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Bilosespens A and B: Two Novel Cytotoxic Sesterpenes from the Marine Sponge *Dysidea cinerea*

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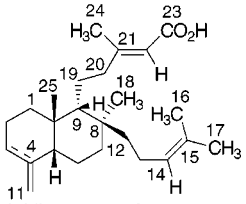
ABSTRACT



1 bilosespene A
2 bilosespene B; 3 ene

Two novel sesterpenes, bilosespens A and B (1 and 2) were isolated from the Red Sea sponge *Dysidea cinerea* collected in the Dahlak archipelago, Eritrea. The structure of the mixture of the two inseparable compounds was established by spectroscopic analysis, mainly by 1D and 2D NMR measurements. The mixture of bilosespens A and B is cytotoxic to a few human cancer cells.

In our continuous search for biologically active metabolites from marine invertebrates,[1−3] we found that the EtOAc extract of the marine sponge *Dysidea cinerea* collected from the Dahlak archipelago, Eritrea, possesses cytotoxicity to a few human cells.[4]

Fractionation resulted in the isolation of an unstable, inseparable mixture of two sesterpenes, bilosespens A and B (**1** and **2**). We here report the isolation and structure elucidation of these two new compounds, which are of an unprecedented group of sesterpenes.

Freeze-dried sponge tissue (16 g dry wt) was extracted with ethyl acetate to give a brown gum (0.5 g) after evaporation. The latter residue was separated first on a Sephadex LH-20 column, eluted with hexane/$CHCl_3$/MeOH, 3:1:1 mixture, and then by chromatography on silica gel (eluting with petroleum ether/EtOAc, 4:1) to give a 1:1 mixture of bilosespens A and B (35 mg). Tedious attempts to separate the mixture caused a change in the ratio of **1** and **2** but mostly the decomposition of the two.[5] A single molecular ion of $C_{25}H_{40}O_2$ established by HREIMS [m/z 372.3025 M^+, Δ 0.3 mmu] and 50 carbon atom resonances in the NMR spectrum suggested a mixture of two sesterpenes, a suggestion that agreed well with the six methyl groups of **2** and five methyls and an *exo*-methylene group of **1**. From the IR spectrum (ν 3500−2500, 1691, 1640 cm^{-1}) and the carbon chemical shifts (δ_C 172.9, 173.0 (CO_2H) and the

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[1] Kashman, Y.; Koren-Goldshlager, G.; Garcia Gravalos, M. D.; Schleyer, M. *Tetrahedron* **1999**, *40*, 997.

[2] Koren-Goldshlager, G.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *J. Org. Chem.* ***1998***, 63, *4601*.

[3] Kelman, D.; Kushmaro, A.; Loya, Y.; Kashman, Y.; Benayahu, Y. *Mar. Ecol. Prog. Ser.* **1998**, *169*, 87.

[4] Earlier investigation of this sponge collected in the northern part of the Red Sea yielded only avarol derivatives: Hirsh, S.; Rudi, A.; Loya, Y.; Kashman, Y. *J. Nat. Prod.* **1991**, *54*, 92.

[5] The decomposition seems to be autocatalyzed by the carboxylic group.

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Table 1. NMR Data (^1H NMR 500 MHz, ^{13}C NMR 125 MHz, in $CDCl_3$) of Bilosespens A and B (**1** and **2**)

	1			**2**[b]			**1**			**2**	
no.	δ_c	δ_H	HMBC[a]	δ_c	δ_H	no.	δ_c	δ_H	HMBC	δ_c	δ_H
1	39.3 t	1.82, 1.05	25	39.3 t	1.75, 0.95	14	125.5 d	5.10 t	16, 17	125.5 d	5.07 t
2	21.5 t	1.65, 1.48		23.3 t	1.90, 1.90	15	131.0 s		14, 16, 17	131.1 s	
3	39.1 t	2.38, 1.95	11a, 11b	123.0 d	5.40 bs	16	17.5 q	1.58 s	14, 17	17.5 q	1.59 s
4	148.1 s		11a, 11b	134.4 s		17	25.7 q	1.67 s	16	25.7 q	1.66 s
5	56.5 d	1.58	11a, 11b, 25	54.0 d	1.62	18	29.3 q	0.87 s		28.8 q	0.85 s
6	19.1 t	1.55, 1.45		18.5 t	1.40, 1.40	19	25.3 t	1.62, 1.30		24.3 t	1.72, 1.38
7	37.9 t	1.72, 0.93	18	37.8 t	1.72, 0.95	20	43.5 t	2.38, 2.10	22, 24	40.1t	2.28, 1.98
8	37.6 s		18	37.0 s		21	164.0 s		20a, 20b, 24	163.5 s	
9	57.7 d	1.12	18, 25	52.5 d	1.25	22	114.7 d	5.70 s	20a, 20b, 24	115.0 d	5.67 s
10	39.8 s		25	36.2 s		23	173.0 s		22	172.9 s	
11	106.2 t	4.82, 4.47	3, 5	22.1 q	1.67 s	24	19.3 q	2.18 s	22	19.2 q	2.16 s
12	32.8 t	1.40, 1.10	14, 18	32.5 t	1.58, 1.20	25	15.4 q	0.72 s		14.4 q	0.78 s
13	23.0 t	1.80, 1.80	14	23.0 t	1.80, 1.80						

[a] C to H correlations. [b] The only difference in the HMBC spectra of **2**, in comparison with **1**, was replacement of the H-11 to C-3 and -5 correlations by CH_3-11 to C-3, -4, and -5 and H-3 and -5 to CH_3-11 ones.

polarized double bond 114.7 d, 164.0 s and 115.0 d, 163.5 s ppm), it became clear that **1** and **2** are carboxylic acids and, moreover, most likely α,β-unsaturated acids. The latter functionality was further confirmed by the preparation of the methyl esters by CH_2N_2 (δ_H 3.70, 3H).

Applying 2D NMR experiments (HMQC, HMBC, and COSY spectra) (Table 1) determined for **1** a pentasubstituted Decalin system (C1−10, ═CH_2(11), Me's 18 and 19 and CH_2's 12 and 19), as well as two segments −CH_2(19)CH_2C-(CH_3)═CH−CO_2H(23) and −CH_2(12)CH_2−CH═C(CH_3)$_2$-(16,17).[6] The latter three parts could be joined together from long-range H,H-couplings observed in a TOCSY experiment, i.e., correlations between H-9 and H_2-20 and between CH_3-18 and H_2-12 as well as a CH− correlation between CH_3-18 and CH_2(12). The complete resonance-lines assignment (Table 1) was also assisted by a selective-SPT experiment[7] which determined the correlations between the various methyl protons and their neighbor carbon atoms. It became clear that the only difference between bilosespens A and B is the replacement of the *exo*-methylene group (C-11) of **1** by a methylvinyl group (−CH(3)═C(CH_3(11))−) in **2**. The *cis* Decalin ring junction as well as the stereochemistry of the two other chiral centers C-8 and C-9 and the 21*E*-geometry of the 21,22-double bond were established by NOEs (Figure 1).

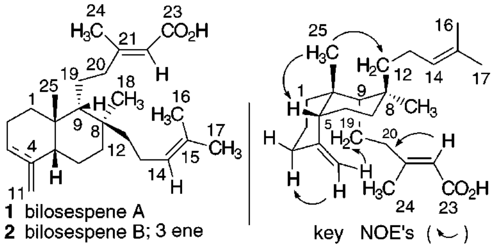

Figure 1.

The mixture of bilosespen A and B has been found to have cytotoxicity against several cancer cells: P-388 Mouse lymphoma, A-549 Human lung carcinoma, HT-29 Human colon carcinoma, MEL-28 Human melanoma in a concentration of 2.5 μg/mL (IC_{50}).

Although polycyclic sesterpenes from marine origin, and mainly sponges, are known,[8] the bilosespens (**1** and **2**) are the first of a new class of sesterpenes in which the middle three units of a penta-isoprenoid acid cyclize into a bicyclic system, leaving the first and last isoprenoids to substitute the *cis* decalin.

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(6) Besides the MH^+ peak (100%) in the CIMS spectrum, the following fragments were seen (*m/z*, %): 291(57) [M − C_6N_{10}] and 273(37) [M − $C_5H_7O_2$], of the two side chains, and 205(28) and 191(22) of $C_{15}H_{25}^+$ and $C_{14}H_{23}^+$, respectively, of the Decalin portion.

(7) (a) Bax, A. *J. Magn. Reson.* **1984,** *57, 314*. (b) Bax, A.; Ferrett, J. A.; Nashed, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029. The selective-SPT experiment gives a better resolution than the HMBC one.

(8) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259, and earlier reports in this series.