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Structure Elucidation for New Sestertepene Alkaloids from the Sponge *Sacotragus* sp.

Jung-Rae Rho[1*], Jongheon Shin[2], and Hyi-Seung Lee[1]

[1]Marine Natural Products Chemistry Laboratory, Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 425-600, Korea
[2]Natural Products Research Institute, College of Pharmacy, Seoul National University, #28 Yungun, Jongro, Seoul 110-460, Korea
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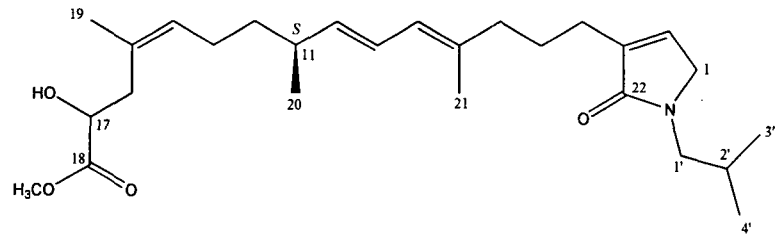
Abstract : Sarcotragins C (**1**) and D (**2**), two novel compounds, have been isolated from the sponge *Sarcotragus* sp. collected from Jaeju Island, Korea. The structures of these compounds have been determined to be linear sesterterpene alkaloids on the basis of combined 1D and 2D NMR experiments. The stereochemistry involved was established by comparison of the NMR data with those reported for a similar compound.

INTRODUCTION

Marine sponges have been a prolific source of biologically active and structurally diverse secondary metabolites.[1] Among the sponge-derived natural products, sesterterpenes, C_{25} metabolites consisted of five isoprenyl moieties are frequently reported from animals of the order Dictyoceratida.[1-2] Recently linear sesterterpenes terminated by a furan ring and tetronic acid moiety at the terminus of the molecule were isolated from the sponge *Sarcotragus* sp. (family Thorectidae, order Dictyoceratida).[3-5] In our continuing search for these compounds, new compounds were isolated from the same sponge. We describe herein the isolation and structure elucidation of sarcotragins C (**1**) and D (**2**), new members of this structural group.[6-7]

* To whom correspondence should be addressed. E-mail: jrrho@kordi.re.kr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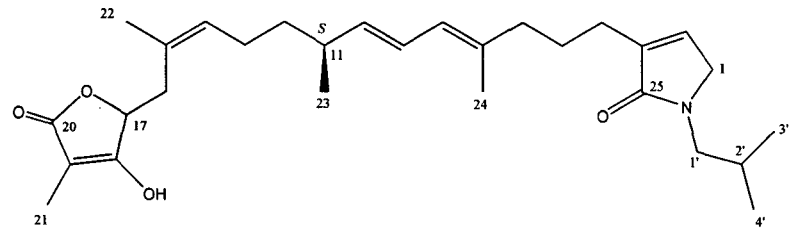
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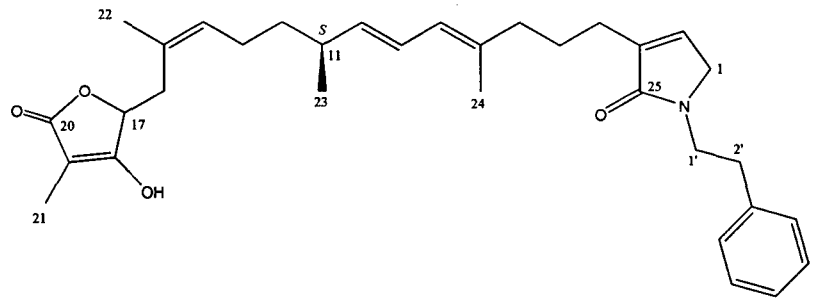
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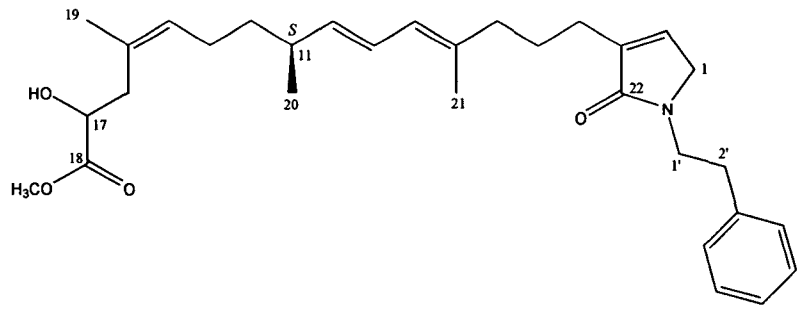
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EXPERIMENTAL SECTION

Animal material

The sponge was collected at 15-25m depth off the coast of Seoguipo, Jaeju Island in 1997. The specimen was taxonomically identified as an unidentified species of the genus *Sarcotragus*. This is massive sponge and oscules are very rare. The color is dark brown in life and the texture is elastic. The surface is covered with irregularly disposed, sharply pointed conules. A voucher specimen (registry No. Por. 35) is deposited at the Natural History Museum , Hannam University, Korea under the curatorship of Prof. Chung Ja Sim.

Extraction and Isolation

The freeze-dried sponge(700g) was repeatedly extracted with MeOH at room temperature. The crude extract was partitioned between H_2O and CH_2Cl_2 and then the latter layer re-partitioned between 20% aqueous MeOH and *n*-hexane to give 80% MeOH (4.6g) and *n*-hexane(10.1g) as soluble portions. The 20% MeOH fraction was subjected to reversed-phase vacuum flash chromatography eluting with stepped gradient mixtures of H_2O and MeOH to yield six fractions. The fraction eluted with 10% MeOH was further separated by silica flash chromatography using gradient mixtures of MeOH and $CHCl_3$ as eluents. The 100% $CHCl_3$ fraction (19.6mg) was separated by reversed-phase HPLC(YMC ODS-A column, 17% aqueous MeOH) to afford compound **1** (2.3mg). The 5% MeOH in $CHCl_3$ fraction showing similar characteristic signals as **1** in the ^1H NMR spectrum was separated under the same HPLC condition, followed by reversed phase HPLC(35% aqueous MeOH) to afford **2** and **3**.

NMR experiment

The 1D and 2D NMR spectra were obtained on a Varian UNITY500 spectrometer working at 500MHz for proton and 125MHz for carbon, respectively. The 1H and 13C NMR chemical shifts refer to CD_3OD at 3.30 and 49.0 ppm, respectively. For all experiments, temperature was stabilized at 297K. The parameters used for 2D NMR spectra were as follows; The gradient COSY spectra were collected with a spectral width 2567 Hz in a 512(t1) × 1024 (t2) matrix applying the pulse gradient of 1ms duration with a strength 10G/m and processed with a sinebell function. The HSQCDEPT spectra were measured in a 128(t1) × 1024(t2) matrix with J_{CH}=140 Hz and processed in a 256(t1) × 1024(t2) matrix by a linear prediction method for a higher resolution. The HMBC experiment was optimized for the long-range coupling constant of 8Hz. The HSQCDEPT and HMBC experiments were utilized by the pulse gradients of 1ms duration and 10G/m strength to reduce the artifact in the spectra.

RESULTS AND DISCUSSION

Sarcotragin C (**1**) was isolated as a colorless gum which analyzed for $C_{27}H_{43}O_4N$ by HRFABMS measurement (*m/z*=446.3273 for $[M+H]^+$) and NMR spectrum. The ^{13}C NMR spectrum of this compound showed the presence of two carbonyl carbon signals (δ 170 - 180) and eight olefinic ones (δ 120 - 140). A prominent singlet resonance at δ 3.70 in the ^1H spectra implied that there is one methoxy group present. Having this information, one remaining degree of unsaturation suggested the presence of one cyclic moiety in this molecule.

The gross structure of **1** was established from the combination of 1D and 2D NMR experiments (Table 1 and 2). The combined analyses of COSY and HMBC data revealed that the substructure of **1** contains a linear chain that incorporates a conjugated diene, a vinyl methyl, and a methyl ester group. In addition, ^1H- ^1H and ^1H -^{13}C correlations indicated that the α-hydroxyl -methyl ester was placed at one end of the chain. Methylene protons (δ 2.22) in the other terminus of this chain were correlated with neighboring carbons including a carbonyl and two olefinic carbons at δ 173.3, 139.5 and 136.8 on the basis of HMBC data. The proton spin coupling between the proton on C-2 (δ136.8) and methylene protons at δ 3.97 suggested the attachment of the chain at C-3 (δ 139.5). Moreover, the HMBC correlations between the carbonyl resonance at δ 173.3 and H-2, H-3 and the adjacent H-1' (δ 3.26) indicated that **1** contained an α, β - unsaturated γ- lactam group. This characterization was confirmed by the consideration of the molecular formula and chemical shifts of key carbons. Compound **1** was structurally similar to sarcotragin A (**4**) separated from the same organisms previously, except for the replacement of phenethyl unit with isobutyl group.[3]

The geometries of olefinic bonds in **1** were assigned as 7*E*, 9*E*, and 14*Z* by a combination of proton coupling constant analyses and carbon chemical shifts. The $J_{7,8}$ vicinal coupling constant of 15.1 Hz and upfield chemical shift of the C-21 methyl carbon (δ15.7), were indicative of the *E* configuration at both of C-7 and 9, while the geometry at C-14 was determined as *Z* from the downfield shift of the C-19 methyl carbon (δ 23.3). In addition, the configuration of C-11 was assigned as *S* by comparison of the carbon chemical shifts with those derived from the same biogenetic origin.[3]

Sarcotragin D (**2**) had a molecular formula of $C_{29}H_{43}O_4N$ as deduced from HRFABMS and ^{13}C NMR spectra. The ^1H NMR spectrum of **2** was very similar to that of **1** with an exception of the upfield shift of a singlet methyl resonance (δ 3.70 → 1.57) (Table 1). However, the ^{13}C spectrum contained signals of two additional quaternary carbons (δ 88.0 and 193.1) as listed in Table 2. These carbon chemical shifts along with the carbonyl carbon (δ 183.6) and an upfield methyl carbon were reminiscent of the tetronic acid moiety, which was supported by the ^1H-^{13}C long-range couplings between the methyl protons (δ 1.57) and three quaternary carbons (δ 183.6, 88.0, 193.1). Thus, compound **2** contained a tetronic acid

moiety instead of α-hydroxy methyl ester of **1**. A literature survey showed that **2** is structurally very close to sarcotrine A isolated from the same sponge. The only difference is the replacement of dimethyl pentyl of sarcotrine A with dimethyl butyl moiety of **2**. The C-17 configuration of **2** was determined to be *R* by comparison of the NMR data with the same partial structure.[5]

Table 1. ^{1}H NMR data for Sarcotragins C (**1**) and D (**2**) in CD_3OD

position	1	2
1	3.97 (2H, d, 1.5)	3.96 (2H, d, 1.5)
2	6.85 (1H, dd, 1.5, 1.5)	6.86 (1H, dd, 1.5, 1.5)
4	2.22 (2H, dt, 1.5, 7.3)	2.22 (2H, dt, 1.5, 7.3)
5	1.69 (2H, p, 7.3)	1.69 (2H, p, 7.3)
6	2.09 (2H, t, 7.3)	2.07 (2H, t, 7.3)
8	5.79 (1H, br d, 10.7)	5.78 (1H, br d, 10.7)
9	6.19 (1H, dd, 15.1, 10.7)	6.20 (1H, dd, 15.1, 10.7)
10	5.39 (1H, dd, 15.1, 8.3)	5.40 (1H, dd, 15.1, 8.3)
11	2.15 (1H, m)	2.17 (1H, m)
12	1.32 (2H, dt, m)	1.32 (2H, dt, m)
13	1.97(2H, m)	2.00 (2H, m)
14	5.27(1H, br t, 7.3)	5.25(1H, br t, 7.3)
16	2.44 (1H, dd, 13.7, 5.9) 2.38 (1H, dd. 13.7, 7.8)	2.15 (1H, dd, 14.2, 10.3) 2.58 (1H, dd, 14.2, 2.4)
17	4.25(1H, dd, 7.8, 5.9)	4.37 (1H, dd, 10.3, 2.4)
19	1.73(3H, s)	
20	0.99(3H, d, 6.8)	
21	1.72 (3H, s)	1.57 (3H, s)
22		1.77 (3H, s)
23		0.99 (3H, d, 6.8)
24		1.71 (3H, s)
1'	3.26(2H, t, 7.3)	3.26(2H, t, 7.3)
2'	1.95 (1H, m)	1.95 (1H, m)
3', 4'	0.89 (6H, d, 6.8)	0.89 (6H, d, 6.3)
OCH_3	3.70(3H, s)	

Table 2. ^{13}C NMR data for sarcotragins C (**1**), D(**2**) and compound **3** measured at 50MHz in CD_3OD.

position	1	2	3
1	52.2, t	52.9, t	51.6, t
2	136.8, d	137.6, d	138.1, d
3	139.5, s	140.3, s	139.1, s
4	25.6, t	26.4, t	25.1, t
5	26.2, t	26.9, t	25.7, t
6	39.6, t	40.4, t	39.1, t
7	135.8, s	136.4, s	135.1, s
8	126.0, d	127.8, d	125.5, d
9	125.8, d	126.4, d	125.1, d
10	138.4, d	139.4, d	136.4, d
11	37.3, d	37.9, d	36.8, d
12	37.7, t	38.5, t	37.3, t
13	26.1, t	26.9, t	25.6, t
14	129.0, d	128.9, d	127.7, d
15	130.9, s	132.6, s	131.4, s
16	37.2, t	36.1, t	34.8, t
17	70.2, d	81.4, d	80.1, d
18	175.4, s	193.1, s	191.8, s
19	23.3, q	88.0, s	86.8, s
20	20.6, q	183.6, s	182.4, s
21	15.7, q	5.9, q	4.7, q
22	173.3, s	24.3, q	23.0, q
23		21.4, q	20.1, q
24		16.5, q	16.4, q
25		174.1, s	172.5, s
1'	50.3, t	51.0, t	43.9, t
2'	28.4, d	29.1, t	34.5, t
3'	19.6, q	20.4, q	139.0, s
4'	19.6, q	20.4, q	128.3, d
5'(7')			128.6, d
6'			126.3, d
8'			128.3, d
OCH_3	51.4, q		

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