Minor pyrroloterpenoids from the marine sponge *Cacospongia mollior*[1]

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Summary. 2 pyrroloterpenes, molliorin-d and -e, have been isolated from the sponge *Cacospongia mollior* and their structures have been established by chemical and spectroscopic methods.

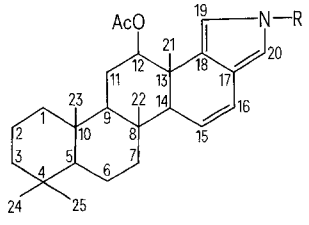
3 pyrroloterpenes with a pentacyclic skeleton, molliorin-a (**I**), -b (**II**) and -c (**III**), have recently been isolated from the marine sponge *Cacospongia mollior*[2-4]. Careful re-examination of the chromatographic pattern of the lipid extract of this sponge has shown the presence of a number of compounds with a positive (purple blue) Ehrlich test. We were not able to isolate all these compounds owing to the small quantities; only 2 of them, molliorin-d and -e, could be investigated.

This paper reports chemical and spectral evidence which led us to establish structures **IV** and **V** for molliorin-d and -e, respectively.

Material and methods. The sample of sponges (500 g, dry weight) collected in the Bay of Taranto (during autumn 1977), was repeatedly extracted following essentially the same procedure as previously reported[5]. The crude combined extracts were fractionated on silica gel column by elution with benzene. Fractions were tested by TLC and the eluates were appropriately recombined.

Subsequent purification on SiO_2-TLC afforded **IV**, $C_{35}H_{47}NO_2$ (31 mg, 40-70° light petroleum-Et_2O 4:1, R_f 0.51, M^+ 513.3603, $[\alpha]_D$ +11.6°, $\nu_{max}^{CCl_4}$ 1735 cm^{-1}, λ_{max}^{EtOH} 262 nm, ε 11760) and **V**, $C_{28}H_{41}NO_2$ (14 mg, 40-70° light petroleum-benzene 4:1, R_f 0.47, M^+ 423.3134, $[\alpha]_D$ +6.5°, $\nu_{max}^{CCl_4}$ 1738 cm^{-1}, λ_{max}^{EtOH} 263 nm, ε 11900). MS and NMR-data of molliorin-d and -e are reported in the table.

Results and discussion. The above data suggested the presence in **IV** and **V** of the pentacyclic skeleton previously found in molliorin-a, -b and -c. The nature of the chain

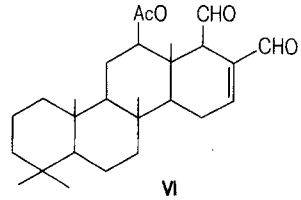


I R = CH_2–CH(CH_3)–CH_2–CH_3

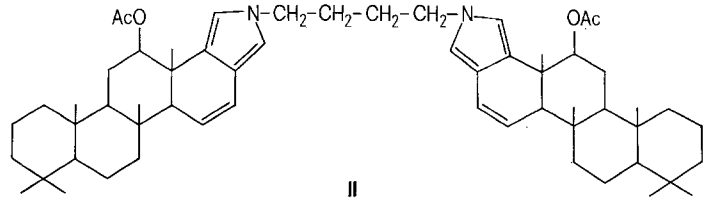
III R = CH_2–C(CH_3)(CH_3)

IV R = $^{26}CH_2$–$^{27}CH_2$–C$_6$H$_5$

V R = $^{26}CH_3$



VI



II

MS		
	IV	**V**
	513 (M^+), 423 ($M^+ - C_6H_5CH_2 + H$), 408 ($M^+ - C_6H_5CH_2CH_2$), 363 ($M^+ - C_6H_5CH_2 - CH_3COOH + H$), 348 ($M^+ - C_6H_5CH_2CH_2 - CH_3COOH$).	423 (M^+), 422 ($M^+ - 1$), 408 ($M^+ - CH_3$), 363 ($M^+ - CH_3COOH$), 348 ($M^+ - CH_3COOH - CH_3$).
NMR		
	IV[a]	**V**[b]
	0.87 [6 H, s] 1.05 [3 H, s] H–C(21)–H–C(25) 1.28 [6 H, s] 1.92 [CH_3CO, s] 2.98 [2 H, t, J 8 Hz, H–C(27)] 3.94 [2 H, t, J 8 Hz, H–C(26)] 5.31 [1 H, b, H–C(12)] 5.43 [1 H, m, H–C(15)] 5.99 [1 H, bs, H–C(19)] 6.23 [1 H, bs, H–C(20)] 6.30 [1 H, m, H–C(16)] 6.98-7.34 [5 H, m]	0.89 [3 H, s] 0.93 [6 H, s] H–C(21)–H–C(25) 1.15 [3 H, s] 1.28 [3 H, s] 1.95 [CH_3CO, s] 2.90 [3 H, s, H–C(26)] 5.48 [1 H, b, H–C(12)] 5.82 [1 H, m, H–C(15)] overlapped 5.87 [1 H, bs, H–C(19)] overlapped 6.17 [1 H, bs, H–C(20)] 6.33 [1 H, bs, H–C(16)]

[a] Run at 90 MHz on a Perkin Elmer R 32 apparatus in CCl_4. [b] Run at 270 MHz on a Brucker WH 270 apparatus in C_6D_6. Values are in ppm (δ-scale).

linked to the pyrrolic nitrogen was established taking into account the characteristic fragmentation of N-alkylpyrroles[6] and the signals in the NMR-spectra. In fact the fragment ion at M^+-1 and the 3H broad singlet at δ 2.90 are diagnostic for a N-methylpyrrole unit in **V**. Likewise the fragment ions at m/e 423 and 408, together with the signals at δ 6.98–7.34, 2.98 and 3.94 are indicative for a $C_6H_5CH_2CH_2$-unit linked to the nitrogen atom in **IV**. The structures of **IV** and **V** were definitively established by their partial synthesis.

The reaction of scalaradial[7] (**VI**) with β-phenylethylamine hydrochloride and methylamine hydrochloride in ethanol at 60 °C for 20 min yielded **IV** and **V**, respectively (identified by comparison of their chromatographic and spectral properties).

The chains linked to the pyrrole ring in **IV** and **V** can be considered to derive from phenylalanine and glycine. This further supports the hypothesis previously reported[2-4] for the biogenetic origin of pyrroloterpenes from mevalonate and an amino acidic precursor.

1 This work has been carried out in the frame of the 'Progetto Finalizzato per l'Oceanografia e i Fondi Marini', C.N.R., Roma.
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Ontogenetic changes in relative levels of cyclic AMP-dependent and cyclic GMP-dependent protein kinases in prostates, epididymides and testes from guinea-pigs

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Summary. Changes in realtive levels of cyclic AMP-dependent protein kinase (A-PK) and cyclic GMP-dependent protein kinase (G-PK) in prostates, epididymides and testes from guinea-pigs were examined at 3 different ages. During postnatal development, a decrease in the ratio of the 2 classes of protein kinases was seen in prostates, whereas increases of the ratios of the enzymes were found in epididymides and testes.

Several lines of recent evidence have suggested that adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) may independently mediate opposing effects of certain physiological agents in the lungs[3] and other tissues[4], presumably via the phosphorylation activity of cyclic AMP-dependent and cyclic GMP-dependent protein kinases respectively[5,6]. Kuo has shown that cyclic GMP-dependent protein kinase exists in high levels in some mammalian tissues[7], and that the changes in relative levels of the 2 classes of protein kinases occur in the lung, heart and brain of developing guinea-pigs[8]. Here we report our observations on the changes of these 2 kinases in male reproductive system during the course of postnatal sexual maturation.

Materials and methods. (γ-^{32}P) ATP was purchased from New England Nuclear. Cyclic GMP and cyclic AMP were obtained from Boehringer, Mannheim (BRD); arginine-rich histone (HA) was from Worthington.

Male guinea-pigs of 3 different ages (7 days old, weighing 80–150 g; 30 days old, weighing 400–550 g; and 100 days old, weighing 950 to 1200 g), were used. Testes, epididymides and prostates were dissected immediately after decapitation of the animals and homogenized in 3 volumes of 50 mM potassium phosphate buffer (pH 7.0) with glass-Teflon homogenizers. The homogenates were centrifuged for 20 min at 30,000 × g, and the supernatant solutions were used as the sources of the cytosol enzymes. The pellets were resuspended in the original volume of the same buffer. To

Age-related changes in the estimated ratios of cyclic AMP-dependent to cyclic GMP-dependent protein kinase activity in cytosols and particulates of prostates, epididymides and testes from guinea-pigs

Age (days)	(A-PK/G-PK) ratio Cytosol Prostate	Epididymis	Testis	Particulate Prostate	Epididymis	Testis
7	1.53 ± 0.14	2.89 ± 0.24	4.26 ± 0.61	2.34 ± 0.26	4.02 ± 0.75	3.93 ± 1.02
30	1.02 ± 0.08[a]	4.02 ± 0.26[b]	9.46 ± 1.10[c]	1.82 ± 0.13	5.78 ± 0.41	6.66 ± 0.85
100	0.81 ± 0.04[c]	9.11 ± 0.58[c]	14.52 ± 1.67[c]	1.22 ± 0.16[a]	8.29 ± 0.94[b]	7.98 ± 0.89[a]

Assay conditions were as described in the text. The (A-PK/G-PK) ratio is defined as: (activity in the presence of cyclic AMP - basal activity): (activity in the presence of cyclic GMP - basal activity). The means (± SE) of the values obtained from 3 to 4 animals for each group were shown. Significantly different from the 7-day-old animals. [a] $p<0.05$; [b] $p<0.02$; [c] $p<0.01$.