# Molliorin-B, a second scalarin-like pyrroloterpene from the sponge Cacospongia mollior[1]

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*Summary.* On the basis of chemical and spectral evidence, structure **IV** was assigned to molliorin-b, a scalarin-like pyrroloterpene present in the marine sponge Cacospongia mollior. Structure **IV** was confirmed by synthesis.

Very recently[2] we reported the isolation and structure determination of molliorin-a (**I**), the first scalarin-like pyrroloterpene, present in the marine sponge Cacospongia mollior. The biosynthesis of **I** was also thought to take place from a C-25 terpenoid intermediate (e.g. scalaradial[3], **III**) by reaction with 2-methylbutylamine, probably deriving from isoleucine by loss of carbon dioxide.

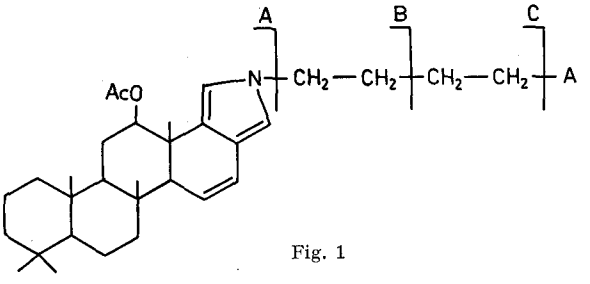


Fig. 1

From a further investigation of the extracts of the same sponge, we have now isolated molliorin-b (**IV**), another pyrroloterpene which appears to be formed by a biosynthetic process very similar to that hypothesized for **I**. Repeated silica-gel chromatography of the ether-soluble extract of the fresh material[4] resulted in the isolation of **IV** as a colourless solid, which was recrystallized from 80–100° light petroleum; m.p. 173–174 °C; $[\alpha]_D$ + 14.6° (c 1.2 in $CHCl_3$). Elemental analysis[5] combined with mass spectrum[6] ($M^+$/e 872) indicated the molecular formula $C_{58}H_{84}N_2O_4$; **IV**[6] shows $\lambda_{max}$ 257, 263 and 275 nm ($\varepsilon$ 27663, 28264 and 17440) and $\nu_{max}$ 1735 and 1240 $cm^{-1}$.

The NMR-spectra of **IV** and **V** (m.p. 124–126 °C; obtained by alkaline hydrolysis of **IV**) are strongly reminiscent of those of **I** and **II** respectively (table).

This is indicative for a close relationship between molliorin-a and -b and hints at the dimeric structure of molliorin-b, the 2 halves containing the partial structure A

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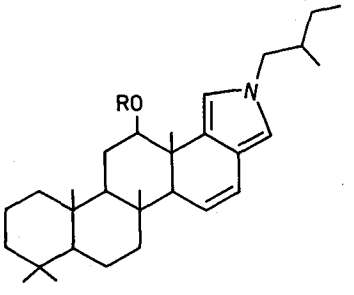
4 The sponge Cacospongia mollior was collected in the bay of Taranto (May 1975) and identified by Prof. M. Sarà (Università di Genova, Italia).

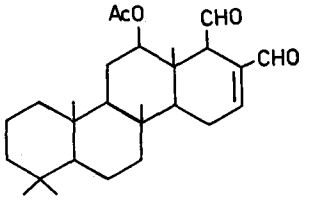
5 Found C 79.2%; H 10.0%; N 3.1%; $C_{58}H_{84}N_2O_4$ requires C 79.6%; H 9.9%; N 3.2%.

6 UV spectra were recorded in cyclohexane using a Perkin-Elmer 402 instrument. IR spectra were taken in $CCl_4$ on a Perkin-Elmer 157 instrument. Mass spectra were obtained with an AEI MS 902 spectrometer using the direct inlet technique.

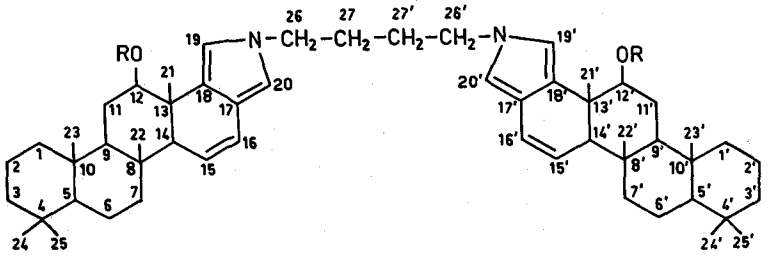



**I** R=Ac

**II** R=H




**III**




**IV** R=Ac

**V** R=H

$^1$H-NMR spectra[a]) of **I**, **II**, **IV** and **V**

| Proton position | I[b]) | II[b]) | IV | V |
|---|---|---|---|---|
| $CH_3CO$ | 1.90 (s) | | 1.86 (s) | |
| H-C(12) | 5.31 (m) | 4.04 (m) | 5.29 (m) | 4.10 (m) |
| H-C(14) | 2.43 (bm) | 2.42 (bm) | 2.39 (bm) | 2.46 (bm) |
| H-C(15) | 5.45 (dd, 9.5, 3) | 5.53 (dd, 9.5, 3) | 5.45 (dd, 10, 3) | 5.57 (dd, 10, 3) |
| H-C(16) | 6.28[c]) | 6.26[d]) | 6.27[c]) | 6.28[d]) |
| H-C(19) | 5.94 (bs) | 6.21[d]) | 5.93 (bs) | 6.21[d]) |
| H-C(20) | 6.22[c]) | 6.32[d]) | 6.22[c]) | 6.32[d]) |
| H-C(21) | 1.25 (s) | 1.01–0.85 (s) | 1.25 (s) | 1.03–0.87 (s) |
| H-C(22)–H-C(25) | 1.02–0.85 (s) | | 1.02–0.85 (s) | |
| H-C(26) | 3.57 (m) | 3.72 (m) | 3.65 (bt, 6) | 3.70 (bt, 6) |
| H-C(29) | 1.02–0.85 | 0.58 (t, 6)[e]) | | |
| H-C(30) | | 0.64 (d, 7)[e]) | | |

[a]) Run at 90 MHz on a Perkin-Elmer R 32 apparatus in $CDCl_3$, using TMS as internal standard. Values are in ppm ($\delta$-scale). Multiplicities are indicated by the usual symbols. Figures in parentheses are coupling constants in Hz. Assignments were confirmed by decoupling; [b]) Added for comparison (see [2]); [c]) H-C(16) and H-C(20) overlap; [d]) H-C(16), H-C(19) and H-C(20) overlap; [e]) In $C_6D_6$.

(figure 1). The presence of a $C_4$-saturated chain joining the 2 pentacyclic units A through the nitrogen atoms was deduced from the NMR-spectrum of **IV** which shows a 4 H broad triplet at $\delta$ 3.65 [$H_2$–C(26), $H_2$–C(26′)].

All these data indicate that molliorin-b is represented most favourably by formula **IV**. This was confirmed by its mass spectrum in which the following peaks are present: 872, $M^+$; 857, $M^+$–$CH_3$; 812, $M^+$ –$CH_3COOH$; 797, $M^+$–$CH_3$ COOH–$CH_3$; 752, $M^+$ –2 $CH_3COOH$; 737, $M^+$ –2 $CH_3COOH$–$CH_3$; 464, A; 437, B–H; 404, A–$CH_3COOH$; 377, B–$CH_3COOH$–H (figure 1).

Definite proof for the proposed structure **IV** was provided by comparison of its properties (IR, NMR, UV, m.p., $[\alpha]_D$) with those of a synthetic sample obtained by reaction of 1,4-diaminebutane with an excess of scalaradial (**III**) (at 60 °C for 5 min), followed by addition of conc. $H_2SO_4$ (at 60 °C for 5 min.) and chromatography on PLC ($SiO_2$, eluent benzene/40–70° light petroleum 17:3). It seems reasonable that scalaradial may be a precursor of both molliorin-a and -b; in addition, the central $C_4N_2$ unit of molliorin-b could derive from ornitine as previously hypothesized for aerothionin[7], a dimeric metabolite present in the sponge Verongia aerophoba.

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## Dissociation of obesity, hypercholesterolemia and diabetes from atherosclerosis in ob/ob mice

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*Summary.* Genetically obese, diabetic and hypercholesterolemic C57BL/6J-ob/ob mice were placed on Purina Laboratory Chow containing 2% cholesterol for up to 4 months. They developed higher plasma cholesterol levels and accumulated an increased quantity of cholesterol in the liver but failed to develop atherosclerotic lesions in the aorta as would be expected in an obese, diabetic and hypercholesterolemic human adult.

The C57BL/6J-ob/ob mice are known to have genetic hypercholesterolemia in addition to being obese[2] and are being used in many laboratories as a model for adult-onset diabetes. Feeding high levels of cholesterol to normal mice has not resulted in aortic atherosclerosis. It is not known, however, if atherosclerosis can be induced in ob/ob mice which have spontaneous hypercholesterolemia. This communication reports an attempt to induce atherosclerotic lesions in this possibly susceptible genotype of mice using high cholesterol diet.

*Materials and methods.* Male C57BL/6J-ob/ob mice and normal mice (+/+) from the Jackson Laboratory, Bar Harbor, Maine, were 4–6 months of age at the beginning of the experiment. 9 mice per genotype, evenly distributed according to age, were fed either Purina Laboratory Chow or Purina Laboratory Chow containing 2% cholesterol. The diet containing cholesterol was prepared by mixing cholesterol with Purina Chow powder and repelletizing the mixture. The regular diet was processed in the same manner to assure the same consistency as the cholesterol diet.

Mice were housed 3 to a cage in transparent, polycarbonate cages covered with filter bonnets (Filtek, Appleton, Wisconsin). They were fed one of the above 2 diets and water ad libitum. The ambient temperature in the animal room was maintained at about 25 °C. The photoperiod was controlled to provide light from 06.00 h–18.00 h and dark from 18.00 h–06.00 h.

3 mice of each group were sacrificed at the end of 2, 3 and 4 months of treatment, respectively. Blood was collected in heparinized tubes. Total cholesterol in plasma was assayed according to the method of Glick, Fell and Sjølin[3].

1 Acknowledgments. We wish to thank Mr. Willis M. Overton for his excellent technical assistance.

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