Pergamon

Tetrahedron Letters 42 (2001) 3005–3007

TETRAHEDRON LETTERS

Sarcotragins A and B, new sesterterpenoid alkaloids from the sponge *Sarcotragus* sp.

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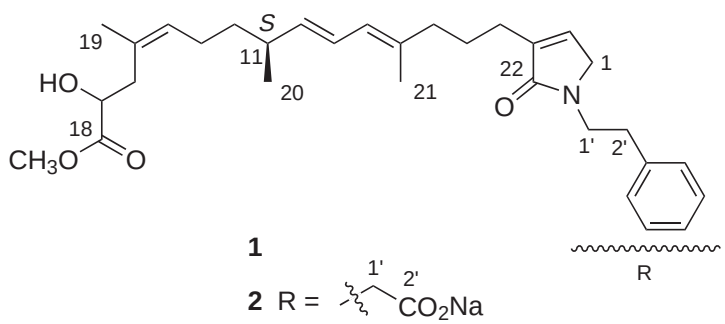
Received 9 January 2001; revised 21 February 2001; accepted 23 February 2001

Abstract—Sarcotragins A (**1**) and B (**2**), two terpenoid alkaloids of an unusual structural class, have been isolated from the sponge *Sarcotragus* sp. collected from Jaeju Island, Korea. The structures of these compounds have been determined as linear trisnorsesterterpenes containing a phenethylamine lactam or the corresponding glycine lactam moiety by combined chemical and spectral methods.

Sponges have produced a wide variety of biologically active and structurally unique metabolites.[1] Of the sponge-derived natural products, terpenoids and mixed biogenetic products containing polyprenyl moieties are frequently encountered in animals of the orders Dictyoceratida and Dendroceratida. These compounds, varying greatly in their carbon frameworks and functionalities, are utilized as biochemical markers for chemosystematics of sponges.[2] In addition, several sponge-derived terpenoids exhibit potent and diverse bioactivities which attract significant biomedical attention.[1,3] During the course of chemical investigation of marine invertebrates, we collected the sponge *Sarcotragus* sp. (family Thorectidae, order Dictyoceratida) from Jaeju Island, Korea.[4] We describe herein the isolation and structure determination of sarcotragins A (**1**) and B (**2**), trisnorsesterterpene alkaloids of an unprecedented structural group.

The sponges were collected by scuba (−25 m) off the coast of Seoguipo, Jaeju Island in July 1997. The lyophilized specimens (dry weight 600 g) were repeatedly extracted with MeOH and CH_2Cl_2. The combined crude extracts were solvent-partitioned between *n*-BuOH and water, then the former layer re-partitioned between 15% aqueous MeOH and *n*-hexane to remove salt and non-polar materials. The aqueous MeOH layer (4.7 g) was subjected to silica vacuum flash chromatography using stepped gradient mixtures of MeOH and CH_2Cl_2 as eluents. The fraction (85 mg) eluted with 10% MeOH in CH_2Cl_2 was separated by silica HPLC (YMC silica column, 40% EtOAc in hexane) followed



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Keywords: marine metabolites; sponge; *Sarcotragus* sp.; trisnorsesterterpene alkaloids.

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0040-4039/01/$ - see front matter
PII: S0040-4039(01)00343-4

by reversed-phase HPLC (YMC ODS-A column, 20% aqueous MeOH) to yield 11.3 mg of **1**. The fractions (350 mg) eluted with 50–60% MeOH in CH_2Cl_2 were combined and separated by successive use of reversed-phase HPLC (YMC ODS-A column, 25% aqueous MeOH then YMC C_8 column, 35% aqueous MeOH) to afford 8.0 mg of **2**.

The molecular formula of sarcotragin A (**1**), a colorless gum, was deduced as $C_{31}H_{43}NO_4$ by HRFABMS analysis. The ^{13}C NMR data of this compound showed 29 carbon signals; 6×C, 10×CH, 9×CH_2, and 4×CH_3. The presence of a phenyl ring was disclosed on the basis of characteristic carbon signals in the region of δ 125–130 and the corresponding proton signals at $\delta \sim 7.2$ in the ^{13}C and ^1H NMR data, respectively. Coupled with the molecular formula and the IR absorption bands at 1735 and 1665 cm^{-1}, the carbonyl signals at δ 176.2 and 173.7 were interpreted as an ester and lactam functionalities.[5]

With the aid of this information, the structure of **1** was elucidated by a combination of 2 D NMR experiments. All of the NMR signals of the linear polyprenyl portion was assigned by detailed interpretation of the ^1H COSY and gradient HMBC data (Table 1). The long-range correlations between the carbonyl carbon at δ 176.2 and neighboring protons placed a α-hydroxy-methyl ester group at the terminus of the polyprenyl chain. Similarly the presence of an α,β-unsaturated-γ-lactam as well as its connection to the aromatic ring via a -CH_2CH_2- unit was also determined by combined 2 D NMR experiments. The key evidence for this interpretation was provided by the gradient HMBC data in which correlations were observed for H-1/C-3, H-2/C-22, H-1′/C-1, and H-1′/C-22. Thus, the planar structure of sarcotragin A (**1**) was determined as an alkaloid consisting of a linear trisnorsesterpene and a phenethylamine lactam moiety.

The geometries of double bonds were assigned as 7*E*, 9*E*, and 14*Z* on the basis of the proton–proton coupling constant ($J_{9,10} = 15.1$ Hz) and carbon chemical shifts (δ_C 24.0 and 16.4 for C-19 and C-21, respectively). The absolute configuration at C-11 was determined by chemical degradation. Treatment of **1** with $NaIO_4$ in the presence of $RuCl_3$ as a catalyst yielded (*S*)-2-methylglutaric acid that was confirmed by comparison of the ^1H NMR and GC analysis data with an authentic sample and measurement of specific rotation {$[\alpha]_D$ +17.1° (*c* 0.03, MeOH), Lit. +22°}.[6–8] Due to the unstable nature of compound **1**, however, the absolute configuration at the C-17 asymmetric center still remains to be determined.[9]

The molecular formula of sarcotragin B (**2**), a colorless gum, was deduced as $C_{25}H_{36}NO_6Na$ by combined HRFABMS and ^{13}C NMR spectroscopy. The spectral data of this compound were highly compatible with those of compound **1**.[10] In particular the NMR signals for the linear norsesterterpene portion were almost identical to each other. However, signals of the benzylic group (C-2′ ~ C-8′) were replaced by a new carboxylic carbon at δ 176.9 in the ^{13}C NMR spectrum. Thus, the phenethylamine moiety of **1** was replaced by Na salt of glycine that was confirmed by combined 2D NMR experiments.

The trisnorsesterterpene portion of sarcotragins, highly uncommon among the sponge metabolites, was undoubtedly derived from a linear sesterterpene precursor frequently found in Dictyoceratid sponges.[1] Oxidative cleavage of an oxygenated functionality, e.g. furan, hydroxylactone, or butenolide, would result in the loss of terminal isopropyl group, thus forming an α-hydroxy-methyl ester group of sarcotragins. The occurrence of an α,β-unsaturated-γ-lactam moiety by condensation between a terpene and an amino acid-derived unit is also highly unusual among marine natural products. To the best of our knowledge, coupling of a terpene and phenethyl amine, derived from phenylalanine, in sarcotragin A is precedented only by molliorin-d and haumanamide from the sponges *Cacospongia mollior* and *Spongia* sp., respectively.[11,12]

The crude extract containing sarcotragins showed moderate cytotoxicity (LC_{50} 207 μg/mL) toward the

Table 1. ^1H and ^{13}C NMR data of sarcotragins A (**1**) in CD_3OD

Position	δ_H	δ_C	Position	δ_H	δ_C
1	3.78, d (1.5)	52.9 (t)	16	2.44, dd (13.7, 5.9)	38.1 (t)
2	6.75, p (1.5)	137.6 (d)		2.38, dd (13.7, 7.8)	
3		140.3 (s)	17	4.23, dd (7.8, 5.9)	71.0 (d)
4	2.19, dt (1.5, 7.3)	26.3 (t)	18		176.2 (s)
5	1.66, p (7.3)	27.0 (t)	19	1.73, d (1.5)	24.0 (q)
6	2.06, t (7.3)	40.3 (t)	20	0.99, d (6.8)	21.4 (q)
7		136.5 (s)	21	1.71, d (1.0)	16.4 (q)
8	5.78, br d (11.2)	126.7 (d)	22		173.7 (s)
9	6.19, dd (15.1, 11.2)	126.5 (d)	1′	3.69, t (7.3)	45.1 (t)
10	5.39, dd (15.1, 8.3)	139.1 (d)	2′	2.89, t (7.3)	35.7 (t)
11	2.15, m	38.0 (d)	3′		140.2 (s)
12	1.31, dt (7.8, 7.3)	38.5 (t)	4′(8′)	7.25, d (7.3)	129.7 (d)
13	1.98, dt (7.8, 7.3)	26.9 (t)	5′(7′)	7.19, m	129.8 (d)
14	5.25, br t (7.3)	129.7 (d)	6′	7.18, m	127.5 (d)
15		131.7 (s)	OMe	3.69, s	52.4 (q)

leukemia cell-line K562. However, the same measurement using pure metabolites showed that compounds **1** and **2** were not the active ingredients (LC_{50}>100 μg/mL).

Acknowledgements

Mass spectral data were kindly provided by Dr. Kun Cho, Korea Basic Science Institute, Taejeon, Korea. Special thanks go to Ms. Mi-Sun Oh for assistance with laboratory work. This research was financially supported by Ministry of Science and Technology (grants PN-99384 and -00410).

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3. *Marine Biotechnology*; Attaway, D. H.; Zaborsky, O. R. Eds.; Plenum Press: New York, 1993; Vol. 1. 500 pp.
4. The sponges are taxonomically identified as a species of the genus *Sarcotragus*. These are massive sponges 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 C.J.S.
5. Compound **1**: $[\alpha]_D^{25}$ +16.0° (*c* 0.11, MeOH); IR (KBr) ν_{max} 3400 (br), 2920, 2850, 1735, 1665, 1640, 1540, 1455, 1410, 1375, 1245 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.10), 236 (4.07) nm; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 494.3274 $[M+H]^+$ (calcd for $C_{31}H_{44}NO_4$, 494.3270, Δ 0.4 mmu).
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10. Compound **2**: $[\alpha]_D^{25}$ +17.5° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3350 (br), 2920, 2850, 1735, 1655, 1625, 1540, 1455, 1395, 1310 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (4.12), 232 (4.15) nm; ^1H NMR (CD_3OD): δ 6.86 (1H, br s), 6.18 (1H, dd, J=14.7, 10.7 Hz), 5.79 (1H, br d, J=10.7 Hz), 5.39 (1H, dd, J=14.7, 8.3 Hz), 5.25 (1H, br t, J=7.3 Hz), 4.24 (1H, dd, J=8.3, 5.9 Hz), 4.06 (2H, br s), 4.02 (2H, s), 3.69 (3H, s), 2.44 (1H, dd, J=13.7, 5.9 Hz), 2.39 (1H, dd, J=13.7, 8.3 Hz), 2.22 (2H, br t, J=7.3 Hz), 2.15 (1H, m), 2.09 (2H, t, J=7.3 Hz), 1.97 (2H, dt, J=7.8, 7.3 Hz), 1.73 (3H, d, J=1.0 Hz), 1.72 (3H, br s), 1.69 (2H, p, J=7.3 Hz), 1.31 (2H, dt, J=7.8, 7.3 Hz), 0.99 (3H, d, J=6.8 Hz); ^{13}C NMR (CD_3OD): δ 176.9 (C), 176.3 (C), 174.1 (C), 140.1 (C), 139.1 (CH), 138.1 (CH), 136.6 (C), 131.7 (C), 129.7 (CH), 126.7 (CH), 126.5 (CH), 71.0 (CH), 53.3 (CH_2), 52.4 (CH_3), 47.1 (CH_2), 40.4 (CH_2), 38.5 (CH_2), 38.04 (CH_2), 38.01 (CH), 27.0 (CH_2), 26.9 (CH_2), 26.4 (CH_2), 24.1 (CH_3), 21.4 (CH_3), 16.5 (CH_3); HRFABMS *m*/*z* 492.2336 $[M+Na]^+$ (calcd for $C_{25}H_{36}NO_6Na_2$, 492.2338, Δ −0.2 mmu).
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