SPECIALIA

Minor sesquiterpenoids from the sponge Axinella cannabina[1]

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Summary. On the basis of chemical and spectral evidence, the structure **IV** has been assigned to Axisonitrile-4, an axane-sesquiterpenoid isonitrile, isolated from the sponge *Axinella cannabina;* in the same organism have been also found Axisothiocyanate-4 (**V**) and Axammide-4 (**VI**).

In a previous paper[2], we reported the isolation and structure elucidation of Axisonitrile-1 (**I**) and Axisothiocyanate-1 (**II**), two sesquiterpenoids with a novel skeleton (axane), from the marine sponge *Axinella cannabina*. More recently[3] we also isolated from the same marine organism Axamide-1 (**III**) which differs from **I** in having a NHCHO group instead of the isonitrile function.

From a further investigation of the extracts of the sponge *Axinella cannabina*, we have now found three novel strictly related axane-sesquiterpenoids, Axisonitrile-4 (**IV**), Axisothiocyanate-4 (**V**), and Axamide-4 (**VI**).

The extraction of the fresh material (1 kg, dry weight after extraction), was carried out as previously described[2]. The crude extract (14.8 g) was chromatographed on a SiO_2 column, thus obtaining three fractions (A, B and C).

Axisonitrile-4 (**IV**). Fraction B was further chromatographed on silica gel (eluent 40–70° light petroleum) and successively on SiO_2-PLC (eluent 40–70° light petroleum-C_6H_6 7:3, R_f 0.48, visualized by heating a thin strip of the plate sprayed with a 5% ceric sulphate in a 10% acqueous H_2SO_4), to give a crystalline product (**IV**, 154 mg), $C_{16}H_{23}N$ (from accurate mass measurement[4] on the molecular ion at m/e 229.1832; $C_{16}H_{23}N$ requires: 229.1830), m.p. 56–58°, $[\alpha]_D$ + 51.4 (c 1, $CHCl_3$).

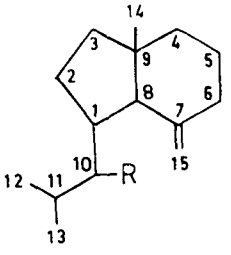
Both IR[5] (ν_{max} 3020, 1640 and 893 cm^{-1}) and NMR[6] spectra (δ 4.62, bs) indicate that a $>C=CH_2$ group is present in **IV**. A further feature revealed by NMR is the presence of a tertiary methyl group (δ 1.03, 3H, s) and two vinylic methyls (δ 1.67 and 1.89, 6H, singlets).

Ir spectrum (ν_{max} 2055 cm^{-1}), coupled with mass spectral data (absence of fragment arising from molecular ion by loss of HCN), agrees with the presence in **IV** of a vinylic isonitrile function.

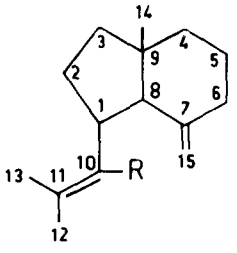
The above data, considering the structure of Axisonitrile-1 (**I**), led us tentatively to assign the structure **IV** to the compound under investigation. This was confirmed by Na/NH_3 reduction of **IV**, which afforded **VIII**, identified by comparing its properties (IR, NMR, MS, co-GLC, $[\alpha]_D$) with those of an authentic sample[2].

Axisothiocyanate-4 (**V**). Fraction A was shown, by GLC, to contain a complex mixture, from which, after reaction with $MeNH_2$ in chloroform at room temperature and subsequent chromatography on SiO_2 (eluent C_6H_6-ether 9:1, R_f 0.45, UV light), we isolated **VII**, clearly deriving from **V**.

VII (amorphous solid), $[\alpha]_D$ + 54.7, ν_{max} 3360 cm^{-1}, δ 3.03 (3H, d, J = 5.5 Hz, collapsed to a singlet by D_2O exchange), 1.0 (3H, s), 1.64 (3H, s), 1.72 (3H, s), 4.58 (2H, m) has molecular formula $C_{17}H_{28}N_2S$ (accurate mass measurement). Its spectral data indicated a structural analogy with **IV**, as proved. **IV**, by treatment with sulphur at 120 °C, yielded **V**, M^+ 261, n_D 1.5493, $[\alpha]_D$ – 35.9 (c 1.2, $CHCl_3$), ν_{max} 2096 cm^{-1}, δ 0.98 (3H, s), 1.60 (3H, s), 1.78 (3H, 1), 4.61 (2H, m). **V**, after reaction with $MeNH_2$ in chloroform, afforded the corresponding thiourea, which was revealed as identical with **VII** by comparison of their properties. The isolation of **VII** pointed to the presence of **V** in the initial mixture; this was proved by GLC



I: R=N≡C
II: R=N=C=S
III: R=N–C–H (H, O)
VIII: R=H



IV: R=N≡C
V: R=N=C=S
VI: R=N–C–H (H, O)
VII: R=N–C–NCH$_3$ (H, S, H)

[1] This investigation was supported by a grant of the Consiglio Nazionale delle Ricerche, Rome (NA 75.00770.03).

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

[3] E. Fattorusso, S. Magno, L. Mayol, C. Santacroce and D. Sica, Tetrahedron *31*, 269 (1975).

[4] Mass spectra and accurate mass measurements were obtained with an AEI MS 902 spectrometer by using the direct inlet technique.

[5] IR spectra were recorded on a Perkin-Elmer 157 instrument in CCl_4.

[6] NMR spectra were taken in CCl_4 on a Perkin-Elmer R 32 apparatus (internal reference TMS); s denotes a singlet, d a doublet, m a multiplet, b broad.

[7] GLC's were run using a Perkin-Elmer F 30 instrument with glass column 2 m × 0.4 cm, on 2.5% SE30.

experiments performed on the fraction A, using as a reference a sample of **V**, prepared as above.

Axamide-4 (**VI**). The occurrence of isonitriles in marine sponges, has generally been accompanied by the corresponding isothiocyanates and formamides, and this was considered to be evidence of the strict biogenetic relationship between these three derivatives. Unfortunately our attempts to isolate **VI** were abortive, probably due to the very small quantities of Axamide-4 present in the sponge. However, co-GLC experiments, performed on the fraction C, revealed the presence of a product with the same retention time of a synthetic sample of Axamide-4 [M^+ 247, m.p. 81–84°, $[\alpha]_D$ +63,3, ν_{max} 3380 to 3170, 1683, 1655 cm^{-1}; δ 0.97 (3H, s), 1.67 (3H, s), 1.73 (3H, s), 4.56 (2H, m)], obtained by treatment of **IV** with acetic acid in anhydrous ether, in the previously described experimental conditions[3].

Diketopiperazines containing L-proline from Streptomyces lavendulae and their stereochemistry in solution

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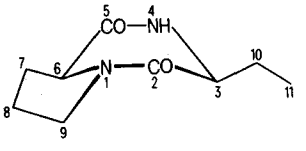
Summary. L-Valyl-L-proline (**I**), L-leucyl-L-proline (**II**), L-phenylalanyl-L-proline (**III**) and L-isoleucyl-L-proline (**IV**) anhydrides were isolated from the cultures of *Streptomyces lavendulae* No. 314 and, on the basis of ^{13}C-NMR and CD spectra, their stereochemistry in solution is described.

In the previous communication[6], we have described the isolation of novel antitumor antibiotics, Chlorocarcin A, B and C from the culture filtrate of *Streptomyces lavendulae* No. 314. We report here the isolation of several diketopiperazines[7] containing L-proline (**I-IV**) as companion substances, and we will discuss their stereochemistry in solution on the basis of ^{13}C-NMR and CD spectra.

The companion substances (**I-IV**) were isolated from the crude basic components by column chromatography over silica gel along with Chlorocarcins having potent antitumor activity. However, four crystalline compounds (**I-IV**) did not exhibit inhibitory activity against experimental L1210 lymphocytic leukemia. These optically active substances (**I-IV**) were shown to have the molecular formula: I, $C_{10}H_{16}O_2N_2$ (M^+, 196) m.p. 186.5–187.5 °C; II, $C_{11}H_{18}O_2N_2$ (M^+, 210) m.p. 159–161 °C; **III**, $C_{14}H_{16}O_2N_2$ (M^+, 244) m.p. 134–134.5 °C; IV, $C_{11}H_{18}O_2N_2$ (M^+, 210) m.p. 124–127 °C, $[\alpha]_D^{20}$ −167.2 °C (c, 0.67, $CHCl_3$) and were identified as L-valyl-L-proline (**I**)[8–12], L-leucyl-L-proline (**II**)[8–11,13–17], L-phenylalanyl-L-proline (**III**)[8,11] and L-isoleucyl-L-proline (**IV**) anhydrides respectively. Among them, **IV** was a novel diketopiperazine and its structure was elucidated by the examination of mass[18] and ^{1}H-NMR spectra and the direct comparison of the synthetic material prepared from L-proline and L-isoleucine. This is, to the best of our knowledge, the first time **IV** has been isolated from *Streptomyces* sp. or indeed from any natural source.

There has been considerable interest in the stereochemistry of diketopiperazine ring through ^{1}H-NMR (in solution)[19–22] and X-ray crystallographic (in the solid state)[18,23–25] studies. As to proline derivatives, glycyl-L-proline[18] and L-leucyl-L-proline (**II**)[24] anhydrides were recently shown to adopt the bowsprit boat conformation by X-ray analysis, and L-valyl-L-proline anhydride (**I**)[12] assumed the same conformation in solution by NMR studies. Then we further examined the ^{13}C-NMR and CD

^{13}C-NMR chemical shifts (δ^{TMS}) of diketopiperazines (**I–III**) in $CDCl_3$[a]



bowsprit boat

DKP	Number of carbon (off resonance)									
	2 (s)	3 (d)	5 (s)	6 (d)	7 (t)	8 (t)	9 (t)	10	11	$\underline{C}H_3$ (q)
I	170.41	58.78	165.08	60.47	28.50	22.27	45.01	28.50 (d)		16.03,19.02
II	170.54	53.58	166.38	59.04	28.11	23.31	45.53	38.64 (t)	24.61 (d)	21.36,22.79
III	169.43	56.31	165.07	59.04	28.30	22.46	45.33	36.82 (t)		
Leucine[26]		55.5						45.2 (t)	25.2 (d)	22.3,23.5

[a] ^{1}H-Noise decoupled and off resonance ^{13}C-NMR spectra were taken with Jeol FX-60 FT-NMR spectrometer operating at 15.0 MHz.