Tetrahedron. Vol 31, pp. 269 to 270. Pergamon Press 1975. Printed in Great Britain

NEW SESQUITERPENOIDS FROM THE SPONGE *AXINELLA CANNABINA*[a]

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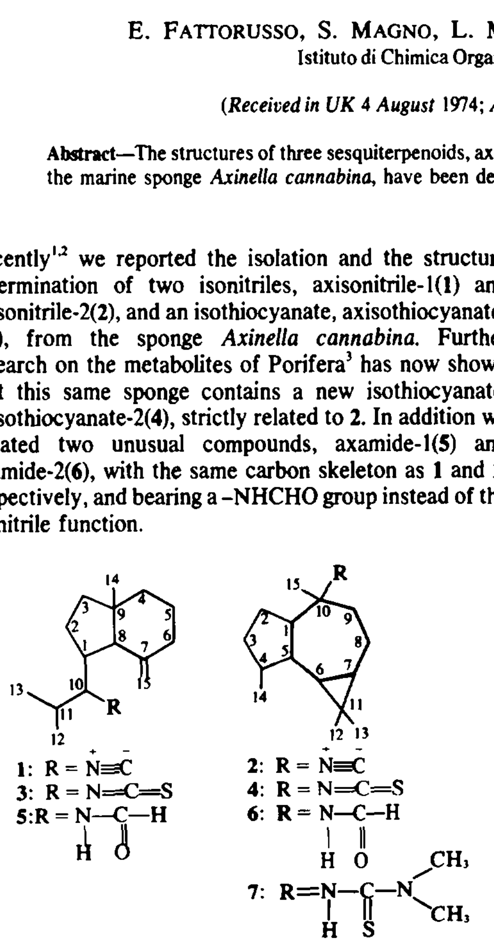
(*Received in UK* 4 *August* 1974; *Accepted for publication* 3 *October* 1974)

Abstract—The structures of three sesquiterpenoids, axisothiocyanate-2(4), axamide-1(5) and axamide-2(6), present in the marine sponge *Axinella cannabina*, have been determined on the basis of chemical and spectral evidences.

Recently[1,2] we reported the isolation and the structure determination of two isonitriles, axisonitrile-1(**1**) and axisonitrile-2(**2**), and an isothiocyanate, axisothiocyanate-1(**3**), from the sponge *Axinella cannabina*. Further research on the metabolites of Porifera[3] has now shown that this same sponge contains a new isothiocyanate, axisothiocyanate-2(**4**), strictly related to **2**. In addition we isolated two unusual compounds, axamide-1(**5**) and axamide-2(**6**), with the same carbon skeleton as **1** and **2**, respectively, and bearing a -NHCHO group instead of the isonitrile function.



1: R = $\overset{+}{N}\equiv\overset{-}{C}$
3: R = N=C=S
5: R = $\underset{H}{N}-\underset{O}{C}-H$

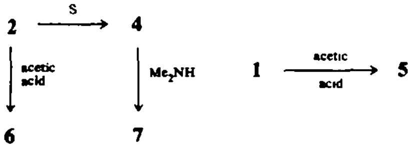
2: R = $\overset{+}{N}\equiv\overset{-}{C}$
4: R = N=C=S
6: R = $\underset{H}{N}-\underset{O}{C}-H$
7: R = $\underset{H}{N}-\underset{S}{C}-N(CH_3)_2$

Axisothiocyanate-2(**4**). Fresh material was extracted with acetone and the ether soluble fraction, after chromatography on silica gel, afforded an oily product (ν_{max} 2120, -N=C=S), which by GLC proved to be at least four compounds; from this mixture, after reaction with Me_2NH and subsequent chromatography on SiO_2, it was possible to isolate **7**, clearly deriving from **4**.

Compound **7** has molecular formula $C_{18}H_{32}N_2S$ (mass spectrum and elemental analyses); IR (ν_{max} 3405 cm^{-1}); NMR (δ 3·15, 6H, s) and mass [intense ion at *m/e* 204 ($M^+ - NH_2CSN(Me_2)$)] spectra agree with the presence in **7** of the -NHCSN(Me_2) group. **7** also contains a secondary Me group (δ 1·03, 3H, d, J 6Hz) and two tertiary Me groups (δ 0·99, 3H, s and δ 0·95, 3H, s) as shown by its NMR spectrum, which suggests, also the presence of another Me group linked to the C atom bearing the functional group (δ 1·6, 3H, s). A further feature, revealed by NMR spectrum, is the presence of the hydrogens of a cyclopropyl group by a high field complex signal, spread between δ 0·8–0·4 (2H).

All these data strongly suggest a close relationship of **7** with axisonitrile-2(**2**) as proved: compound **2**, by treatment with sulphur at 120°, afforded **4**, $[\alpha]_D + 12·8$, n_D 1·5402, ν_{max} 2120, which, by treatment with (Me_2)NH, gave the corresponding thiourea which resulted to be identical to **7** by comparison of $[\alpha]_D$, spectral (NMR, IR and MS) and chromatographic properties.

This identification points to the presence of **4** in the initial mixture. This was confirmed by GLC (2·5% SE 30 on chromosorb W at 180°, 170° and 160°) using as a reference a pure sample of **4** synthesised as described above.



SCHEME 1

Axamide-1 (**5**). This compound present in *Axinella cannabina* in relatively small amounts was isolated from the ether soluble fraction by PLC as a colourless oil, $[\alpha]_D + 10·0°$, n_D 1·5087, $C_{16}H_{27}NO$ (mass spectrum and elemental analyses). The presence of the -NHCHO function was deduced by IR (ν_{max} 3350–3150, 1687 cm^{-1}) and mass [intense ion at *m/e* 204 ($M^+ - HCONH_2$)] spectra. Spectral data also showed the presence of a $\gt C=CH_2$ group [ν_{max} 3050, 1640 and 895 cm^{-1}, δ 4·68 (2H, bm)]; a tertiary Me group (δ 0·94, 3H, s) and two secondary Me groups (δ 0·88, 3H, d, J 6 Hz and δ 0·80, 3H d, J 6 Hz), probably belonging to an isopropyl group as indicated by IR spectrum (ν_{max} 1385 and 1375 cm^{-1}).

All these data indicated a close structural relationship of **5** with axisonitrile-1 (**1**); this was confirmed by

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[a] This work was supported by a grant from Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Arco Felice, Napoli, Italy.

hydratation of **1** which afforded the corresponding formamide identified as **5** by comparison of $[\alpha]_D$, n_D, spectral (IR, NMR, MS) and chromatographic [TLC in Et_2O, GLC (2·5% SE 30 on chromosorb W at 195°, 175° and 160°)] properties.

Axamide-2 (**6**). Like **5**, this compound was isolated from the ether soluble fraction by PLC as an oily substance, $C_{16}H_{27}NO$ (elemental analyses and mass spectrum), $[\alpha]_D + 37·5°$, n_D 1·5618. IR (ν_{max} 3320–3170, 1684 cm^{-1}) and mass [*m/e* 204 ($M^+ - HCONH_2$)] spectra pointed to the presence of a –NHCHO function. Spectral analogies (Experimental) between **6** and **2**, induced us to correlate the two metabolites as follows: hydration of **2** gave **6**, identified by comparison of $[\alpha]_D$, n_D, spectral (IR, NMR, MS) and chromatographic [TLC in Et_2O, GLC (2·5% SE 30 on chromosorb W at 200°, 180° and 160°)] properties.

The co-occurrence of the compounds **1**–**6** in the sponge *Axinella cannabina* could be of biogenetic interest considering that very little is known about the formation and the role of the isonitrile function. We can now suppose that this group derives from a –NHCHO function by dehydration and successively evolves to a –N=C=S group.

EXPERIMENTAL

The IR spectra (CCl_4 solns) were recorded on a Perkin-Elmer 157 spectrophotometer. The NMR spectra were determined on a Perkin-Elmer R32 spectrometer in CCl_4 solns using TMS as internal reference (δ = 0). The mass spectra were taken on an AEI MS 902 instrument. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Mr. S. De Rosa (Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico–Arco Felice–Napoli). TLC and PLC were effected using glass packed precoated silica gel F 254 plates (E. Merck). Compounds **5**, **6** were visualized on PLC by heating a thin strip of each plate sprayed with a 5% ceric sulphate in a 10% aqueous H_2SO_4. GLC's were run using a Perkin-Elmer F 30 instrument with columns 2m × 0·4 cm.

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

Isolation of 7. Fresh sponges (800 g, dry after extraction) were extracted 4 times with Me_2CO at room temp for 2 days. The combined extracts (14 l) were concentrated under red press 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 (12 g) which was chromatographed on a SiO_2 column using benzene-Et_2O (3:2) as eluent. Fractions of 500 ml were collected. Fractions 16–18, on evaporation of the solvent, afforded 150 mg of a residue (fraction A) which was used, as described below, for the isolation of **5** and **6**.

Fractions 1-2 (3·5 g) were rechromatographed on a SiO_2 column (200 g), eluent: 40–70° light petroleum. Fractions of 200 ml were collected. Fractions 9–12 were evaporated to dryness to give 175 mg of an oily residue which migrates as a single spot on TLC (SiO_2 and Al_2O_3; eluent *n*-hexane). GLC (2·5% SE 30 on chromosorb W at 180°, 170° and 160°; flow of N_2 30 ml/min) revealed the presence of at least four products with very similar retention times. This mixture (80 mg) and excess of a 15% Me_2NH in benzene were kept at room temp for 24 hr. The soln was taken to dryness and the residue was chromatographed on PLC (eluent: benzene–Et_2O, 95 – 5). After two migrations the band R_f 0·45 (UV light), scraped and eluted with Et_2O, gave 14 mg of **7** as amorphous solid, $[\alpha]_D - 12·5°$ (*c* 1, $CHCl_3$); M^+ 308 *m/e* (Found: C, 69·95; H, 10·65; N, 9·21, S, 10·35. Calc. for $C_{18}H_{32}N_2S$: C, 70·09; H, 10·46; N, 9·08; S, 10·37%). The significant features of IR and NMR spectra are reported in the introduction.

Isolation of axamide-1(**5**) *and axamide*-2(**6**) *from the sponge* Axinella cannabina. Fraction A, isolated as above described, was rechromatographed on PLC (2 plates) using Et_2O as eluent. The bands R_f 0·7 and R_f 0·6, scraped and eluted with Et_2O, afforded respectively mg 25 of **5**, $[\alpha]_D + 10·0°$ (*c* 1·2, $CHCl_3$); n_D 1·5087; M^+ 249 *m/e*; (Found: C, 77·21; H, 10·85; N, 5·45. Calc. for $C_{16}H_{27}NO$: C, 77·06; H, 10·91; N, 5·62%), and mg 15 of **6**, $[\alpha]_D + 37·5°$ (*c* 0·9, $CHCl_3$); n_D 1·5618; M^+ 249 *m/e*; ν_{max} 3320–3170, 1684; δ 1·0 (6H, bs, H_3–C_{12} and H_3–C_{13}), 1·21 (3H, s, H_3–C_{15}), 0·92 (3H, d, J 6 Hz, H_3–C_{14}), 0·8–0·4 (2H, bm, H–C_6 and H–C_7. (Found: C, 77·25; H, 10·77; N, 5·49%).

Treatment of **2** *with sulphur to obtain* **4**. Compound **2** (50 mg) and excess S were heated at 120° for 16 hr; after addition of 40–70° light petroleum (10 ml) and filtration the soln was taken to dryness and the residue was purified by PLC (eluent: n-hexane). The band R_f 0·4 (UV light) eluted with Et_2O, afforded 30 mg of **4**, $[\alpha]_D + 12·8°$ (*c* 1·5, $CHCl_3$); n_D 1·5402; ν_{max} 2120; M^+ 263 *m/e*; δ 1·02 (3H, s, H_3–C_{12} or H_3–C_{13}), 0·98 (3H, s, H_3–C_{12} or H_3–C_{13}), 1·3 (3H, s, H_3–C_{15}), 0·90 (3H, d, J 6 Hz, H_3–C_{14}), 0·8–0·4 (2H, bm, H–C_6 and H–C_7). (Found: C, 72·84; H, 9·71; N, 5·28; S, 12·18. Calc. for $C_{16}H_{25}NS$: C, 72·95; H, 9·57; N, 5·32; S, 12·16%).

Treatment of **4** *with* Me_2NH *to obtain* **7**. Compound **4** (30 mg) and excess of a 15% Me_2NH in benzene were kept at room temp for 24 hr. After removal of solvent and excess of Me_2NH *in vacuo*, the crude product was purified on PLC, using as eluent benzene–Et_2O (95–5). After two migrations the band R_f 0·45 (UV light), eluted with Et_2O, afforded mg 20 of **7**.

Hydratation of **1**. A soln of **1** (50 mg) in anhydrous Et_2O (6 ml) and AcOH (5 ml) was kept at room temp for 2 hr. After washing with 10% Na_2CO_3 aq and then with H_2O, the organic phase was dried and taken to dryness. The residue was chromatographed on PLC (eluent: Et_2O); the band R_f 0·7, eluted with Et_2O, gave mg 24 of **5**.

Hydratation of **2** *to obtain* **6**. Compound **2** (50 mg) in Et_2O (6 ml) was treated with AcOH (5 ml) in the same experimental conditions described for **1**. The crude product was purified on PLC (eluent: Et_2O). The band R_f 0·6 yielded mg 21 of **6**.

Acknowledgement—The authors wish to thank Prof. M. Sarà (Università di Genova) for identifying the sponge.

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