Molliorin-c, a further pyrroloterpene present in the sponge *Cacospongia mollior*[1]

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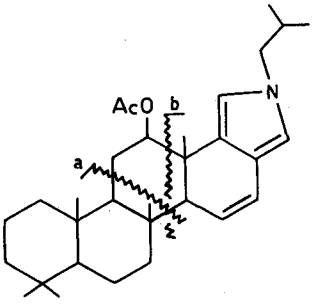
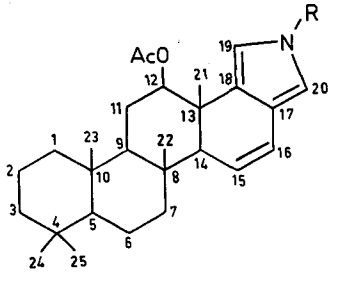
Summary. A new pyrroloterpene, molliorin-c (**III**), has been isolated from the sponge *Cacospongia mollior*. Structure **III** was assigned to molliorin-c on spectral grounds and confirmed by synthesis.

The marine sponge *Cacospongia mollior* was previously shown to contain 2 scalarin-like pyrroloterpenes, molliorin-a (**I**)[2] and molliorin-b (**II**)[3]. It was also hypothesized that these compounds originate biogenetically from mevalonate and an amino acidic precursor.

We now report the occurrence in the same marine organism of a further pyrroloterpene component, molliorin-c (**III**), which differs from molliorin-a only in the aliphatic chain linked to the pyrrolic nitrogen.



Extraction of fresh material[4] (500 g, dry weight after extraction), carried out as previously described[5], and a subsequent combination of SiO_2 column and TLC, resulted in the isolation of a colourless amorphous solid (**III**) 105 mg, ν_{max} ($CHCl_3$)[6] 1735 and 1240 cm^{-1}, λ_{max} (EtOH)[7] 257 nm, ε 11900, $[\alpha]_D$ −46.9 °C (c 0.9; $CHCl_3$).



I $R = CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-CH_3$

III $R = CH_2-CH\langle^{CH_3}_{CH_3}$

High resolution mass spectrometry[8] of **III** established the formula $C_{31}H_{47}NO_2$ (found M^+ 465.3602; $C_{31}H_{47}NO_2$ requires 465.3607), with major ions at m/e 450 (M^+-CH_3), 423 ($M^+ -C_3H_6$), 405 ($M^+ -CH_3COOH$), 390 ($M^+ -CH_3 -CH_3COOH$), 363 ($M^+ -CH_3COOH -C_3H_6$), 191 (fragment a −H), 188 (fragment b + H) (figure).

The 90 MHz ^{1}HNMR-spectrum[9] showed the following resonances: δ 0.63 (6 H, d, J 7 Hz), 0.84 (3H, s), 0.96 (3H, s), 1.08 (3H, s), 1.27 (3H, s), 1.90 (3H, s), 3.12 (2H, d, J 7 Hz), 5.51 (1H, m, H–C_{12}), 5.68 (1H, dd, J 10 and 2 Hz), 6.27 and 6.32 (1H each, bs, H–C_{19} and H–C_{20}), 6.75 (1H, dd, J 10 and 3 Hz, H–C_{16}).

The fragment ions at 423 and 363 m/e in the mass spectrum of **III** indicated the presence of a C_4 unit

1 This investigation was supported by a grant of the Consiglio Nazionale delle Ricerche, Rome.
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4 Sponges, collected in the bay of Taranto, were obtained by Stazione di Biologia Marina del Salento, Porto Cesareo, Italy.
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6 IR-spectra were recorded on a Perkin-Elmer 157 instrument.
7 UV-spectra were recorded using a Perkin-Elmer 402 instrument.
8 Mass spectra and accurate mass measurements were obtained with an AEI MS 902 spectrometer, using the direct inlet technique.
9 NMR-spectra were taken in C_6D_6 on a Perkin-Elmer R 32 apparatus (internal reference TMS, multiplicities are indicated by the usual symbols).
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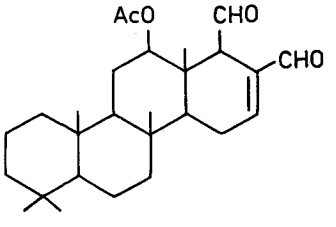
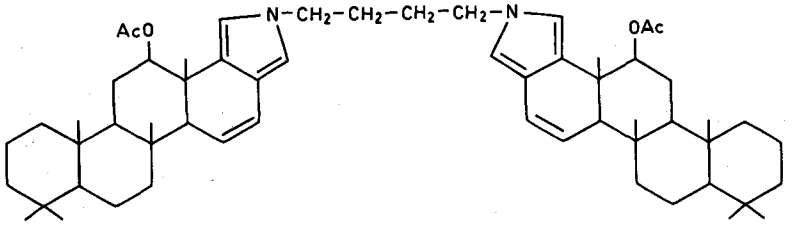


IV



II

linked to the pyrrolic nitrogen[10]. This unit was shown to be $CH_2CH(CH_3)_2$, on the basis of spin-decoupling experiments: irradiation at δ 1.79 simplified the doublet at δ 3.12 into a singlet and caused collapse of the doublet at δ 0.63 to a sharp singlet.

The spectral data of **III**, considering those of molliorin-a, led us tentatively to assign structure **III** to the compound under investigation. Confirmatory evidence for this structure was provided by synthesis: condensation of scalaradial (**IV**)[11] with 2-methylpropylamine gave **III** in good yield, identical in all respects with the natural product.

Molliorin-c represents another example of mixed biogenesis and may be formally considered to be derived by a combination of a sesterterpenoid moiety and the isobuthylamine arising from valine by loss of CO_2.

The effect of γ-irradiation on soil enzyme stability*

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Summary. Arylsulphatase, β-1,3 glucanase, phosphatase and urease responded differently to γ-irradiation (5–50 Mrad) in air-dried and moist soils. In all instances phosphatase was the most stable. The variability between enzymes may be due to inherent biochemical and stuctural characteristics or to their location within the soil microenvironment.

The value of γ-irradiation in the study of soil microbiology and biochemistry has been discussed at length by Cawse[1] and McLaren[2]. A major *differential advantage* of this technique is that doses of ~ 5 Mrad can eliminate microbial proliferation whilst still allowing colloid-bound enzymes to function. Higher levels of irradiation will progressively denature soil enzymes. The protective characteristics of soil organic and inorganic colloids on extracellular enzymes has been reviewed by many[3–5], and includes resistance to temperature extremes, storage, and proteolysis as well as irradiation. There is little doubt that the accumulated and persistent soil enzyme fraction is crucial to the mineralization of organic matter. The work reported in this paper illustrates the differential stability of 4 soil enzymes when subjected to a range of γ-irradiation doses. In addition, some suggestions are made to account for the differences.

Materials and methods. A silt loam soil (< 2 mm) was used for all experiments. Its characteristics, fully described elsewhere[6], were: sand 16%; silt 64%; clay 20%; organic matter 5.4%; c.e.c. 14.8 mEq. 100 g soil^{-1}; pH 5.4; w.h.c. 0.72 ml · g soil^{-1}.

Soil samples (25 g) either air-dried or at field wetness (29% w.h.c.) were sealed in polyethylene bags and subjected to 5, 10, 15, 20 or 50 Mrad doses of γ-irradiation (approx. 4 Mrad · h^{-1}) at the AERE Harwell Fuel Pond Assembly. Prior and subsequent to irradiation soils were stored at 4 °C. Control soils were also sealed and refrigerated but did not receive irradiation treatment.

Arylsulphatase and phosphatase were assayed using p-nitrophenyl ester substrates[7]; β-1,3 glucanase using laminarin[8]; and urease after the method of Pettit et al.[9] but using 0.5 M tris-maleate buffer (pH 7.0) and adding 0.5 ml $AgSO_4$ (10 mM) to terminate the reaction. All the activities plotted in figures 1 and 2 at the means of at least 3 replicates, SD: arylsulphatase 2.5%; β-1,3 glucanase 6.5%; phosphatase 6.1%; urease 3.0%.

Results and discussion. From figures 1 and 2 it can be seen that in both the dry and wet soil phosphatase was the most resistent enzyme to γ-irradiation. From this data the levels of irradiation required to induce a 90% loss in activity were a) in the dry soil: phosphatase 48 Mrad; urease 19.5 Mrad; β-1,3 glucanase 18 Mrad; arylsulphatase 14 Mrad; b) in the wet soil: phosphatase 29 Mrad; β-1,3 glucanase 15 Mrad; arylsulphatase 9 Mrad; urease 7 Mrad. Following 50 Mrad treatment 7.5% of the phosphatase activity survived in the dry soil (next best was urease with 2.7%); 3.1% in the wet soil (all others zero). Ramirez-Martinez and McLaren[11] found that phosphatase was inactivated more rapidly in wet than dry soil and it is well known that the radio-sensitivity of microorganisms as well as enzymes generally increases in wet soil[12] due, in part, to the reactive free radicals (OH, H, HO_2) produced when water is ionized[13,14]. Skujins et al.[10] have described an inactivation coefficient (k) for enzymes: $N/N_0 = e^{-kD}$ where N = activity at irradiation dose D and N_0 = activity of nonirradiated soil. Using a graphical representation of equation (1) the computed values of k in dry soil were arylsulphatase 0.031 (correlation coefficient r = 0.99); β-1,3 glucanase, 0.020 (r = 0.96); phosphatase 0.009 (r = 0.99); urease 0.024 (r = 0.89). The equivalent values in wet soil were: arylsulphatase 0.049 (r = 1.0); β-1,3 glucanase 0.027 (r = 1.0); phosphatase 0.019 (0.97); urease 0.042 (0.99).

$$\log_{10}\frac{N}{N_0} = \frac{-k}{2.303} D \qquad (1)$$

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