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ISOLATION AND STRUCTURE OF AXISONITRILE-2

A NEW SESQUITERPENOID ISONITRILE FROM THE SPONGE *AXINELLA CANNABINA*[a]

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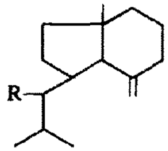
Abstract—On the basis of chemical and physico-chemical evidence, structure **3** has been assigned to axisonitrile-2, a new sesquiterpenoid isonitrile from the marine sponge *Axinella cannabina*.

As a part of a study on the metabolite of Porifera[1] we have been investigating the marine sponge *Axinella cannabina*. In an earlier paper we described two new compounds from this sponge, a sesquiterpenoid isonitrile, axisonitrile-1 (**1**), and an isothiocyanate, axisothiocyanate-1 (**2**), strictly related to it.[2] We wish to report here that this marine organism also produces another sesquiterpenoid isonitrile, axisonitrile-2 (**3**), possessing an aromadendrane skeleton.



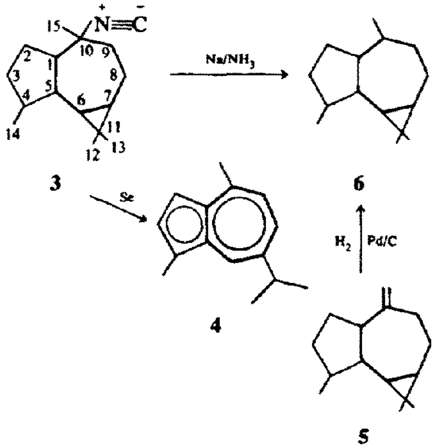
1: R = $\overset{+}{N} \equiv \overset{-}{C}$
2: R = N = C = S

Column chromatography of the ether soluble fraction of the acetonic extract of *Axinella cannabina* resulted in the isolation of **3**, as colourless oil $[\alpha]_D + 29{\cdot}0$, $n_D^{20°}$ 1·5180. The molecular formula, $C_{16}H_{25}N$, was deduced from mass spectrum and elemental analyses.

IR spectrum (λ_{max} 2140 cm^{-1}) coupled with the mass spectral data [intense ions at *m/e* 205 ($M^+ - NC$) and 204 ($M^+ - HCN$)] indicated the presence in **3** of an isonitrile function. Isonitrile **3** contains a secondary methyl group (δ 0·91, 3H, d, J = 6 Hz) and two tertiary methyl groups (δ 0·97, 3H, s and δ 1·02, 3H, s) as shown by its NMR spectrum, which suggests also the presence of another tertiary methyl group linked to the carbon atom bearing the isonitrile function (δ 1·27, 3H). This latter attribution was clearly confirmed by the splitting of the signal, which appears as a 1:1:1 triplet (J = 2·0 Hz) because of the long range $N^{14} - H^{1}$ coupling characteristic of the alkyl isonitriles.[3]

The NMR spectrum indicated also the presence of the hydrogens of the cyclopropyl group by a high field complex signal, spread between δ 0·8–0·4(2H).

The NMR assignments were confirmed by the spectrum performed in the presence of the shift reagent 2,2,6,6-tetramethylheptanedionate Praseodymium (III) (Prashift); in this case the methyl group on C-4 resonates at δ 0·73 while the CH_3 linked to C − 10 was at δ 0·47; finally the remaining methyl groups also were shifted to high field (δ 0·95 and 0·84).



All these data suggested that the compound under investigation was a sesquiterpenoid isonitrile possessing a tricyclic carbon skeleton. In order to obtain additional information on the structure of **3**, this compound was dehydrogenated in the presence of selenium, thus obtaining guaiazulene (**4**) in relatively high yields.

This result indicated for **3** a perhydroazulene skeleton bearing methyl groups on C-4 and C-10; furthermore it is evident that the ciclopropyl group

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must be linked to C-6 and C-7 or to C-7 and C-8. Aromadendrane, the well known terpenoid carbon ring system, can meet these requirements.

The assignments of 3 to this ring system was made based on the Birch reduction of axisonitrile-2, which afforded in relatively high yields an oily product with molecular formula $C_{15}H_{26}$ (mass spectrum and elemental analysis) which migrates as a single spot on SiO_2 chromatography. This was identified as aromadendrane (6) by comparison of its spectral (IR, NMR and MS) and chromatographic (SiO_2 and $SiO_2/AgNO_3$) properties with those of a sample synthetized by catalytic hydrogenation of aromadendrene (5).

GLC experiences, performed also with capillary column, confirmed this identification and revealed that both products were a mixture of two compounds, which must be evidently the two epimeric dihydroaromadendrenes differing for the stereochemistry at C-10.

This result indicated also that the isonitrile function must be linked to C-10, as it can be deduced from the fact that the Birch reduction of 3 afforded the two dihydroaromadendrenes epimers at this carbon atom.

From the above reported data we can conclude also that, except for the configuration at C-10, the stereochemistry of axisonitrile-2* must be identical to that of aromadendrene.[4]

EXPERIMENTAL

The IR spectra (CCl_4 solns) were recorded on a Perkin–Elmer 157 Spectrophotometer. The NMR spectra were determined on a Perkin–Elmer R12A Spectrometer in CCl_4 solns using TMS as internal reference ($\delta = 0$). The mass spectra were taken on an AEI MS 902 instrument. The optical rotation of 3 was 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 were effected using glass packed precoated silica gel F_{254} plates (E. Merck). TLC on $SiO_2/AgNO_3$ were performed on glass packed precoated SiO_2 F_{254} plates sprayed with 30% $AgNO_3$ in Me_2CO-H_2O (1:1) and dried at 120° for 2h.GLC's were run using a Perkin–Elmer F30 instrument with columns 2m × 0·4 cm (flow of N_2 30 ml/min); for the experiences with capillary column a C. Erba Fractovap GV chromatograph was used.

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 axisonitrile-*2 (3) *from the sponge* Axinella cannabina. 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 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 (15 g), which was chromatographed on a SiO_2 (900 g) column using benzene as eluent. Fractions of 750 ml were collected. Fractions 3–5 (3·6 g) were rechromatographed on a SiO_2 column (210 g, eluent: 40–70° light petroleum-benzene 85:15). Fractions of 200 ml were collected; fractions 13-21, on evaporation, afforded 200 mg of 3; $n_D^{20°}$ 1·5180; $[\alpha]_D + 29{\cdot}0$ (*c*, 0·5; $CHCl_3$); M^+ 231 *m/e*; the significant features of IR and NMR spectra are reported in the general part. For the experience in the presence of Prashift (Pierce) a solution of 3 (25 mg) and Prashift (15 mg) in CCl_4 was used (Found C, 83·21; H, 10·94; N, 5·90. Calc. for $C_{16}H_{25}N$ C, 83·05; H, 10·89; N, 6·05%).

Dehydrogenation of 3. A mixture of 62 mg of 3 and 120 mg of Se was heated at 280° for 30 min. After cooling, 40–70° light petroleum was added and the filtered soln was extracted with 60% aq H_2SO_4. The acid phase was diluted with water and extracted with 40–70° light petroleum. After washing with H_2O the organic phase was taken to dryness to give 9 mg of guaiazulene, identified by comparison of its spectral (UV, IR, NMR) and chromatographic (TLC using as eluents 40–70° light petroleum and 40–70° light petroleum-benzene 8:2) properties with those of an authentic specimen.

Catalytic hydrogenation of aromadendrene (5). 5 (200 mg, isolated from Eucalyptus globulus oil according to L. Dolejš *et al.*[5]) in MeOH (30 ml) was overnight hydrogenated over 10% Pd/C (20 mg) at 45° and 3 atm. After removal of the catalyst by filtration, the soln was evaporated to dryness and the residue was chromatographed on a 3:1 $SiO_2/AgNO_3$ column (10 g) using as eluent 40–70° light petroleum. Fractions of 10 ml were collected; fractions 2–3 were evaporated to dryness to give 80 mg of an oily residue which migrates as a single spot on TLC (SiO_2 and $SiO_2/AgNO_3$; eluent: n-hexane). GLC (10% DC 550 on Chromosorb W at 120°, 150° and 170°; 20% DEGS on Chromosorb W HMDS at 60°, 70° and 90°; 8% Carbowax 1540 on Chromosorb W AW-DMCS at 70°, 80° and 100°; 2·5% SE 30 on Chromosorb W at 90° and 110°; capillary column 2·5% SE 52 on carbon black, length 14 m. at 110° and 130°) revealed the presence of two peaks with very similar retention times.

Reduction of 3 *with* Na/NH_3. To a stirred soln of 3 (100 mg) in Et_2O (10 ml) and liquid NH_3 (20 ml) at −45°, Na (30 mg) was added in 2 hr. 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 40–70° light petroleum. The organic phase was dried over $CaSO_4$ and evaporated to dryness to give 60 mg of an oily residue, which was chromatographed on a 3:1 $SiO_2/AgNO_3$ column (2·5 g) using as eluent 40–70° light petroleum; fractions of 3 ml were collected. Fractions 2–3, taken to dryness, gave 50 mg of an oily product (Found C, 87·09; H, 12·54. Calc. for $C_{15}H_{26}$ C, 87·30; H, 12·70%) with the same spectral (IR, NMR, MS) and chromatographic (in the same experimental conditions reported above for the catalytic hydrogenation product of aromadendrene) properties of those of the catalytic hydrogenation product of aromadendrene.

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*Because the relative abundances of the two epimers obtained both from the catalytic hydrogenation of aromadendrene and from the Birch reduction of 3 are different, it cannot be excluded from the $[\alpha]_D$ values that the compounds deriving from 3 are enantiomers of the hydrogenation products of aromadendrene.

REFERENCES

Inter alia G. Cimino, S. De Stefano, L. Minale and E. Fattorusso, *Tetrahedron* **28**, 267 (1972); G. Cimino, S. De Stefano, L. Minale and E. Fattorusso, *Ibid.* **28**, 333 (1972); F. Cafieri, E. Fattorusso, C. Santacroce and L. Minale, *Ibid.* **28**, 1579 (1972); E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Ibid.* **28**, 5993 (1972); E. Fattorusso, L. Minale and G. Sodano, *J. Chem. Soc.* Perkin Trans **I**, 16 (1972); K. Moody, R. H. Thomson, E. Fattorusso, L. Minale and G. Sodano, *Ibid.* **18**, (1972); E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Gazz. Chim. It.* **104**, 409 (1974)

[2] F. Cafieri, E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Tetrahedron* **29**, 4259 (1973)

[3] I. D. Kuntz, P. Von R. Schleyer and A. Allerhand, *J. Chem. Phys.* **35**, 1533 (1961)

[4] G. Büchi, W. Hofheinz and J. V. Pankstelis, *J. Am. Chem. Soc.* **88**, 4113 (1966)

[5] L. Dolejš, O. Motl, M. Souček, V. Herout and E. Sŏrm, *Coll. Czech. Chem. Comm.* **25**, 1483 (1960)