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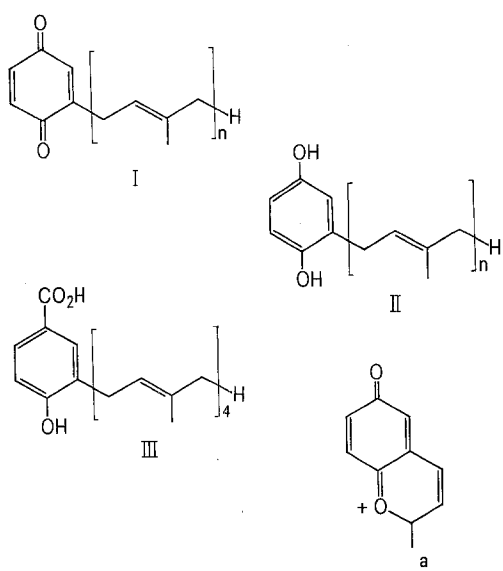
Prenylated Quinones in Marine Sponges: *Ircinia sp.*

In a previous paper[1], the occurrence in the marine sponge, *Ircinia spinosula*, of the unsubstituted prenylated benzoquinones (I; $n = 6, 7, 8$), a novel group of terpenoid quinones, and the corresponding quinols (II; $n = 6, 7, 8$), present in much larger quantities, was reported. In pursuing our chemical investigations on the metabolites of Porifera, we have now investigated another species, *Ircinia muscarum*[2], from which 2-tetraprenyl-1,4-benzoquinone (I; $n = 4$), the corresponding quinol (II; $n = 4$) and 4-hydroxy-3-tetraprenylbenzoic acid (III) have been isolated.



Fresh material (300 g, dry weight after extraction) was extracted with acetone and subsequently with methanol; the combined extracts were concentrated, and the remaining aqueous solution was extracted with ether to give, after removal of solvents, oily material (45 g), which was chromatographed on a silica gel column (100 g, Merck). Elution with benzene gave, first *all-trans*-squalene, which was rechromatographed in light petroleum (b.p. 40–70°) on silica gel and identified (90 mg) by direct comparison with an authentic specimen, and then 2-tetraprenyl-1,4-benzoquinone (I; $n = 4$) (0.8 g) which was further purified by preparative TLC (Merck precoated silica gel F_{254} plates) in benzene. Further elution of the column with benzene : ether (9:1) afforded the quinol II ($n = 4$) (16.5 g), m.p. 47–48°, after crystallization first from light petroleum (b.p. 80–100°) and then from cyclohexane. Finally, elution with benzene : ether (8:2) gave 4-hydroxy-3-tetraprenylbenzoic acid (III) (5.2 g), further purified by chromatography on Merck precoated silica gel F_{254} plates in benzene : ether (8:2) followed by crystallization from methanol at –20°; III had m.p. 61–63°.

The structures of the quinone, quinol and the benzoic acid were mainly deduced from their UV-, NMR-, IR- and mass spectra[3] and comparison with higher isoprenologues.

2-Tetraprenyl-1,4-benzoquinone. The UV-(λ_{max} 245, 315 and 440 nm; log ε 3.26, 2.13 and 1.55) and IR-(ν_{max} 1660, 1600 cm^{-1}) spectra indicated a benzoquinone structure; the NMR-spectrum showed signals from 3 quinonoid protons (2H broad singlet at δ 6.64 and 1H broad singlet at δ 6.43), while in the mass spectrum the fragmentation pattern [ions at M^+-69-(68)n, $n = 0$ to 2; base peak at *m/e* 161 due to ion $a^{1,4}$] pointed to the presence of a polyprenyl chain, the length of which was deduced from the molecular ion at *m/e* 380. The chemical shifts of the methyl groups suggested that they are *all-trans* (6H singlet at δ 1.64 for *trans*-Me on the first isoprene unit counting from the ring and *cis*-Me of the terminal isoprene unit; 9H singlet at δ 1.58 for 3 *trans*-Me)[5], except for the *cis*-methyl group at the end of the chain.

1,4-Dihydroxy-2-Tetraprenylbenzene. The UV- (λ_{max} 293 nm, log ε 3.64), IR- (ν_{max} 3350, 1500, 1445, 910, 785 and 730 cm^{-1}) and NMR- (3H multiplet at δ 6.46) spectra are consistent with a monosubstituted –1,4-quinol structure, which was confirmed by oxidation with silver oxide,

[1] G. Cimino, S. De Stefano and L. Minale, Tetrahedron *28*, 1315 (1972).

[2] The sponge collected in the bay of Naples was obtained from the supply department of the Zoological Station (Naples) and identified by Professor M. Sarà (University of Genova), to whom the authors express their thanks.

[3] UV-spectra were recorded in cyclohexane solutions (unless otherwise indicated) with a Bausch and Lomb Spectronic 505 spectorphotometer. IR-spectra were determined in nujol with a Perkin-Elmer 257 Infracord spectrophotometer. NMR-spectra were recorded in CCl_4 solutions on a Varian HA-100 apparatus operating at 100 MHz with TMS as internal standard. Mass spectra were recorded on an A.E.I. MS-9 spectrometer. Melting points, taken on a Kofler block, are uncorrected. All compounds gave satisfactory elementary analyses.

[4] B. C. Das, M. Loveasmaa, C. Tendille and E. Lederer, Biochem. biophys. Res. Commun. *21*, 318 (1965).

[5] A. Langemann and O. Isler, in *Biochemistry of Quinones* (Ed. R. O. Morton; Academic Press, London and New York 1965), p. 134.

to a quinone identical in all respects with I ($n = 4$). The mass spectrum showed, as expected, the molecular ion at *m/e* 382, ions at *m/e* M^+-69-(68)n ($n = 0$ to 2) and the base peak at *m/e* 123 [dihydroxytropylium ion]. Ozonolysis yielded acetone, characterized as its 2,4-dinitrophenylhydrazone and malonic and levulinic acids, identified as their methyl esters by GLC (5% SE-30 and 10% DEGS at 100° and 175°, respectively) by direct comparison with authentic samples.

4-Hydroxy-3-tetraprenylbenzoic acid. It showed UV- [λ_{max} (MeOH) 257 nm, log ε 4.1; λ_{max} (MeOH + OH^-) 283 nm, log ε 4.24] and IR- (ν_{max} 3420, 1670 and 1600 cm^{-1}) spectra indistinguishable from those of 4-hydroxy-3-octaprenylbenzoic acid isolated from mutant strains of *E. coli* by Cox et al.[6]. The NMR-spectra are also very similar; in the aromatic region signals from 3 protons were observed, 2 of which occurred at relative low field (δ 7.89, m) and are therefore assigned to the deshielded protons *ortho* to the carboxyl group. The 3rd signal appeared as a doublet at δ 6.81 ($Jo = 8$ Hz) which further supports the location of the prenyl chain at C-3. The rest of the spectrum is very similar to that of (II; $n = 4$) at higher field indicating the *all-trans* configuration for the side chain. The mass spectrum of 4-hydroxy-3-tetraprenylbenzoic acid showed the molecular ion at *m/e* 410 and the expected sequential loss of isoprene units; an intense ion at *m/e* 151, attributed to a carboxyhydroxytropylium ion[6], is also observed. Ozonolysis of III afforded acetone, malonic and levulinic acids.

The co-occurrence of 4-hydroxy-3-tetraprenylbenzoic acid, 2-tetraprenyl-1,4-benzoquinone and 2-tetraprenyl-1,4-dihydroxybenzene in *Ircinia muscarum* strongly suggests that *p*-hydroxybenzoic acid is the ring precusor as in ubiquinone biogenesis. Furthermore these quinones and quinols could conceivably be the precursors of ubiquinones in sponges.

The biosynthesis of ubiquinones has been extensively investigated[7], mainly in *Rhodospirillum rubrum*, and 2 biosynthetic pathways from *p*-hydroxybenzoic, neither of which involve 2-polyprenyl-1,4-benzoquinones, have been proposed[8,9]. More recently WHISTANCE et al.[10], after a tentative identification of a quinone fraction in *Pseudomonas ovalis* as 2-polyprenyl-1,4-benzoquinones and incorporation[11] of *p*-hydroxy [U-^{14}C] benzoic acid in this fraction, suggested that an alternative pathway involving 2-polyprenyl-1,4-benzoquinones could be operative in that organism.

However we could not detect ubiquinones in *I. muscarum* (45 g of extract has been worked) and could only found ubiquinone-10[12] (ca. 1 mg from 12 g of extract) in *I. spinosula* which contains I and II ($n = 6,7$ and 8) (the absence of Q-6, Q-7 and Q-8 was established by reverse phase chromatography and comparison with authentic samples[12]).

Although further work is necessary, it seems that in the sponges the unsubstituted prenylated quinones are probably not biogenetically related to ubiquinones. Furthermore the possibility that ubiquinone-10 is derived from an external origin (e.g. symbionts) cannot be excluded.

Riassunto. Viene descritto l'isolamento del 2-tetraprenil-1,4-benzochinone, del corrispondente idrochinone e dell'acido 4-idrossi-3-tetraprenilbenzoico dalla spugna *Ircinia muscarum*. Il rinvenimento di quest'ultimo suggerisce che il precursore dell'anello dei chinoni prenilati non sostituiti e dei loro corrispondenti idrochinoni, ritrovati nelle spugne, sia l'acido *p*-idrossibenzoico, come nel caso degli ubichinoni. Vengono, inoltre, riportate alcune considerazioni sulle possibili relazioni biogenetiche nelle spugne tra gli ubichinoni e questi insoliti chinoni.

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[6] G. B. COX, I. G. YOUNG, L. M. MCCANN and F. GIBSON, J. Bact. *99*, 450 (1969).
[7] D. R. THRELFALL and G. R. WHISTANCE, in *Aspect of Terpenoid Chemistry and Biochemistry* (Ed. T. W. GOODWIN; Academic Press, London and New York 1971), p. 357.
[8] G. D. DAVES JR., P. FRIIS, R. K. OLSEN and K. FOLKERS, Vitams Horm. *24*, 427 (1966).
[9] P. FRIIS, J. L. G. NILSON, G. D. DAVES JR. and K. FOLKERS, Biochem. biophys. Res. Commun. *28*, 234 (1967).
[10] G. R. WHISTANCE, J. F. DILLON and D. R. THRELFALL, Biochem. J. *111*, 461 (1969).
[11] G. R. WHISTANCE, B. S. BROWN and D. R. THRELFALL, Biochem. J. *117*, 119 (1970).
[12] Ubiquinone-10 has been identified by direct comparison with an authentic sample kindly given by Dr. R. AZERARD (University of Paris, F-91 Orsay, France), who also supplied samples of Q-6 and Q-9.

Optical Rotatory Dispersion and Antibacterial Activity of the Macro-Ring Analogues of Gramicidin S

We have synthesized a series of cyclopeptides with rings smaller than the 30-membered one found in gramicidin S (GS), but none of them showed antibacterial activity[1]. For example, we found that the 15-membered semigramicidin S synthesized possesses no activity[2]. In order to determine further the influence of a ring size on the activity, we synthesized a 45-membered sesquigramicidin S and a 60-membered digramicidin S. We wish to report also their antibacterial properties together with the conformations by optical rotatory dispersion (ORD).

Synthesis. Boc-Val-Orn(δ-Z)-Leu-D-Phe-Pro-$NHNH_2$ (VI)[3] was prepared in 90% yield by treatment of Boc-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OEt[4] with hydrazine hydrate. Condensation of the azide using isoamylnitrite derived from VI with H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH[4] gave Boc-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_2$-OH (VII), 83%, which was converted to H-(Val-Orn (δ-Z)-Leu-D-Phe-Pro)$_2$-OH (VIII), 87%, by the action of formic acid on VII. Condensation of the azide derived from VI with VIII gave Boc-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_3$-OH (IX), 80%, which was converted to an amorphous Boc-pentadecapeptide N-hydroxysuccinimide ester (X) by the action of N-hydroxysuccinimide and dicyclo-

[1] T. KATO and N. IZUMIYA, *Chemistry and Biochemistry of Amino Acids, Peptides, and Protein* (Ed. B. WEINSTEIN; Marcel Dekker, New York), vol. 2, in press.
[2] M. WAKI and N. IZUMIYA, J. Am. chem. Soc. *89*, 1278 (1967).
[3] Satisfactory elemental analyses and chromatographic data were obtained for all crystalline compounds described here. Boc, t-butyloxycarbonyl; Z-, benzyloxycarbonyl. Amino acid symbols except D-Phe denote the L-configuration.
[4] M. OHNO and N. IZUMIYA, J. Am. chem. Soc. *88*, 376 (1966).