

SESQUITERPENOIDS BASED ON THE EPI-MAALIANE SKELETON
FROM THE MARINE SPONGE *AXINELLA CANNABINA*

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ABSTRACT.—From a further investigation of the extracts from the marine sponge *Axinella cannabina*, three novel sesquiterpenes based on the epi-maaliane skeleton, carrying an isonitrile, isothiocyanate, or formylamino function were isolated in addition to bicyclogermacrene (**4**), and their structures determined on the basis of chemical and spectroscopic evidence.

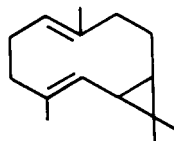
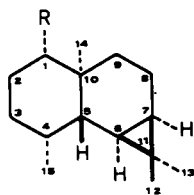
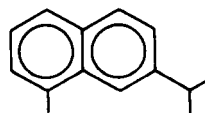
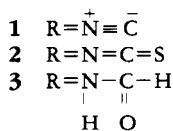
During the last decade, marine sponges belonging to the order Halicondrida have been the subject of a large number of chemical investigations. These animals have provided a host of terpenoids carrying functionalities very rare in nature, such as isocyanide, isothiocyanate, or formylamino groups (1-10). Some of these compounds exhibit *in vitro* cytotoxic properties¹ as well as antifeedant activities (9), thus generating much interest among marine ecologists.

A species particularly rich in these compounds has been established to be the Mediterranean sponge *Axinella cannabina* Esper. whose investigation has resulted to date in the isolation and characterization of six different isocyanide-isothiocyanate-formylamino series of sesquiterpenes (1,3,4,6,7,10), including three compounds based on a unique spiro [4,5]-decane ring system (6).

Careful reexamination of the minor constituents of this organism has now led to the isolation of a further isonitrile sesquiterpene accompanied by the corresponding isothiocyanate and formamide, for which formulae **1-3** have been established on the basis of their chemical and spectroscopic behavior, along with the hydrocarbon **4**, previously isolated from the extractives of *Citrus junos* (11).

Samples of *A. cannabina* were collected in the Bay of Taranto during the autumn of 1983 and homogenized in MeOH. The CHCl₃ soluble material from the methanolic extract was chromatographed on silica gel, thus obtaining three principal fractions (A,B,C) in order of increasing polarity.

Isonitrile 1.—Rechromatography of fraction B on silica gel gave a mixture of

**4****5**

¹Samples of Axisonitrile-1 (**1**) and Axisothiocyanate-3 (**6**), tested in the Laboratories of Farmitalia-Carlo Erba (Milano, Italy), showed cytotoxic activity *in vitro* on KB and/or P388 cells (unpublished results).

isocyanides from which compound **1** was obtained in pure form by hplc on a reverse phase column. The compound showed $[\alpha]_D -34.4$ and analyzed for $C_{16}H_{25}N$. The nitrogen atom was readily assigned to an isocyanide group by interpretation of various spectral features. Specifically, an ir absorption at 2120 cm^{-1} , a 1-proton signal in the ^1H -nmr spectrum (C_6D_6) at δ 2.61 dd, $J=12.5$ and 3.7 Hz, each line being split into a 1:1:1 triplet ($J=1.8$ Hz) by coupling with a nitrogen atom, and finally fragmentation in the mass spectrum illustrating the loss of HCN were in strong support of the $-\text{N}^+\equiv\bar{\text{C}}$ assignment.

The ^{13}C -nmr features (see Table 1) reinforced the presence of an isonitrile function [δ 63.04 (C-1) and 158.58 (C-16)] and provided significant additional structural information. Carbon-carbon double bonds were ruled out by the absence of sp^2 carbon signals, and hence a tricyclic structure was established for **1**.

TABLE 1. Nuclear Magnetic Resonance Data (C_6D_6) for **1**

^{13}C chemical shift	assignment	^1H chemical shift
63.04 ^a	1	2.61 (dd) ^b
25.17	2 _{ax}	1.80 (dddd)
	2 _{eq}	c
	3 _{ax}	1.1-1.3 (m)
3 _{eq}		
31.52	4	c
32.20	5	0.71 (dd)
40.52	6	0.45 (dd)
22.53	7	0.54 (bdd)
19.38	8 α	c
	8 β	
	9 α	
15.73	9 β	0.50 (ddd)
	10	
37.23	11	
36.38	12	1.05 (s)
17.73	13	0.92 (s)
29.47	14	1.01 (s)
15.29	15	0.89 (d)
14.93	16	
158.58		

J (Hz) 1-2_{ax}=12.5; 1-2_{eq}=3.7; 2_{ax}-2_{eq}=12.5; 2_{ax}-3_{ax}=12.5;
 2_{ax}-3_{eq}=4.3; 4-15=7.0; 4-5=5.0; 5-6=5.6; 6-7=10.0;
 7-8 α =4.5; 7-8 β =0; 8 α -9 β =2.5; 8 β -9 β =2.5; 8 β -9 α =8.9

^aThe signal appears broadened by coupling with the nitrogen atom.

^bEach line is split as a 1:1:1 triplet ($J=1.8$ Hz).

^cOverlapped with other signals in the region 1.45-1.70 δ .

On the other hand, three signals at δ 22.53 (C-6), 19.58 (C-7), and 17.73 (C-11) pointed to the presence in the molecule of a cyclopropane ring. This was confirmed by the ^1H -nmr spectrum, which includes two high-field signals at δ 0.54 (1H, bdd, 7-H) and 0.45 (1H, dd, 6-H). Four Me groups were also discerned by ^1H nmr: three *t*-Me groups were observed as singlets at δ 0.95, 1.05 and 1.01 (12-H₃, 13-H₃, and 14-H₃, respectively) and a *sec*-Me group resonated as a doublet at δ 0.89 (15-H₃).

Further information on the structure of **1** was obtained by its dehydrogenation on Pd/C at 350°, which afforded as the principal compound eudalene (**5**), thus establishing the decalin nature of **1**, the position of the *sec*-Me group, and the location of a dimethyl-substituted cyclopropane ring on C₆ and C₇ or, alternatively, on C₇ and C₈. The second possibility was ruled out by an accurate analysis of the 250 MHz ^1H nmr of **1** and spin

decoupling experiments: the cyclopropyl proton at δ 0.45 was coupled with the proton linked to the carbon of the ring junction, resonating at δ 0.71 (dd), in turn coupled to H-4 resonating in the complex region 1.45-1.70 δ . These characteristics allowed also the location of the last *t*-Me group at C-10.

The multiplicity of the signal at δ 2.61 is only consistent with the location of the $^+ \text{N} \equiv \text{C}^-$ group on C₁ or C₉. Decoupling experiments supported the first hypothesis. The signal at δ 2.61 was coupled with the proton resonating at δ 1.82, which appears as a double double doublet. Since this signal was unaffected by irradiation at δ 0.54 (H-7 resonance), it was assigned to the axial proton linked to C-2 and, consequently, the $^+ \text{N} \equiv \text{C}^-$ group was located at C-1. Difference decoupled spectra confirmed this assignment and permitted the identification of the signals of the equatorial H-2 proton and those of the H₂-8 methylene protons resonating as complex multiplets in the region 1.45-1.70 δ .

Additional evidence to confirm structure **1** was obtained by ¹³C-nmr spectra which showed the signals reported in Table 1, assigned on the basis of selective decoupling experiments.

The absolute stereochemistry of **1** has not been established; its relative stereochemistry was determined as follows. The values of the coupling constants of the signal at δ 2.61 required an axial orientation for H-1. The presence of nOe's between H-1 and H-5 and H_{ax}-2 and Me-10, respectively, indicated that the junction of the two rings must be *trans*-diequatorial. The equatorial nature of H-4 was deduced from the values of the coupling constant of the pertinent signal with H-5 (5.5 Hz), and the relative stereochemistry of C₆ and C₇ was deduced from the presence of a nOe between Me-10 and H-6 and Me-4 and H-6.

Isothiocyanate 2 and bicyclogermacrene (4).—Fraction A was separated by hplc on a silica gel column to obtain **2**, and a hydrocarbon which was identified on the basis of its physical data ([α]_D, nmr, ir, ms) as bicyclogermacrene (**4**), a sesquiterpene previously isolated from *C. junos* (11).

The isothiocyanate **2** had the molecular formula C₁₆H₂₅NS (hrms). The ir spectrum contained a strong isothiocyanate band at 2100 cm⁻¹. The ¹H-nmr spectrum showed that this function was linked to a methine group [δ 3.16, dd, *J*=11.5 and 3.7 Hz]. The spectrum also contained three Me singlets at δ 0.90, 0.99, and 1.00, one Me-doublet at δ 1.02 (*J*=7.0 Hz) and two cyclopropyl signals at δ 0.49 (dd, *J*=9.5 and 4.5 Hz, 6-H) and 0.62 (bdd, *J*=9.5 and 6.5 Hz, 7-H). These data strongly suggested a close similarity between **1** and **2**; this was proved by treatment of **1** with sulfur at 120° for 16 h, which gave **2** in good yield.

Amide 3.—This compound, present in *A. cannabina* in a smaller amount, was isolated from fraction C by hplc on a silica gel column. Spectral analogies (Experimental) between **3** and **1** led us to correlate these two compounds by hydration of **1** to a formamide which was identified as **3** by comparison of [α]_D and spectral (nmr, ir, ms) and chromatographic properties.

EXPERIMENTAL

Ir spectra (CCl₄) were recorded on a Perkin Elmer 157 spectrometer. Mass spectra were taken on a AEI MS-902 instrument. Optical rotations were measured on a Perkin Elmer 191 polarimeter with a 10 cm microcell in CHCl₃ solns.

Nmr spectra were determined on a Bruker 250 WM spectrometer in CDCl₃ unless otherwise indicated, using TMS as internal reference (δ =0). Determination of nOe's and decoupling difference experiments were performed with the aid of Aspect 2000 microprograms which allowed direct accumulations of difference FID's. The sample used for nOe measurements was previously degassed by bubbling argon through the solution for 40 min.

EXTRACTION AND ISOLATION OF 1-4.—Fresh sponge (350 g, dry weight after extraction), was collected in the Bay of Taranto during the autumn of 1983 and identified by Prof. M. Sarà (University of Genova, Italy). The methanolic extract of the homogenized material was concentrated, and the resulting aqueous solution was extracted three times with CHCl_3 . The organic phase, taken to dryness, was chromatographed under pressure on a silica gel column using solvent mixtures of increasing polarities from light petroleum to Et_2O through C_6H_6 . The appropriate fractions which emerged from the column with light petroleum- C_6H_6 8:2 (fraction A, 1.6 g), light petroleum- C_6H_6 1:1 (fraction B, 5 g) and Et_2O (fraction C, 0.3 g) were used for the isolation of **1-4** as follows.

Fraction A was rechromatographed on a silica gel column (eluent light petroleum) thus yielding 96 mg of **4** [identified by comparison of its physico-chemical properties ($[\alpha]_D$, nmr, ir, ms) with those reported in the literature (11)] and 94 mg of a mixture of isothiocyanates which were further separated by hplc (LiChrosorb Si 60, *n*-hexane) to give 14 mg of **2**, $[\alpha]_D -56^\circ$ (c 0.04); hrms m/z 263.1715 ($\text{C}_{16}\text{H}_{25}\text{NS}$ requires 263.1709); ir ν max 2100 cm^{-1} ; ^1H nmr δ 3.16 (1H, dd, $J=1.5$ and 3.7 Hz, 1-H), 1.02 (3H, d, $J=7.0$ Hz, 15- H_3), 1.00, 0.99 and 0.90 (3H each, singlets, 12- H_3 , 13- H_3 and 14- H_3), 0.62 (1H, bdd, $J=9.5$ and 6.5 Hz, 7-H) and 0.49 (1H, dd, $J=9.5$ and 4.5 Hz, 6-H).

Fraction B contained an isocyanide mixture, and isolation of compound **1** in pure form required extensive use of chromatography over silica gel. Later eluates which emerged from the column with light petroleum- C_6H_6 (1:1), on further purification by hplc (ODS-2, 20% H_2O in CH_3CN), gave 28 mg of **1**, $[\alpha]_D -34.4^\circ$ (c 0.04); hrms m/z 231.1996, $\text{C}_{16}\text{H}_{25}\text{N}$ requires 231.1988; ir ν max 2135 cm^{-1} ; nmr data are reported in Table 1.

Fraction C was applied to a silica gel column which was eluted with Et_2O , thus obtaining crude **3**, which was further purified by hplc (LiChrosorb Si 60, EtOAc) (7 mg); $[\alpha]_D +14^\circ$ (c 0.03); hrms m/z 249.2099, $\text{C}_{16}\text{H}_{27}\text{NO}$ requires 249.2094; ir ν max 3440 and 1680 cm^{-1} ; ^1H -nmr spectrum shows signals due to two rotational isomers of the formamide group in a ratio **4** (*cis*): **1** (*trans*); *cis* isomer: δ 8.17 (bs, H-C=O), 5.27 (bm, NH), 3.70 (ddd, $J=10.5$, 10.5 and 3.7 Hz, 1-H), 1.04 (d, $J=7.0$ Hz, 15- H_3), 1.02, 0.95 and 0.91 (singlets, 12- H_3 , 13- H_3 and 14- H_3); *trans* isomer: δ 7.99 (d, $J=10.0$ Hz, H-C=O), 5.41 (bm, NH), 2.90 (ddd, $J=10.5$, 10.5 and 3.7 Hz, 1-H), 1.04 (d, $J=7.0$ Hz, 15- H_3), 1.02, 0.95 and 0.89 (singlets 12- H_3 , 13- H_3 and 14- H_3). In the complex region 0.44-0.70 ppm, the cyclopropyl signals of the two isomers are present.

DEHYDROGENATION OF 1 TO EUDALENE (5).—A mixture of 5 mg of **1** and 10 mg of 10% Pd/C was heated at 280° under N_2 for 1 h. After cooling, the mixture was extracted with CHCl_3 and chromatographed by tlc (silica gel, *n*-hexane, uv), thus giving 2 mg of eudalene, identified by comparison of its spectral (nmr, uv) and chromatographic (tlc; silica gel, *n*-hexane) properties with those of an authentic specimen.

TREATMENT OF 1 WITH SULPHUR TO OBTAIN 2.—Isocyanide **1** (10 mg) and excess S were heated at 120° for 16 h; after cooling, 40-70° light petroleum (5 ml) was added and the filtered solution was taken to dryness and chromatographed by tlc (silica gel, *n*-hexane, uv). The band with Rf 0.6, scraped and eluted with Et_2O , afforded 4 mg of **2**, whose spectral (nmr, ms) and chromatographic (tlc; silica gel, *n*-hexane) properties matched those of natural **2**.

HYDRATION OF 1 TO OBTAIN 3.—A solution of **1** (11 mg) in anhydrous Et_2O (5 ml) and HOAc (5 ml) was kept at room temperature for 2 h. After washing with 10% Na_2CO_3 solution and then with H_2O , the organic phase was dried and taken to dryness, thus yielding crude **3** which was purified by tlc (SiO_2 , Et_2O); the band with Rf 0.5 was eluted with Et_2O to give 6 mg of **3** identical with **3** isolated from *A. canabina* on the basis of physical properties (nmr, ms, $[\alpha]_D$).

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