-6
6	34.6 t	2.25 ddd (13.2, 12.9, 4.1), 1.70 m	H-7
7	46.0 d	2.86 ddd (12.9, 6.5, 5.2)	H-14, H-9, H-5
8	43.2 d[c]	2.07[d] m	H-6, H-5
9	37.7 d	2.00 m	H-11, H-7
10	43.1 d[c]	2.07[d] m	H-5
11	14.3 q	0.85 d (6.6)	H-2
12	13.1 q	0.89 s	H-10, H-8
13	143.6 s	–	H-17, H-7, H-6
14	112.0 t	4.84 brs, 4.76 brs	H-15, H-7
15	41.5 t	2.29 dd (14.4, 7.6), 2.16 (14.4, 6.9)	H-17, H-14
16	70.7 d	5.61 ddd (8.45, 7.6, 6.9)	H-15
17	123.5 d	5.06 brd (8.45)	H-20, H-19, H-15
18	137.3 s	–	H-20, H-19, H-17
19	18.4 q	1.68 brs	H-17
20	25.7 q	1.71 brs	H-17
*C*OCH$_3$	170.6 s	–	H-5
*C*OCH$_3$	170.4 s	–	H-16
CO*C*H$_3$	21.3 q	2.01	–
CO*C*H$_3$	21.2 q	2.01	–

[a] Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses. Assignments determined by COSY-45 and HETCOR.
[b] HMBC (J_{C-H} = 10 Hz).
[c] Interchangeable signals.
[d] Overlapped signals.

45 data it had been also possible to define the spin system corresponding to the protons H-2/H-3/H-10/H-9 and the presence of a 1-methylene-3-acetoxy-5-methyl-4-hexenyl side chain. The side chain was located at C-7 by presence of HMBC correlation between the exomethylene at δ 4.84 and 4.76 (H-14) and the carbon at δ 46.0 (d), which was correlated (HETCOR) with the proton at δ 2.86. HMBC correlations observed between the H-5 α-acetoxy proton (δ 4.88) and the carbons observed at δ 46.1 (C-4), 46.0 (C-7) and 43.2 (C-8) defined the location of the acetoxy group at C-5. A combination of COSY, HETCOR and HMBC experiments allowed us to assign all the chemical shifts in the ^{1}H and ^{13}C NMR spectra Table 1 and led to structure **2**, without stereochemical implications. Two HETCOR experiments, with different D2 values (3.57 and 4.17 ms), were indispensable to obtain all $^{1}H/^{13}C$ correlations. Extensive ^{1}H–^{1}H decoupling experiments enabled us to assign approximate coupling constants for those signals which overlapped.

The relative stereochemistry of **2** was deduced by a NOESY spectrum, which exhibited the presence of nOes indicating that the Me-1 (δ 0.85) and H-8 (δ 2.07) are oriented on the same side (α) of the molecule, while H-9 has the same orientation (β) of the side chain. These data are in accordance with the relative stereochemistry reported for spatane diterpenoids.

Hydrolysis of **2** yielded a diol, identical in all respects to the natural compound **3**.

5(*R*),16(*S*)-Dihydroxyspata-13,17-diene (**3**) had $[\alpha]_D$ $+5.9^{\circ}$ (literature value, 6.3°). It is the most abundant diterpene isolated from *S. marginatum*, and it has been previously reported from the same alga collected in the Indian Ocean (Kurata et al., 1990) and from the related alga *S. schmitii* (Gerwick & Fenical, 1983). The spectral data of **3** are in agreement with published data (Gerwick et al., 1981).

Acetylation of compound **3**, under mild conditions, yielded a diacetyl derivative, identical in all respects to the natural compound **2**. The compound **2** was therefore the diacetyl derivative of **3**.

Gerwick et al. established the absolute stereochemistry only at C-5 in **3**, while the stereochemistry at C-16 was not reported. The absolute stereochemistry of compound **3** was determined by application of modified Mosher's method (Ohtani, Kusumi, Ishitsuka, & Kakisawa, 1989; Ohtani, Kusumi, Kashman, & Kakisawa, 1991). Treatment of **3** 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 selected protons are reported in Table 2. From the MTPA determination rule (Ohtani et al., 1989, 1991), the positive and negative $\Delta\delta$ 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 configurations at C-16 is *S*, and confirmed the *R* absolute configurations at C-5.

Table 2
Selected ^{1}H NMR chemical shifts differences[a] of MTPA esters (**4** and **5**)

H	$\Delta\delta^{b} = \delta_{S\text{-MTPA ester}} - \delta_{R\text{-MTPA ester}}$
5	+21.1
6a	+8.8
6b	−7.0
7	+37.0
8	+0.5
11	+5.0
12	−57.0
14a	−18.8
14b	−15.6
15a	−11.0
15b	−13.8
16	+25.9
17	+54.6
19	+13.3
20	+17.6

[a] The assignments were aided by COSY-45.
[b] $\Delta\delta$ values are given in Hz.

3. Experimental

3.1. General experimental procedures

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. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-50 spectrometer. ^{1}H 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 2-D NMR spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using precoated Merck F_{254} plates and Merck Kieselgel 60 powder.

3.2. Extraction and isolation of compounds

S. marginatum was collected in shallow water along the coast of Iran in the gulf of Oman, and frozen at $-20^{\circ}C$ until extracted and was identified by Mr S. R. Abhari, University of Sistan, Baluchestan, Faculty of Marine Sciences, Zahedan, Iran. A voucher specimen is maintained in the Islamic Azad University collection (voucher No. A.R.101). The alga (240 g dry wt after extraction) was extracted with MeOH–CH_2Cl_2 (1:1). The diethyl ether extract (5.3 g) was applied on a column of

Si gel. The column was eluted with a solvent gradient system from petrol ether (40–70°C) to Et_2O. From fractions eluted with petrol ether–Et_2O (4:1) was recovered compound **2** (25 mg, 0.01% dry weight). Compound **3** (40 mg, 0.017% dry weight) was eluted with 40% of Et_2O.

3.3. 5(R),16(S)-*Diacetoxyspata*-13,17-*diene* (*2*)

$[\alpha]_D$ +10.5° (*c* 2.0; $CHCl_3$); IR ν_{max} $CHCl_3$ cm^{-1}: 2980, 1720, 1645, 1410, 1370; EIMS *m*/*z* (rel. int.) 388.2610 $[M]^+$ (1) ($C_{24}H_{36}O_4$ requires 388.2613), 328 $[M–HAc]^+$ (5), 268 $[M–2HAc]^+$ (15), 161 (33), 135 (48), 107 (30), 105 (40), 81 (100); cross peaks were observed in a NOESY spectrum between the following signals: δ 5.61–1.68 (H-16, H_3-19), 5.06–1.71 (H-17, H_3-20), 4.88–2.07 (H-5, H-10), 4.76–2.25 (H-14a, H-6$_\beta$), 4.76–2.00 (H-14a, H-9), 2.07–0.85 (H-8, H_3-11). The ^{1}H NOESY spectrum was recorded at 500 MHz; only cross peaks not sensitive to strong filtering are reported.

3.4. 5(R),16(S)-*Dihydroxyspata*-13,17-*diene* (*3*)

$[\alpha]_D$ +5.9° (*c* 3.1; $CHCl_3$); EIMS *m*/*z* 304.2412 [M+] ($C_{20}H_{32}O_2$ requires 304.2402); ^{1}H and ^{13}C NMR data are in agreement with literature values (Gerwick et al., 1981).

3.5. *Hydrolysis of compound 2*

Compound **2** (3 mg) was dissolved in a 5% solution (1 mL) of KOH in MeOH and was kept, under stirring, at room temperature over night. The reaction mixture was diluted with water and extracted with Et_2O. The Et_2O extract, after elimination of the solvent in vacuo, was subjected to preparative TLC on Si gel plate (petroleum ether–Et_2O; 1:1) to give the diol **3** (2 mg); $[\alpha]_D$ +6.0° (*c* 0.15; $CHCl_3$); MS; ^{1}H and ^{13}C NMR data are identical with those of natural compound **3**.

3.6. *Acetylation of compound 3*

A solution of compound **3** (5 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 diacetate **2** (4 mg), identical in all respect with the natural compound.

3.7. *Preparation of* R- *and* S-*MTPA esters of compound 3*

S-(−)-MTPA chloride (Aldrich) (20 μL) was added to a solution of compound **3** (2.5 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 **3** (2 mg). The *S*-MTPA ester **5** was obtained in the same manner, starting from *R*-(+)-MTPA chloride.

3.8. R-*MTPA ester 4*

$[\alpha]_D$ +25.1° (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.5–7.3 (5H, m, Ph), 5.81 (1H, ddd, *J* = 8.45, 7.65, 6.88 Hz, H-16), 5.05 (1H, *br d*, *J* = 4.1 Hz, H-5), 4.97 (1H, br d, *J* = 8.4 Hz, H-17), 4.90 (1H, s, H-14a), 4.81 (1H, s, H-14b), 3.49 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 2.66 (1H, m, H-7), 2.35 (1H, m, H-6a), 2.30 (1H, dd, *J* = 14.4, 6.5 Hz, H-15), 2.21 (1H, dd, *J* = 14.4, 7.6 Hz, H-15), 2.07 (2H, m, H-8 and H-9),1.67 (3H, s, H-20), 1.64 (3H, s, H-19), 0.90 (3H, s, H-12), 0.82 (3H, d, *J* = 6.5 Hz, H-11).

3.9. S-*MTPA ester 5*

$[\alpha]_D$ −34.5° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.5–7.3 (5H, m, Ph), 5.87 (1H, ddd, *J* = 8.45, 7.65, 6.88 Hz, H-16), 5.11 (2H, m, H-5 and H-17), 4.85 (1H, s, H-14a), 4.77 (1H, s, H-14b), 3.53 (3H, s, OCH_3), 3.52 (3H, s, OCH_3), 2.75 (1H, m, H-7), 2.37 (1H, m, H-6a), 2.31 (1H, dd, *J* = 14.4, 6.5 Hz, H-15), 2.18 (1H, dd, *J* = 14.4, 7.6 Hz, H-15), 2.08 (2H, m, H-8 and H-1), 1.72 (3H, s, H-20), 1.69 (3H, s, H-19), 0.84 (3H, d, *J* = 6.5 Hz, H-11), 0.76 (3H, s, H-12).

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