Pergamon

Tetrahedron Letters 43 (2002) 315–317

TETRAHEDRON LETTERS

New diterpenoids from the far-eastern gorgonian coral *Plumarella* sp.

Valentin A. Stonik,* Irina I. Kapustina, Anatoly I. Kalinovsky, Pavel S. Dmitrenok and Boris B. Grebnev

Pacific Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Vladivostok-22, Russia

Received 18 September 2001; revised 25 October 2001; accepted 5 November 2001

Abstract—Two new cytotoxic diterpenoids, plumarellide (**1**) and the ethyl ester of plumarellic acid (**2**), were isolated from the alcoholic extract of the gorgonian coral *Plumarella* sp. and their structures were established by NMR, EIMS, MALDI TOF MS, IR and UV spectroscopy.

Marine gorgonian corals are an extremely rich source of new diterpenoids[1] that often have interesting biological activities. For instance, diterpene glycosides pseudopterosins and *seco*-pseudopterosins from *Pseudopterogorgia elisabethae*[2,3] demonstrate anti-inflammatory and analgesic properties, superior to those of indomethacin.[4] Another diterpenoid, eleutherobin from *Eleutherobia* sp. is a tubulin-stabilizing antimitotic agent.[5]

The gorgonian corals belonging to the genus *Plumarella* have not been previously studied for natural products. In the course of our continuing investigations into marine invertebrates inhabiting the North-western part of the Pacific Ocean,[6] we have studied extracts of a *Plumarella* sp. collected from the Kuril Islands region.† We report here the isolation and structure elucidation of two new diterpenoids, plumarellide (**1**) and the ethyl ester of plumarellic acid (**2**) from this species.

Specimens of the octocoral were cut into small pieces and extracted with ethanol immediately after collection. The ethanol-soluble materials were concentrated in vacuo. The dark residue was partitioned between aqueous ethanol and hexane and the ethanol-soluble portion was chromatographed on a Si gel column using $CHCl_3$–EtOH (10:1). A crude diterpenoid fraction was obtained and purified by repeated chromatography on a LH-20 Sephadex column in ethanol. The separation of this fraction by HPLC on an Ultrasphere column using ethyl acetate as the mobile phase gave individual diterpenoids (**1** and **2**) in 0.002 and 0.0009% yields based on the dry weight of the coral, respectively. Plumarellide (**1**), mp 223–225°C (MeOH), $[\alpha]_D^{25}$ +109.6 (*c* 0.23, $CHCl_3$–EtOH, 1:1) has the molecular formula $C_{20}H_{24}O_6$ as determined by high resolution MALDI-TOF mass spectroscopy (found for the $[M+Na]^+$ peak 383.1476, calcd 383.1465). The IR spectrum (KBr) contained bands at 1745 and 1670 cm^{-1} (α,β-unsaturated γ-lactone), 3454 cm^{-1} (hydroxyls) and 1640 cm^{-1} (double bonds). The UV spectrum confirmed the presence of an α,β-unsaturated γ-lactone moiety (λ_{max} 217 nm, ε 3600, EtOH). Based on the above mentioned spectroscopic data and interpretation of NMR experiments (^1H–^1H-COSY, DEPT, HMQC), three connectivity sequences indicative of subunits A, B and C were revealed. Also, identified were the quaternary carbon of a semiacetal type (δ_c 106.6, s) and one connected with oxygen (δ_c 90.1, s). The HMBC experiments (see Table 1 and key correlations on the figure) and the conception of biogenesis of this group from a cembranoid precursor permitted connection of the subunits as shown in structure **1**.

Confirmation of this structure and stereochemical assignments were determined from the analysis of 1D NOE experiments and ^1H NMR coupling constants. In fact, the ddd character of the H-14 signal (δ_H 2.14 ppm) is due to homoallylic coupling between H-14 and H-11. This, along with the HMBC data, confirmed the connection of subunits A and B as C-12 to C-13 and C-6 to C-7. The *cis* relationship between H-7, H-11, H-10 and H-14 was determined through NOE observations (see Table 1). For example, irradiation of H-7 (δ_H 2.68, dd)

Keywords: marine metabolites; terpenes; spectroscopy.

* Corresponding author.

† Collected near Matua Island (48°00'N, 153°20.7'E) from a depth of 100 m by dredging.

0040-4039/02/$ - see front matter
PII: S0040-4039(01)02114-1

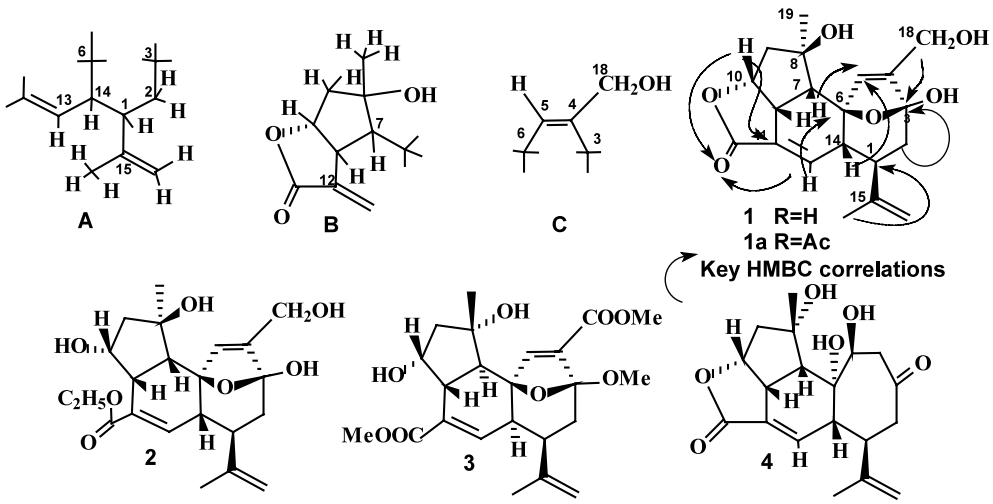

Table 1. Comparative ^{13}C (75.5 MHz) and ^{1}H (300 MHz) NMR spectral data of **1** and **2** (C_6D_6:CD_3OD, 1:1, TMS standard)

	1				**2**			
C	δ C	δ H (*J*)	HMBC	NOE	δ C	δ H (*J*)	HMBC	NOE
1	46.7, d	2.36, td (11.4; 5.6)	C-15, C-16		45.8, d	2.29, m	C-13, C-14, C-15	
2	35.2, t	1.75, dd (11.6; 12.7) 1.89, dd (5.6; 12.7)	C-3, C-4, C-15 C-1, C-3, C-14, C-15	H-2α, 14 H-1, 2β, 18	36.8, t;	1.77, m 1.88, m	C-1, C-3, C-14, C-15 C-1, C-3, C-14, C-15	
3	106.6, s				107.3, s			
4	148.1, s				146.9, s			
5	127.5, d	5.77, t (1.9)	C-3, C-6	H-1, 9α, 13, 18, CH_3-19	128.2, d	6.69, t (1.8)	C-3, C-4, C-6, C-18	H-1, 9α, 18
6	90.1, s				85.3, s			
7	56.2, d	2.68, dd (1.8; 10.3)	C-5, C-6, C-9 C-10, C-11, C-12	H-11, 14, CH_3-19	56.8, d	2.53, dd (1.5; 8.3)	C-5, C-6, C-8, C-9 C-10, C-11, C-14	H-11, 14
8	83.5, s				81. 8, s			
9	47.6, t	1.45, dd (9.0; 13.1) 2.24, ddd (1.8; 6.5; 13.1)	C-10 C-7, C-12		51.5, t	1.96, dd (5.7; 14.6) 2.45, ddd (1.5; 8.3; 14.6)	C-8, C-10 C-7, C-8	H-9β, 5 H-9α, 10
10	83.3, d	5.20, td (9.0; 6.5)	C-12, C-20	H-9β, 11	73.6, d	4.98, td (8.2; 5.7)	C-7, C-8	H-9β, 11
11	45.6, d	3.61, m		H-7, 10, 14	46.6, d	3.95, tt (2.2; 8.3)	C-6, C-10, C-12, C-13, C-20	H-10, 7
12	133.5, s				132.7, s			
13	141.7, d	6.55, t (3.5)	C-6, C-20	H-1, 5, 14, 16	140.0, d	6.77, t (1.9)	C-1, C-6, C-14, C-20	H-1, 14, 16, 17
14	43.9, d	2.14, ddd (2.9; 3.5; 11.4)	C-5, C-6, C-7, C-12, C-15, C-20		42.9, d	2.32, brd (≈12.0)	C-1	
15	146.6, s				146.3, s			
16	113.8, t	4.83, m	C-15, C-17	H-1, 13, 14, CH_3-17	114.6, t	4.88, m	C-1, C-17	H-1, 13, 14, CH_3-17
17	20.7, q	1.67, s	C-1, C-15, C-16		20.5, q	1.67, s	C-1, C-15, C-16	H-1, 13, 14, 16
18	58.5, t	4.38, dd (1.9; 15.7) 4.24, dd (1.9; 15.7)	C-3, C-4, C-5 C-3, C-4, C-5	H-1, 2α	58.7, t	4.39, dd (1.8; 15.4) 4.24, dd (1.8; 15.4)	C-3, C-4, C-5 C-3, C-4, C-5	H-5, 2α
19	25.0, q	1.54, s	C-7, C-8, C-9	H-5, 7, 9β	26.9, q	1.67, s	C-7, C-8, C-9	H-5, 7, 9α, 9β
20	171.6, s				169.3, s			
1′					61.8, t	4.12, q (7.1)	C-20, C-2′	
2′					15.0, q	1.12, t (7.1)	C-1′	

resulted in an NOE to H-11 (δ_H 3.61, m) and H-14 (δ_H 2.14, ddd), while irradiation of H-10 (δ_H 5.20, td) returned an enhancement to H-11 (δ_H 3.61, m) requiring the *cis* fusion of the lactone moiety and the five-membered ring and the same for the five-membered and six-membered rings. Conversely, $J_{1,14}$ (11.4 Hz) was consistent with a *trans* relationship between these protons. Irradiation of the protons of the CH_2OH group (δ_H 4.24, dd and 4.38, dd) resulted in an NOE to H-1 (δ_H 2.36, td) and H-2α (δ_H 1.89, dd), requiring *cis* orientation of the corresponding atoms. Enhancement of the H-1 signal resulting from irradiation of H-5 (δ_H 5.77, t) showed that the C-5 to C-6 bond is *cis*-orientated towards H-1. The $J_{7,9}$ (1.8 Hz) coupling constant probably indicates the E_9 conformation of the five-membered ring and the 'equatorial' orientation of the methyl group at C-8.

Diacetate (**1a**), obtained as the result of treatment of **1** with pyridine–acetic acid mixture (1:1), room temperature, 24 h, mp 67–69°C, $[\alpha]_D^{25}$ +69.6 (*c* 0.25, $CHCl_3$), showed an intensive absorption band at 1740 cm^{-1} (acetoxyl groups) in its IR spectrum. Its UV spectrum demonstrated a λ_{max} at 217 nm, ε 3800 (EtOH), the MALDI TOF (+) mass spectrum demonstrated the $(M+Na)^+$ peak at m/z 467.3. Comparison of the ^{13}C NMR spectra of **1** and **1a** indicated that the hydroxyl group at C-8 was not acetylated in **1a**.

The diterpenoid (**2**) named as the ethyl ester of plumarellic acid, mp 136–138°C, $[\alpha]_D^{25}$ +84.1 (*c* 0.27, EtOAc–EtOH, 4:1), has the molecular formula $C_{22}H_{30}O_7$ as determined by high resolution MALDI-TOF mass spectroscopy (found 429.1869 for the $[M+Na]^+$ peak, calcd 429.1884). Its NMR data (COSY, HMQC, DEPT, HMBC, 1D NOE, see Table 1) showed that **2**, in contrast with **1**, is a tetracyclic ethyl ester and does not contain a γ-lactone moiety. Other structural peculiarities of **2** coincided with those of **1** (see Table 1).

Although the terpenoid **1** is clearly related to mandapamate (**3**)[7] and isomandapamate,[8] a diastereomer of **3** with respect to the C-3 and C-6 chiral centers, isolated from the soft corals belonging to the genus *Sinularia*, it has a more complex pentacyclic skeleton system. Moreover, **1** and tetracyclic terpenoid **2** differ significantly stereochemically from mandapamate and its isomer. All the new terpenoids have a *cis* junction at the five-membered and a six-membered rings as well as a *cis*-orientated proton at H-14 and an isopropenyl group at C-1 in contrast with the transoidal configuration in the diterpenoids from *Sinularia*. On the other hand, the norditerpenoid dissectolide A (**4**) from the Indian Ocean soft coral *Sinularia dissecta*[9] contains four rings with the same conjunctions as in **1**, but differs from the latter in the carbon skeleton system and in the absence of the ether oxygen atom between C-3 and C-6.

It may be suggested that **2** is an artifact, resulting from ethanol addition during extraction of **1**. It has been noted that long-term storage of **1**, in chloroform, led to a partial opening of the lactone. The diterpenoids **1** and **2** show moderate hemolytic activity, inducing 50% hemolysis of mice blood erythrocytes at concentrations of 140 and 250 μM, respectively.

Acknowledgements

The authors are grateful to Dr. T. N. Makarieva for collecting the gorgonian coral and the assistance and Dr. N. G. Prokofieva for bioassay. This work was financially supported by RFBR (grants 00-15-97806, 01-03-96903).

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