Journal of Natural Products
Vol. 50, No. 2, pp. 217-220, Mar-Apr 1987

CIS-EUDESMANE NITROGENOUS METABOLITES FROM THE MARINE SPONGES *AXINELLA CANNABINA* AND *ACANTHELLA ACUTA*

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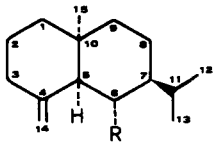
ABSTRACT.—From the sponge *Axinella cannabina* a new isocyanide-isothiocyanate-formamide triad [**1-3**], based on the *cis*-eudesmane skeleton, has been isolated. Two of the new metabolites [**1** and **2**] have also been isolated from *Acanthella acuta*, a sponge belonging to the same order (Axinellida) of *Ax. cannabina*. Their structures have been established on the basis of spectral data, including 2D-nmr and chemical reactions.

Our continuing study (1) of the minor constituents of the sponge *Axinella cannabina* Esper has now led to the isolation and characterization of a new isocyanide-isothiocyanate-formamide series [**1-3**], which is the subject of this paper. Two of the new metabolites [**1** and **2**] have also been isolated from *Acanthella acuta* Schmitt, a sponge belonging to the same family (Axinellidae).

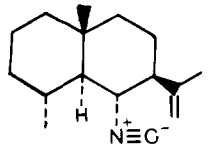
Considering that another isocyanide, acanthellin-1 [**4**], was isolated from both the sponges *Ax. cannabina* (2) and *Ac. acuta* (3), the results presented here suggest that these nitrogenous sesquiterpenes could be of chemotaxonomical interest.

The sponges *Ax. cannabina* and *Ac. acuta* were soaked in MeOH, and the $CHCl_3$-soluble materials from the methanolic extracts were subjected to preliminary fractionations by flash chromatography on Si gel thus giving three major fractions.

ISONITRILE **1** [6α-isocyano-5α-H,7α-H,10α-eudesm-4(14)-ene].—Isonitrile **1** was obtained in pure form by hplc from the fractions of intermediate polarity following two different procedures for the two sponges; it is a colorless crystalline product possessing the molecular formula $C_{16}H_{25}N$. Its ir spectrum contained an isonitrile band at ν max 2135 cm^{-1} and absorptions at 1640 and 895 cm^{-1} attributed to an exo methylene group, which was confirmed by the presence of two narrow multiplets in the ^{1}H-nmr spectrum at δ 4.94 and 4.83, and two olefinic signals at δ113.3 (=CH_2, C-14) and 144.2 (=C<, C-4), in the ^{13}C-nmr spectrum (Table 1). The presence of the isonitrile function was reinforced by the fragmentation peak at *m/z* 204 (M^+-HCN) in the mass spectrum and by the ^{13}C-nmr spectrum [δ 55.8 (−CH, 1:1:1 triplet due to the cou-



1 R=$N^+\equiv C^-$
2 R=N=C=S
3 R=N−C−H (H, O)



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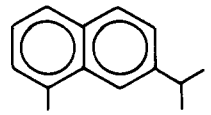

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pling with the nitrogen atom, J=4 Hz, C-6) and 155.2 (C-16)] and its ^{1}H-nmr spectrum, which shows a broad 1-H proton signal at δ 3.58 (6-H) attributable to the methine proton geminal to the $-N^+\equiv C^-$ group.

TABLE 1. ^{1}H- and ^{13}C-nmr Data for **1** (in $CDCl_3$)

Assignment	δ ^{13}C[a]	δ ^{1}H
1a	39.2[d]	1.21-1.33[b,f] (m)
1b		1.42-1.58[b,g] (m)
2	22.6	1.48-1.76[b] (m)
3a	29.9	2.00 (m)
3b		2.17 (bdd, J=3.8, 9.3 Hz)
4	144.2	
5	57.6	1.90 (bd, J=11.3 Hz)
6	55.8[c]	3.58 (bdd, J=11.3, 11.3 Hz)
7	47.7	1.33-1.46[b] (m)
8ax	18.1	1.15-1.23[b] (m)
8eq		1.40-1.53[b] (m)
9ax	30.1[d]	0.91-1.07[b,f] (m)
9eq		1.50-1.70[b,g] (m)
10	34.9	
11	27.3	2.24 (d heptet, J=3.0, 6.8 Hz)
12	15.3[e]	0.84[h] (d, J=6.8 Hz)
13	20.7[e]	0.97[h] (d, J=6.8 Hz)
14	113.3	4.83 and 4.94 (bs)
15	28.0	0.93 (s)
16	155.2	

[a]Assignments based on DEPT sequence and ^{13}C-^{1}H shift correlated 2D-nmr spectroscopy.

[b]Values deduced from the ^{13}C-^{1}H shift correlated 2D-nmr spectrum.

[c]The signal appear as a 1:1:1 triplet (J=4 Hz) due to the coupling with the nitrogen atom.

[d-h]Values with identical superscript within each column may be reversed.

The ^{1}H-nmr spectrum (Table 1) also contained one methyl singlet at δ 0.93 (15-H_3) and two methyl doublets at δ 0.97 and 0.84 (12-H_3 and 13-H_3) belonging to an isopropyl group because they collapsed to singlets on irradiation of the deshielded methine signal at δ 2.24 (11-H).

Since the elemental formula of **1** requires five degrees of unsaturation, it was inferred that the molecule has a bicyclic skeleton, whose decalin nature was established by dehydrogenation of **1** to obtain eudalene [**5**], which was identified by comparison of its properties with those of an authentic sample. From this experiment we also deduced the position of the exo methylene and isopropyl groups. The completion of the gross structure of **1** was carried out by a further analysis of the 500 MHz ^{1}H-nmr spectrum assisted by extensive spin decoupling experiments and by ^{13}C-^{1}H shift correlated 2D-nmr spectroscopy, which allowed the complete assignment of all the resonances to the respective protons and carbon atoms (Table 1). In particular, irradiation at δ 3.58 (6-H) simplified the doublet at δ 1.90 (5-H, broadened by long range coupling with the exo methylene protons) to a broad singlet and modified the complex multiplet centered at δ 1.40 (7-H), which was in turn coupled with the isopropyl proton (11-H).

The absolute stereochemistry of **1** has not been established; its relative stereochemistry was determined as follows. The values of the coupling constants required a diaxial relationship between 6-H and the adjacent protons 5-H and 7-H. The

presence of an nOe effect (12%) between 10-Me and 5-H established the *cis* junction of the two rings as confirmed by the chemical shift of the 10-Me (δ 28.0) in the ^{13}C-nmr spectrum that would be expected to resonate at less than 20 ppm (4) in the *trans* isomer.

ISOTHIOCYANATE **2** [6α-isothiocyano-5αH,7αH,10α-eudesm-4(14)-ene].—Compound **2** was also isolated by hplc from the less polar fractions of the lipid extracts of the two sponges in two different ways. Hrms and ^{13}C nmr indicated a molecular formula $C_{16}H_{25}NS$. Its ir spectrum contained a strong isothiocyanate band at ν max 2100 cm^{-1} and bands of an exo methylene group at 1640 and 895 cm^{-1}. The ^{1}H- and ^{13}C-nmr spectra were strongly reminiscent of those of **1**, except that the signals of the methine carrying the isothiocyano function lacked the fine structure present in **1**.

These spectral features suggested a close structural relationship between **1** and **2**, which was proved by reacting **1** with sulfur at 120° for 16 h to obtain **2**.

AMIDE **3** [6α-formamido-5αH,7αH,10α-eudesm-4(14)-ene].—Formamide **3** is present as a minor component in the most polar fraction obtained from the extract of *Ax. cannabina*, while it is missing in that obtained from *Ac. acuta*. It was isolated by hplc on a Si gel column, and showed spectral similarities with **1** thus suggesting that it was the amide **3**. Hydration of **1** by treatment with glacial HOAc confirmed this hypothesis, affording a product indistinguishable from natural **3** by comparison of their spectral and chromatographic properties.

EXPERIMENTAL

INSTRUMENTAL.—Ir spectra were recorded on a Perkin-Elmer Model 399 spectrophotometer and uv spectra on a Perkin-Elmer Model 550S spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 Polarimeter with a 10 cm microcell in $CHCl_3$ solutions.

^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-500 and a Bruker WM-250 spectrometer, respectively. All chemical shifts are reported with respect to TMS (δ=0). The nature of each carbon signal was deduced through DEPT experiments performed by using polarization transfer pulse of 90° and 135°, respectively, obtaining in the first case only CH groups and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups. The ^{13}C-^{1}H shift correlated 2D-nmr spectrum was performed using a Bruker microprogram. The shift correlation with polarization transfer via ^{1}J coupling experiment was carried out adjusting the fixed delays to give maximum polarization for $J_{C\text{-}H}$= 135 Hz. NOe's were determined with the aid of an Aspect 2000 microprogram on a sample degassed by bubbling argon through the solution for 40 min.

Low resolution mass spectra were recorded on an AEI MS 30 spectrometer. High resolution mass spectra were recorded on an AEI MS 902 spectrometer. Hplc separations were carried out on a Varian 2010 and Varian 5000 apparatus equipped with a differential refractometer using LiChrosorb Si-60 (25×1.0 cm) and μ-Bondapak C_{18} (25×10 cm) columns.

EXTRACTION.—Fresh sponges *Ax. cannabina* (350 g, dry weight after extraction) and *Ac. acuta* (48 g, dry weight after extraction) were collected in the Bay of Taranto near Porto Cesareo in autumn 1985, and in the Bay of Napoli in spring 1985, respectively, and identified by Professor M. Sarà, University of Genova, Italy. Voucher specimens are preserved in the Dipartimento di Chimica delle Sostanze Naturali, Napoli (*Ax. cannabina*) and in the Dipartimento di Chimica Organica e Biologica, Napoli (*Ac. acuta*).

The initial procedure employed for the extraction and fractionation of the lipid mixture was common to both sponges and was performed as follows.

The homogenized material was extracted three times with MeOH at room temperature for 3 days, and the aqueous residue resulting from the concentration under reduced pressure was extracted with $CHCl_3$. The organic phase was taken to dryness and the oily residue (15 g for *Ax. cannabina*, 6.3 g for *Ac. acuta*) was applied to a column of Si gel eluted under a slight N_2 pressure with a solvent gradient system from light petroleum to Et_2O through C_6H_6.

The early fractions (eluent; light petroleum-C_6H_6, 8:2) gave a mixture of isothiocyanates; the middle fractions (eluent; light petroleum-C_6H_6, 2:8) yielded a mixture of isocyanides and the last ones (eluent Et_2O) afforded, only in the case of *Ax. cannabina*, a mixture of amides. These fractions were used for the isolation of compounds **1-3** as described below.

ISOLATION OF **2** FROM *AX. CANNABINA*.—The combined less polar fractions (1.6 g), obtained as above, were further separated on a Si gel column (100 g, eluent; light petroleum-C_6H_6, 8:2) to give 92 mg

of an oily residue that still contained a mixture of isothiocyanates from which **2** was obtained in pure form (15 mg) by hplc (μ-bondapak C_{18}, eluent; MeOH-H_2O, 95:5).

Compound **2**, mp 52-53°, $[\alpha]_D$ +88.4 (*c*, 1.0, $CHCl_3$); ir ($CHCl_3$) ν max 2100 cm^{-1}; hrms *m/z* 263.1712 ($C_{16}H_{25}NS$ requires 263.1709); ^1H nmr ($CDCl_3$) δ 4.96 and 4.86 (1H, each, narrow m's vinyl protons), 3.69 (1H, dd, *J*=11.2 and 11.2 Hz, 6-H), 2.03 (1H, bd, *J*=11.2 Hz, 5-H), 0.96 and 0.84 (3H each d's *J*=6.8 Hz, 11-Me's), 0.92 (3H, s, 10-Me); ^{13}C nmr ($CDCl_3$) δ 145.0 (C-4), 113.8 (C-14), 59.0 (C-5 or C-6), 58.2 (C-6 or C-5), 48.9 (C-7), 39.4 (C-9), 35.6 (C-10), 30.4 (C-1 or C-3), 30.1 (C-3 or C-1), 28.0 (C-11), 27.9 (C-15), 22.8 (C-2), 20.7 (C-12 or C-13), 18.8 (C-8), 15.8 (C-13 or C-12).

ISOLATION OF **2** FROM *AC. ACUTA*.—The less polar fractions obtained as above (1.2 g) were further separated by hplc on a LiChrosorb Si-60 column (eluent; *n*-hexane-EtOAc, 96:4) affording 50 mg of **2**.

ISOLATION OF **1** FROM *AX. CANNABINA*.—The combined middle fractions (5 g), obtained as above, were extensively chromatographed over Si gel. Later eluates emerged from the column with light petroleum-C_6H_6 (1:1), combined, were further purified by hplc (μ-Bondapak C_{18}, eluent MeOH-H_2O, 8:2), thus affording 23 mg of **1**, mp 78-79°; $[\alpha]_D$ +92.9 (*c* 1.8, $CHCl_3$); ir ($CHCl_3$) ν max 2135, 1640, 895 cm^{-1}; hrms *m/z* 231.1991 ($C_{16}H_{25}N$ requires 231.1988); ^1H- and ^{13}C-nmr data are reported in Table 1.

ISOLATION OF **1** FROM *AC. ACUTA*.—A portion (900 mg) of the mixture of isocyanides from the fractions of intermediate polarity (3.9 g) obtained as above, was successfully resolved by hplc giving 60 mg of **1**.

ISOLATION OF **3** FROM *AX. CANNABINA*.—The combined more polar fractions, obtained as above, were applied to a Si gel column that was eluted with Et_2O, thus affording crude **3**. Final purification was achieved by hplc (LiChrosorb Si-60, EtOAc), to give 8 mg of **3**, hrms *m/z* 249.2089 ($C_{16}H_{27}NO$ requires 249.2094); ir ν max 3440 and 1680 cm^{-1}; ^1H-nmr spectrum shows a 2(*cis*): 1(*trans*) ratio of the rotational isomers of the formamide group; *cis* isomer: ^1H nmr δ 8.14 (d, *J*=2.5 Hz, H-C(=O)-), 4.75 (bm, >NH), 4.77 and 4.53 (narrow m's, >C=CH_2), 4.22 (ddd, *J*=11.2, 11.2, and 11.2 Hz, 6-H), 0.90 and 0.87 (d's, *J*=7.0 Hz, 12-H_3 and 13-H_3), 0.90 (s, 15-H_3); *trans* isomer: ^1H nmr δ 7.81 (d, *J*=12.0 Hz, H-C(=O)-), 5.00 (bm, >NH), 4.89 and 4.65 (narrow m's, >C=CH_2), 3.36 (ddd, *J*=11.2, 11.2, and 11.2 Hz, 6-H), 0.92 and 0.80 (d's, *J*=7.0 Hz, 12-H_3 and 13-H_3), 0.92 (s, 15-H_3).

DEHYDROGENATION OF **1** TO EUDALENE [**5**].—Isocyanide **1** (30 mg) and 10% Pd/C (50 mg) were heated at 250° under N_2 for 1 h. After cooling, the mixture was extracted with $CHCl_3$ and purified by tlc (Si gel, *n*-hexane, uv) thus obtaining 10 mg of eudalene [**5**], identified by comparison of its spectral and chromatographic properties with those of an authentic sample.

CONVERSION OF **1** TO **2**.—A mixture of **1** (30 mg) and excess sulfur were heated at 120° for 16 h; after cooling, light petroleum (40-70°) was added, and the solution was filtered and taken to dryness. The residue was chromatographed on tlc (Si gel, *n*-hexane, uv) thus obtaining 20 mg of a product whose spectral and chromatographic properties were identical to those of natural **2**.

HYDRATION OF **1** TO **3**.—To **1** (12 mg), 99.7% glacial HOAc (1 ml) was added, and the solution was kept at room temperature for 10 h. After washing with 10% aqueous Na_2CO_3 solution and then with H_2O, Et_2O was added. The organic phase was dried and taken to dryness, and the residue was purified by tlc (Si gel, Et_2O), to give 6 mg of **3** whose spectral and chromatographic properties matched those of natural **3**.

ACKNOWLEDGMENTS

This work was supported by Ministero della Pubblica Istruzione, Italy, and by CNR (Progetto Finalizzato Chimica Fine e Secondaria). We wish to thank Professor M. Sara for identifying the sponges. Mass spectral data were provided by Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli. The assistance of the staff is gratefully acknowledged.

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Received 8 August 1986