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AXISONITRILE-3, AXISOTHIOCYANATE-3 AND AXAMIDE-3. SESQUITERPENES WITH A NOVEL SPIRO[4,5]DECANE SKELETON FROM THE SPONGE *AXINELLA CANNABINA*[a]

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Abstract—Axisonitrile-3 from the marine sponge *Axinella cannabina* has been shown, on the basis of chemical and spectral data and by single-crystal 3-dimensional X-ray diffraction study, to be **1**. Axisonitrile-3 was found to be a sesquiterpene isonitrile with a novel carbon skeleton ("spiroaxane"). From the same sponge, axisothiocyanate-3 (**2**) and axamide-3 (**3**), closely related to **1**, have been also isolated and their structures determined.

In 1973 we reported[1] the isolation from the marine sponge *Axinella cannabina* and the structure determination of axisonitrile-1 and axisothiocyanate-1, two sesquiterpenes with a novel carbon skeleton (axane) also found in oppositol, a brominated sesquiterpene alcohol from the marine red alga *Laurencia subopposita*.[2] Axisonitrile-1 and axisothiocyanate-1 were the first examples of naturally-occurring isoprenoids having an isonitrile or an isothiocyanate function. Later, a second sesquiterpenoid isonitrile, acanthellin-1, with a 4-*epi*-eudesmane skeleton was isolated from the sponge *Acanthella acuta* by Minale *et al.*[3]

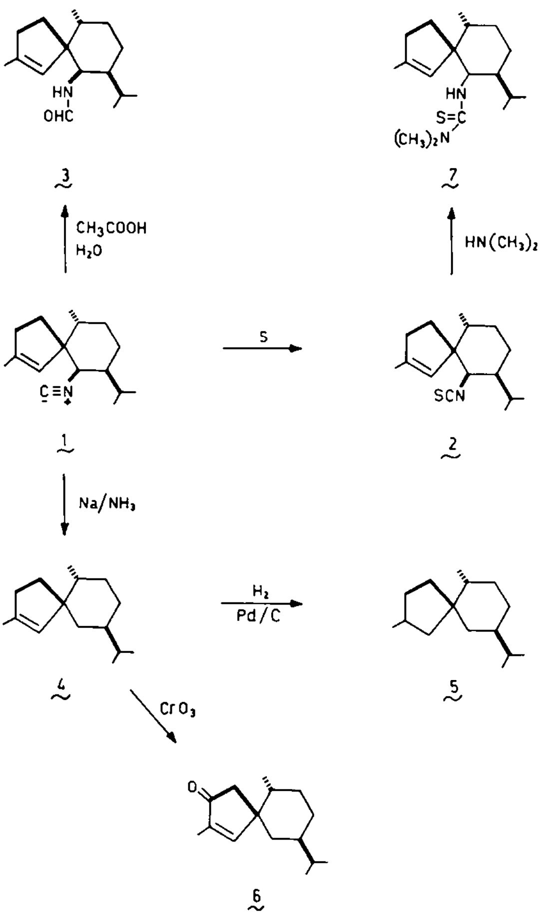
More recently we also found axisonitrile-2[4] and axisothiocyanate-2[5] (two aromadendrane sesquiterpenes bearing an $-\overset{+}{N}\equiv\overset{-}{C}$ and an $-N{=}C{=}S$ function respectively) and axamide-1 and axamide-2[5] (differing from axisonitrile-1 and -2 respectively in having an -NHCHO group instead of the isonitrile function) in *Axinella cannabina*.

Scheuer *et al.*[6] reported the occurrence in a marine sponge of the genus *Halichondria* of an amorphane sesquiterpenoid isonitrile accompanied by its corresponding formamide and isothiocyanate. The same sponge also elaborates a diterpenoid functionalised by the same three groups.[7]

The co-occurrence in the same organism of an isonitrile with its corresponding formamide and accompanied by an isothiocyanate, was considered to be evidence that the formamide is the biogenetic precursor of the isonitrile and isothiocyanate function.[5-7]

We now report the isolation and the structure determination of three novel related sesquiterpenoids, axisonitrile-3 (**1**), axisothiocyanate-3 (**2**) and axamide-3 (**3**), all present in relatively small amounts in the sponge *Axinella cannabina*.

Axisonitrile-3 (**1**). Silica gel chromatography of the ether soluble fraction of the acetone extract of the fresh material afforded an oily product, containing essentially axisonitrile-1, which after repeated silica gel chromatography followed by crystallization from light petroleum afforded small amounts of **1**, m.p. 101-3°, $[\alpha]_D + 68{\cdot}44°$. Mass spectrum and elemental analysis indicated a formula $C_{16}H_{25}N$. The IR absorption at 2115 cm^{-1} suggested the presence of an $-\overset{+}{N}\equiv\overset{-}{C}$ group, confirmed by MS [intense ion at *m/e* 204 $(M^+\text{-HCN})$]. The isonitrile function must be linked to a methine group as indicated by NMR, which showed a slightly broadened singlet at δ 3·51 (1H H-C_6).



Scheme 1.

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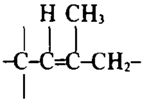
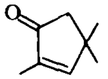
Axisonitrile-3 possesses three secondary Me groups [δ 0·76, (3H, d, J 7 Hz), 0·92 (3H, d, J 7 Hz), 0·95 (3H, d, J 7 Hz)] two of which could be part of an isopropyl function as indicated by mass [*m/e* 188 (M$^+$-43)] and IR (ν_{max} 1378–1373 cm^{-1}) spectra. Further analysis of the NMR spectrum of **1** pointed to the presence of the following partial structure



$$-\underset{|}{\overset{|}{C}}-\overset{H}{\overset{|}{C}}=\overset{CH_3}{\overset{|}{C}}-CH_2-$$

[δ 1·73 (3H, bs, H_3–C_{15}), 2·25 (2H, m, H_2–C_3), 5·1 (1H, bs, H–C_1)].

1 was reduced by Na/NH_3, giving hydrocarbon **4** (spiroaxone), M$^+$ 206, $[\alpha]_D$ − 11·6°, n_D 1·4834. NMR of **4**, when compared with that of **1**, showed as only significant difference the absence of the signal at δ 3·51 due to the methine linked to the isonitrile function. Catalytic hydrogenation of **4** afforded the dihydro-derivative **5** (mixture of diastereoisomers), M$^+$ *m/e* 208, thus indicating that the three unsaturations are due to two cycles and to the trisubstituted double bond.



A

Useful information on the structure of axisonitrile-3 was obtained by allylic oxidation of **4** which gave the α,β-unsaturated ketone **6**, M$^+$ 220, $[\alpha]_D$ − 21·3°, n_D 1·5400. IR [ν_{max} 1716 cm^{-1} (five membered α,β-unsaturated ketone)], UV[λ_{max} 233 nm (ε = 7050)] and NMR[δ 1·75 (3H, bs, H_3-C_{15}), 2·06 (2H, AB system, J 18 Hz, H_2-C_4) 7·17 (1H, bs, H-C_1)] spectra agree with the partial structure A.

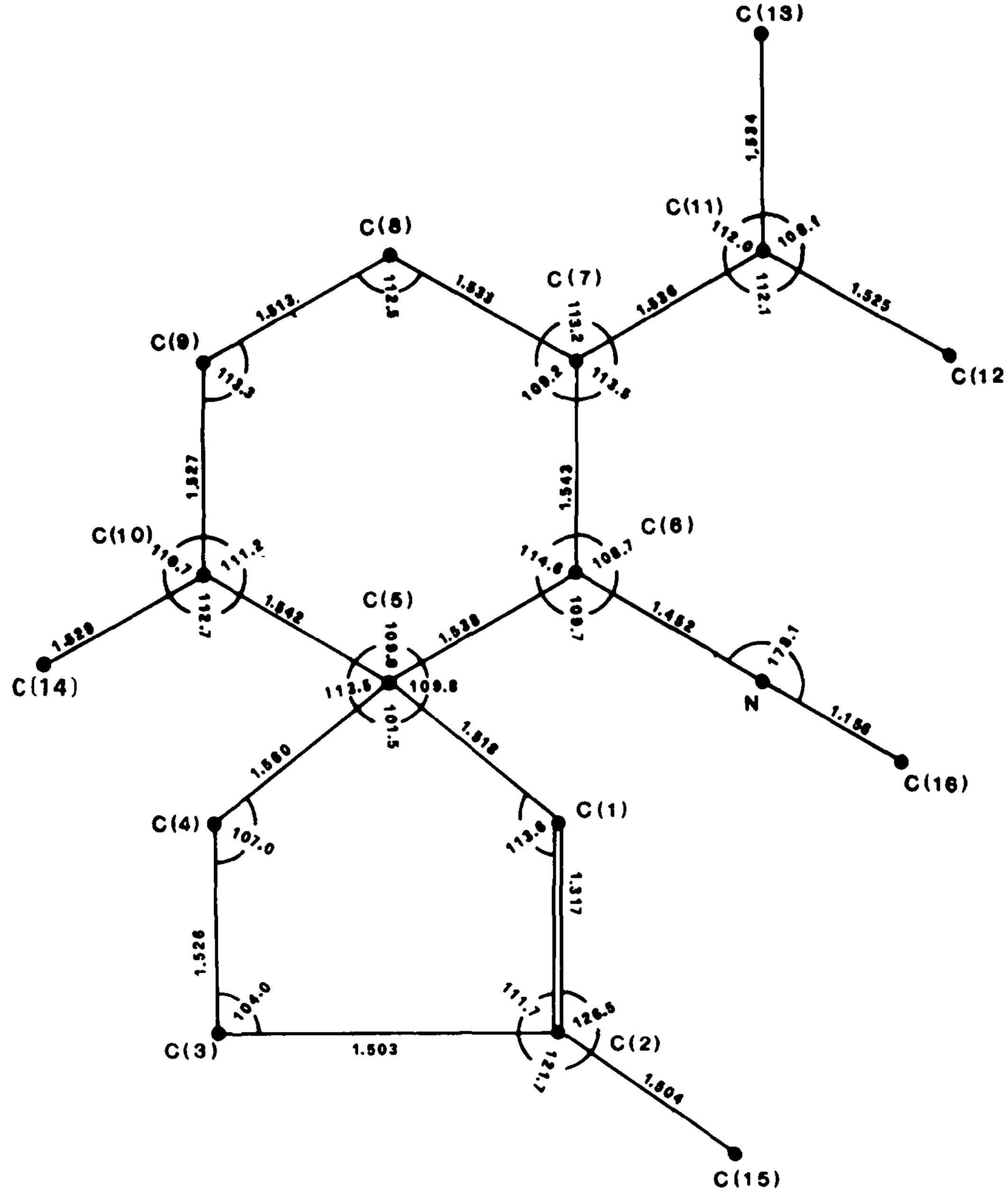


Fig. 1. Bond lengths and bond angles of axisonitrile-3 (1) (σ of the bonds: 0·002 Å, σ of the angles 0·1–0·2).

Table 1. Crystal data of axisonitrile-3 (1)

Molecular formula	$C_{16}H_{25}N$
Molecular weight	231·17 a.m.u.
Space Group	$P_{2_12_12_1}$
Z	4 molecules/unit cell
a	11·600 ± 0·005 Å
b	11·110 ± 0·005 Å
c	11·430 ± 0·005 Å
V	1473·0 Å^3
Density, flotation	1·00 g/cm^3
Density, calculated	1·04 g/cm^3
Radiation	Cu K ᾱ, λ = 1·54179 Å

As to the quaternary carbon atom in A, it appears likely to be a spiro-carbon, considering the substituents linked to the rings deduced from the spectral data of **1** and its derivatives.

The data above reported indicated that **1** must be a spiro[4,5]decane bearing on the 6-membered ring $-\dot{N}\equiv\bar{C}$, isopropyl, and secondary methyl groups. Since the interpretation of the data could not be accommodated by any known sesquiterpene skeleton and because of the small amounts of **1** available, axisonitrile-3 was subjected to single-crystal X-ray analysis which led to the stereostructure **1** depicted without regard to absolute configuration of the molecule.

X-ray study. The molecular model of **1** is shown in Fig. 1 and the final atomic coordinates and thermal parameters are given in Table 2. Bond angles and lengths are all close to the normal values within experimental error. The solid state indicates that the molecule is configurationally and conformationally rigid. The only deformation in the geometry of the molecule may be considered the slight increase of the C(10)-C(5)-C(4) angle (113·5°) probably due to intramolecular interaction between H(1)C(4)···H(1)C(14) (separation 2·2 Å). The cyclopentene ring, with its zero torsion angles imposed by the double bond, adopts an envelope conformation with a high degree of flattening as compared to the most probable conformation for the isolated ring.[8] The cyclohexane ring is in the favoured chair conformation. The C(6) carbon atom brings an axial isonitrile group which is in *cis* position to the isopropyl and *trans* to the secondary methyl on C(10) and to C(1). The corresponding torsion angles are reported in Table 3. In Fig. 2 a molecular packing of the structure as view along the *c* axis is reported; the shortest intramolecular contacts are indicated.

Axisothiocyanate-3 (**2**). The ether soluble fraction of

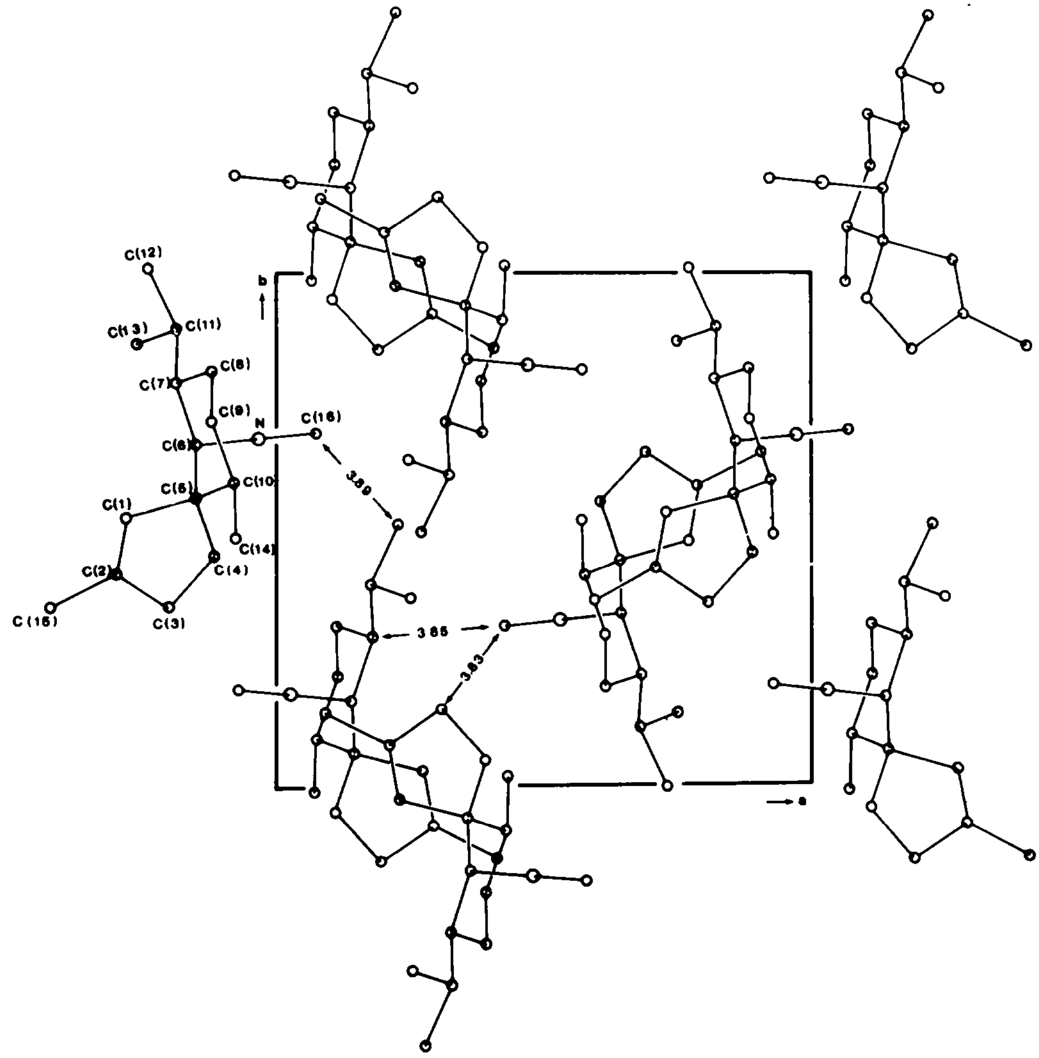


Fig. 2. Packing of *axisonitrile*-3 (**1**) along the *c* axis.

Table 2. Final Atomic Parameters (× 10^4) for axisonitrile 3 with their estimated standard deviations*

A. POSITIONAL PARAMETERS

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
C(1)	2686(2)	292(2)	−5164(2)	H(1)–C(1)	3235(26)	847(26)	−5489(30)
C(2)	2950(2)	−842(2)	−4959(2)	H(1)–C(3)	1694(25)	−2204(25)	−5115(28)
C(3)	1939(3)	−1530(2)	−4480(3)	H(2)–C(3)	2082(23)	−1964(23)	−3705(23)
C(4)	1035(2)	−562(2)	−4241(3)	H(1)–C(4)	250(30)	−828(29)	−4637(34)
C(5)	1444(2)	605(2)	−4877(2)	H(2)–C(4)	1059(33)	−409(33)	−3418(34)
C(6)	1409(2)	1673(2)	−4022(2)	H(1)–C(6)	1857(23)	1464(25)	−3358(26)
C(7)	1803(2)	2884(2)	−4548(2)	H(1)–C(7)	2632(18)	2752(19)	−4823(19)
C(8)	1100(2)	3146(2)	−5655(2)	H(1)–C(8)	283(26)	3349(26)	−5409(30)
C(9)	1112(2)	2106(3)	−6510(2)	H(2)–C(8)	1366(29)	3876(27)	−6078(27)
C(10)	722(2)	917(2)	−5968(2)	H(1)–C(9)	606(27)	2297(27)	−7171(27)
C(11)	1794(2)	3926(2)	−3666(2)	H(2)–C(9)	1904(23)	1957(25)	−6867(25)
C(12)	2282(3)	5083(2)	−4185(3)	H(1)–C(10)	−148(22)	916(23)	−5660(23)
C(13)	2482(4)	3627(3)	−2558(3)	H(1)–C(11)	907(21)	4070(22)	−3478(22)
C(14)	727(3)	−88(3)	−6882(3)	H(1)–C(12)	2177(35)	5830(33)	−3452(37)
C(15)	4101(3)	−1430(3)	−5154(3)	H(2)–C(12)	3176(37)	5020(43)	−4535(44)
C(16)	−84(3)	1424(3)	−3228(3)	H(3)–C(12)	1765(27)	5323(26)	−4808(26)
N	250(1)	1814(1)	−3563(1)	H(1)–C(13)	2429(33)	4354(30)	−1956(34)
				H(2)–C(13)	2010(32)	3032(33)	−2141(34)
				H(3)–C(13)	3297(64)	3506(59)	−2769(76)
				H(1)–C(14)	449(26)	−875(26)	−6588(27)
				H(2)–C(14)	217(26)	189(29)	−7387(25)
				H(3)–C(14)	1558(32)	−262(36)	−7217(34)
				H(1)–C(15)	4686(29)	−945(29)	−5720(30)
				H(2)–C(15)	4401(45)	−1697(38)	−4386(49)
				H(3)–C(15)	4049(28)	−2133(31)	−5612(33)

B. THERMAL FACTORS. Temperature factor in the form:

$$T = \exp\left[-1/4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)\right]$$

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}	Atom	B
C(1)	3·9(1)	3·9(1)	5·2(1)	0·0(1)	−0·4(1)	0·4(1)	H(1)–C(1)	3·9(1)
C(2)	4·8(1)	4·3(1)	5·1(1)	0·7(1)	0·4(1)	0·9(1)	H(1)–C(3)	3·5(1)
C(3)	6·8(1)	3·7(1)	6·3(1)	0·2(1)	0·2(1)	−0·3(1)	H(2)–C(3)	2·4(1)
C(4)	5·4(1)	3·5(1)	6·7(1)	−0·5(1)	−0·6(1)	−0·6(1)	H(1)–C(4)	5·1(1)
C(5)	3·7(1)	3·3(1)	4·6(1)	−0·2(1)	−0·5(1)	−0·2(1)	H(2)–C(4)	6·8(1)
C(6)	3·4(1)	3·7(1)	3·9(1)	−0·0(1)	−0·2(1)	−0·3(1)	H(1)–C(6)	2·6(1)
C(7)	3·8(1)	3·3(1)	4·3(1)	0·0(1)	−0·4(1)	−0·2(1)	H(1)–C(7)	1·4(1)
C(8)	5·6(1)	4·1(1)	4·8(1)	0·4(1)	−0·0(1)	−1·2(1)	H(1)–C(8)	4·8(1)
C(9)	5·7(1)	5·9(1)	3·9(1)	0·0(1)	0·1(1)	−0·7(1)	H(2)–C(8)	4·4(1)
C(10)	4·2(1)	4·8(1)	4·8(1)	−0·1(1)	0·0(1)	0·5(1)	H(1)–C(9)	3·3(1)
C(11)	5·2(1)	4·0(1)	6·1(1)	−0·4(1)	−1·2(1)	0·6(1)	H(2)–C(9)	3·3(1)
C(12)	7·2(2)	3·5(1)	8·1(2)	−0·4(1)	−0·7(2)	0·3(1)	H(1)–C(10)	2·6(1)
C(13)	11·2(3)	6·4(2)	5·3(1)	−2·9(2)	0·7(2)	0·9(1)	H(1)–C(11)	2·3(1)
C(14)	6·6(1)	7·0(2)	6·1(1)	−0·4(1)	0·9(1)	2·1(1)	H(1)–C(12)	5·9(1)
C(15)	6·3(2)	6·3(1)	7·5(2)	2·5(1)	0·7(1)	1·6(1)	H(2)–C(12)	9·4(2)
C(16)	5·1(1)	7·4(2)	6·1(1)	0·1(1)	−1·4(1)	−0·6(1)	H(3)–C(12)	3·7(1)
	4·1(1)	4·3(1)	4·4(1)	−0·1(1)	−0·7(1)	−0·2(1)	H(1)–C(13)	7·1(1)
							H(2)–C(13)	7·4(1)
							H(3)–C(13)	15·9(3)
							H(1)–C(14)	3·2(1)
							H(2)–C(14)	3·9(1)
							H(3)–C(14)	5·7(1)
							H(1)–C(15)	6·3(2)
							H(2)–C(15)	7·9(1)
							H(3)–C(15)	5·8(1)

*E.s. d's in unit of last significant figure.

the acetone extract of *Axinella cannabina* after chromatography on silica gel, afforded an oily product (ν_{max} 2120 cm^{-1}, -NCS) which, by GLC, proved to contain at least four compounds; from this mixture, by treatment with Me_2NH and successive chromatography on silica gel, we isolated **7**, clearly derived from **2**.

Compound **7**, m.p. 137–138°, $[\alpha]_D - 61{\cdot}9°$, has molecular formula $C_{18}H_{32}N_2S$ (elemental analysis and mass spectrum). Spectral data [δ 0·85 (3H, d, J 7 Hz, H_3-C_{14}), 1·00 (6H, d, J 6·5 Hz, H_3–C_{12} and H_3–C_{13}), 1·76 (3H, bs, H_3–C_{15}), 3·22 (6H, s, $-N(CH_3)_2$, 5·2 (1H, bs, H–C_1); ν_{max} 3400 cm^{-1} (NH); *m/e* 308 (M^+), *m/e* 204 ($M^+-NH_2-\underset{\underset{S}{\|}}{C}-N(CH_3)_2$)]

Table 3. Internal rotation angles (degrees)

C(10-C(9)-C(8)-C(7)	−54
C(9)-C(8)-C(7)-C(6)	52
C(8)-C(7)-C(6)-C(5)	−54
C(7)-C(6)-C(5)-C(10)	55
C(6)-C(5)-C(10)-C(9)	−54
C(5)-C(10)-C(9)-C(8)	55
C(5)-C(1)-C(2)-C(3)	0
C(1)-C(2)-C(3)-C(4)	9
C(2)-C(3)-C(4)-C(5)	−14
C(3)-C(4)-C(5)-C(1)	14
C(4)-C(5)-C(1)-C(2)	−9
C(14)-C(10)-C(5)-C(6)	181
C(14)-C(10)-C(5)-C(4)	58
C(14)-C(10)-C(5)-C(1)	−56
C(15)-C(2)-C(3)-C(4)	189
C(5)-C(6)-N-C(16)	48
C(7)-C(6)-N-C(16)	−78
C(12)-C(11)-C(7)-C(8)	59
C(12)-C(11)-C(7)-C(6)	185
C(13)-C(11)-C(7)-C(8)	181
C(13)-C(11)-C(7)-C(6)	−53
C(14)-C(10)-C(9)-C(8)	181
C(11)-C(7)-C(6)-N	−58

indicated the relationship between **7** and **1**. Axisonitrile-3 (**1**), by treatment with sulphur at 120°, yielded **2**, $[\alpha]_D + 165{\cdot}2°$, n_D 1·5415, which, after reaction with Me_2NH, gave the corresponding thiourea, identified as **7** by comparison of $[\alpha]_D$, m.p., spectral (NMR, IR and MS) and chromatographic properties.

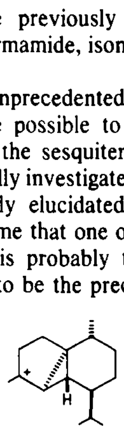
This identification pointed to the presence of **2** in the initial mixture. This was confirmed by GLC (2·5% SE30 on Chromosorb W at 180°, 170° and 160°) using as reference a pure sample of **2** synthetized from **1** as described above.

Axamide-3 (**3**). This compound, present in *Axinella cannabina* in smaller amounts, was also isolated from the ether soluble fraction of the acetone extract by silica gel chromatography as an amorphous solid.

Spectral analogies (Experimental) of **3** with **1** led us to correlate these two compounds as follows: hydration of **1** afforded a formamide which was identified as **3** by comparison of $[\alpha]_D$, spectral (NMR, IR and MS) and chromatographic (GLC, 2·5% SE30 on Chromosorb W at 200°, 180° and 160°) properties.

The co-occurrence in the same sponge of **1**, **2** and **3** further confirms the previously proposed biogenetic relationship of the formamide, isonitrile and isothiocyanate functions.

As far as the unprecedented spirane nucleus is concerned, it will be possible to formulate biogenetic considerations when the sesquiterpene fraction of *Axinella cannabina* is fully investigated and the stereochemical details completely elucidated. However, it is not unreasonable to assume that one of the intermediates in the biogenesis of **1** is probably the carbonium ion **8**, previously proposed to be the precursor of cubebenes.[9]



8

EXPERIMENTAL

IR spectra (CCl_4 solns) were recorded on a Perkin-Elmer 157 spectrophotometer. NMR spectra were determined on a Perkin-Elmer R32 spectrometer in CCl_4 solns using TMS as internal reference ($\delta = 0$). Mass spectra were taken on a AEI MS 902 instrument. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. The UV spectrum of **6** was recorded on a Cary 118 spectrometer. X-Ray data were collected using a single-crystal Siemens X-ray diffractometer and elaborated with a 360/40 IBM computer. TLC was effected using glass-packed precoated silica gel F_{254} plates (Merck). GLC was effected using a Perkin-Elmer F30 instrument with 2m × 0·4 cm columns (flow of N_2 30 ml/min) of 1% SE.30 at 160°, 180° and 200°.

Sponges (*Axinella cannabina*) collected in the Bay of Taranto were supplied by Stazione di Biologia marina del Salento, Porto Cesareo (Direttore Prof. P. Parenzan).

Isolation of **1**. Fresh sponges (1 kg dry weight after extraction) were extracted 4 times with Me_2CO at room temp for 2 days. The combined extracts (16 l) were concentrated under reduced pressure and the remaining aqueous residue was extracted with Et_2O (4 l in 3 portions). The organic phase was taken to dryness leaving an oily residue (15 g), which was chromatographed on a silica gel (900 g) column using C_6H_6-Et_2O (3:2) as eluent. Fractions of 750 ml were collected. Fractions 1 and 2, taken to dryness, afforded 3·8 g of a residue (fraction A), fractions 17–19, on evaporation of the solvent, yielded 200 mg of an oily product (fraction B). Fraction A and fraction B were used, as described below, for the isolation of **7** and **3** respectively.

Fractions 3–5 (5 g) were re-chromatographed on silica gel column (250 g) using light petroleum (40–70°) -C_6H_6 (7:3) as eluent. Fractions of 250 ml were collected. Fractions 9–12 (900 mg) were further purified on a silica gel column (80 g) using light petroleum (40–70°) -C_6H_6 (95:5) as eluent (50 ml fractions). Fractions 85–115, taken to dryness, afforded 120 mg of **1**, recrystallized from 80–100° light petroleum, m.p. 101–3°, $[\alpha]_D + 68{\cdot}4°$ (C = 1; $CHCl_3$), M^+ *m/e* 231; the significant features of IR and NMR spectra are reported in the Discussion. (Found: C, 83·17; H, 10·73; N, 5·94. Calc. for $C_{16}H_{25}N$: C, 83·05; H, 10·89; N, 6·05%).

Reduction of **1** *with* Na/NH_3. To a stirred soln of **1** (100 mg) in anhydrous Et_2O (10 ml) and liquid NH_3 (20 ml) at −45°, Na (30 mg) was added during 2 h. After addition of a little NH_4Cl to destroy excess Na, the mixture was evaporated to dryness and the residue, after addition of H_2O, was extracted with light petroleum (40–70°). The organic phase, taken to dryness, afforded 80 mg of an oily product which was chromatographed on a SiO_2 column (6 g) using light petroleum (40–70°) as eluent (8 ml fractions). Fractions 2 and 3, after evaporation of solvent afforded 60 mg of **4** (spiroaxene) M^+ *m/e* 206), $[\alpha]_D = -11{\cdot}6°$ (c = 1 in $CHCl_3$), $n_D = 1{\cdot}4834$.

Catalytic hydrogenation of **4** *to obtain* **5**. A soln of **4** (50 mg) in EtOH (10 ml) was hydrogenated overnight over 10% Pd/C (15 mg) at 60° and 3 atm. After removal of the catalyst by filtration, the soln was evaporated to dryness leaving an oily residue which was chromatographed on a $SiO_2/AgNO_3$ column (5 g) using light petroleum (40–70°) as eluent. Fractions of 5 ml were collected. Fractions 2 and 3, after removal of the solvent, yielded 27 mg of the dihydro-derivative **5** (mixture of diastereoisomers), M^+ *m/e* 208.

Allylic oxidation of **4** *to obtain* **6**. Spiroaxeme (**4**; 80 mg) in Me_2CO (6 ml) was treated with Jones reagent at room temp for 16 h. Following usual work-up, **6** (35 mg) was obtained and purified by PLC, eluent light petroleum (40–70°) -C_6H_6 1:1, R_f 0·3, under UV light, M^+ *m/e* 220, $n_D = 1{\cdot}5400$, $[\alpha]_D = 21{\cdot}3°$ (c = 1 in $CHCl_3$). Significant features of IR and NMR spectra are reported in the Discussion.

Isolation of **7**. Fraction A, obtained as above, was rechromatographed on a silica gel column (220 g), eluent light petroleum (40–70°). Fractions of 200 ml were collected. Fractions 10–13 were evaporated to dryness to give 190 mg of an oily residue which migrates as a single spot on TLC (SiO_2 and Al_2O_3; eluent n-hexane). GLC revealed the presence of at least four products with very similar retention times. This mixture (150 mg) and excess of a 15% Me_2NH in C_6H_6 were kept at room temp for 24 h. The soln was taken to dryness and the residue was chromatographed on PLC (eluent C_6H_6-Et_2O, 95:5). After two developments, the band R_f 0·5 (UV light), scraped and eluted with Et_2O, afforded 15 mg of **7**, M^+ *m/e* 308; $[\alpha]_D = -61{\cdot}9°$ (c = 1 in $CHCl_3$); m.p. 137–138°C, IR and NMR data are discussed in the Discussion.

(Found: C, 69·92; H, 10·54; N, 8·92; S, 10·45. Calc. for $C_{18}H_{32}N_2S$: C, 70·09; H, 10·46; N, 9·08; S, 10·37%).

Treatment of **1** *with sulphur to obtain* **2**. Axisonitrile-3 (**1**, 100 mg) and excess S were heated at 120° for 16 h; after addition of 40°–70° light petroleum (15 ml) and filtration, the soln was taken to dryness and residue was purified by PLC (eluent: *n*-hexane). The band R_f 0·45 (UV light) eluted with Et_2O, afforded 70 mg of **2**, M^+ *m/e* 263; $[\alpha]_D + 165{\cdot}2°$ (c=1, $CHCl_3$); $n_D = 1{\cdot}5415$; ν_{max} 2120 cm^{-1} (–N=C=S); δ 5·08 (1H, bs, H–C_1); 3·65 (1H, bs, H–C_6), 2·54 (2H, m, H_2–C_3), 1·75 (3H, s H–C_{15}), 0·95 (6H, d, J 6 Hz H_3–C_{12} and H_3–C_{13}); 0·80 (3H, d, J 6 Hz H_3–C_{14}). Found: C, 72·90; H, 9·45; N, 5·40; S, 12·25; Calc. for $C_{16}H_{25}NS$ C, 72·95; H, 9·57; N, 5·32; S, 12·16%.

Treatment of **2** *with* Me_2NH *to obtain* **7**. Axisothiocyanate-3 (**2**) (60 mg) and excess of 15% Me_2NH in C_6H_6 were kept at room temp for 24 h. After removal of the solvent and excess Me_2NH in *vacuo* the crude product was purified by repeated crystallizations from light petroleum (80–100°), giving **7** (30 mg).

Isolation of axamide-3 (**3**). Fraction B, obtained as above, was further purified by silica gel (20 g) column using C_6H_6-Et_2O (4:1) as eluent. Fractions of 20 ml were collected. Fractions 21–23, taken to dryness afforded 15 mg of **3**; $[\alpha]_D - 6{\cdot}86°$ (c=1, $CHCl_3$); M^+ *m/e* 249; the significant features of IR and NMR spectra are reported in the Discussion. Found: C, 77·14; H, 10·65; N, 5·53. Calc. for $C_{16}H_{27}NO$ C, 77·06; H, 10·91, N, 5·62%.

Hydratation of **1** *to obtain* **3**. A soln of **1** (100 mg) in dry Et_2O (8 ml) and AcOH (6 ml) was kept at room temp for 2 h. After washing with 10% aq Na_2CO_3 and then with H_2O, the organic phase was dried and taken to dryness, so giving an oily residue which was chromatographed on a silica gel column (8 g) using C_6H_6-Et_2O (1:1) as eluent. Fractions of 8 ml were collected. Fractions 14–16, on evaporation of the solvent afforded 55 mg of **3**.

Single crystal X-ray structure determination of axisonitrile-3 (**1**). 1334 independent reflections were measured by an "on line" single crystal Siemens diffractometer; 50 of these were not considered in the refinement, their values being less than $2\sigma(I)$. The unit cell parameters were determined by a least-squares procedure[10] of the 2θ, χ; φ setting angles of twelve reflections with large value of 2θ. The parameters obtained are reported in Table 1.

The structure was solved by the multisolution method[11] with MULTAN, the phase-permutation computer program for non-centrosymmetric structures which incorporates the weighted tangent formula.

The signs of 324 factors with $|E| > 1{\cdot}20$ were determined and the E-map (ABS FOM = 1·05) indicated the positions of all atoms present in the molecule, other than hydrogen. The refinement by full-matrix least-squares procedures brought the conventional R to 0·080. A difference Fourier synthesis revealed the positions of all hydrogen atoms not far from the expected stereochemical positions. The light atoms were included in the refinement and the final R value was 0·040. The weighting scheme adopted throughout the refinement corresponded to that suggested by Cruickshank and Philling.[12]

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