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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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-

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[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.
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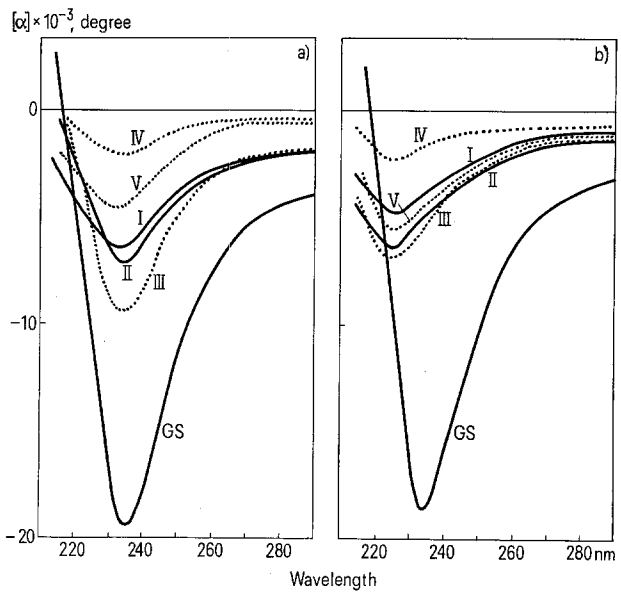
hexylcarbodiimide[5] on IX. The Boc group of (X) was removed by the action of trifluoroacetic acid, the mixture was evaporated, and the residual pentadecapeptide ester trifluoroacetate (XI) was treated with a large amount of pyridine for 2 h at room temperature. The similar treatment[6] of the residue gave cyclo-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_3$ (XII), 47% from IX, mp 128–130°. Hydrogenation of XII yielded sesquiGS·3HCl·8H$_2$O (I) as an air-dried crystalline product, 76%, mp 163–165° (dec), $[\delta]_D^{20}$–33.2° (EtOH). H-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_3$-OH (XIII) was prepared in 90% yield from IX with formic acid. Condensation of the azide derived from VI with XIII gave Boc-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_4$-OH (XIV), 82%, and the cyclization reaction of XIV gave cyclo-(Val-Orn(δ-Z)-Leu-D-Phe-Pro)$_4$ (XV), 50%, from XIV, mp 153–155°. Its hydrogenation yielded diGS·4HCl·9H$_2$O (II), 85%, mp 205–207° (dec), $[\alpha]_D^{20}$-51.6° (EtOH). Open-chain analogue, H-(Val-Orn-Leu-D-Phe-Pro)$_3$-OH·4HCl·8H$_2$O (IV), related to sesquiGS was prepared by hydrogenation of XIII, 74%. The hydrogenation of an amorphous compound, which was obtained by the action of hydrogen chloride in ethyl acetate on XIV, yielded H-(Val-Orn-Leu-D-Phe-Pro)$_4$-OH·5HCl·1OH$_2$O (V), 70%. H-(Val-Orn-Leu-D-Phe-Pro)$_2$-OH·3HCl·6H$_2$O (III) was already prepared in this laboratory[7].

Inhibitory activity of compounds on microorganisms

Compound	Minimum inhibitory concentration (μg)	
	Staphylococcus aureus	*Bacillus subtilis*
semiGS	>100	>100
GS	5	5
I (sesquiGS)	50	50
II (diGS)	20	10
H-Val-Orn-Leu-D-Phe-Pro-OH	>100	>100
III (decapeptide)	50	50
IV (pentadecapeptide)	50	50
V (eicosapeptide)	20	20
GS+I[a]	5	5
GS+II[a]	5	2
GS+III[a]	5	5
GS+IV[a]	5	5
GS+V[a]	5	5

[a] Each mixture is composed of 1:1 (by weight).



ORD of the cyclic and open-chain peptides. Solvent, a) ethanol and b) 8 *M* urea; I, sesquiGS; II, diGS; III, decapeptide; IV, pentadecapetide; V, eicosapeptide.

Structure activity relationship. We reported previously the shapes of ORD curves of GS and several analogues[8]. In this experiment, the ORD curves measured in a solvent of ethanol are shown in Figure a). The macro-ring analogues (I, II) and the open-chain analogues (III–V) have similar shaped curves with a negative trough at 232 nm as GS possesses the same. In a solvent of 8 *M* urea or 1% sodium dodecyl sulfate[9], the troughs of I–V were moved to 225 nm whereas that of GS remained constant, see Figure b). These results indicate that the conformation of the macro-ring analogues is similar to that of the open-chain analogues whereas that of GS is very stable; HODGKIN and OUGHTON[10] suggested that GS has the rigid β-pleated sheet structure having an antiparallel tripeptide sequence with 4 hydrogen bondings[1].

The antibacterial properties of the macro-ring analogues were very similar to that of the corresponding open-chain analogues (Table). For both the analogues, similar synergistic effects with GS were observed. ERLANGER and GOODE[11] observed already that the open-chain decapeptide (III) possesses weaker activity than GS itself but exhibits a synergistic effect when III is combined with GS. The activity of a cyclic (I or II) or open-chain peptide (III, IV or V) increased with an increase in its molecular size. In conclusion, the results presented suggest that a cyclic character in a pentadeca- or eicosapeptide containing a sequence of GS is not essential to exhibit an activity, whereas in a decapeptide sequence of GS, the cyclic character is important to form the rigid structure and consequently to exhibit a strong and specific activity.

Zusammenfassung. Sesquigramicidin S (zyklisches Pentadekapeptid) und Digramicidin S (zyklisches Eikosapeptid) wurden nach den konventionellen Methoden der Peptid-Synthese hergestellt. Die ORD-Messungen dieser makrozyklischen Analoga und offenkettigen Analoga (lineare Pentadeka- und Eikosapeptide) zeigten, dass die Konformation der makrozyklischen Analoga derjenigen der offenkettigen Analoga ähnlich ist. Es wird auch gezeigt, dass die antibakteriellen Eigenschaften der makrozyklischen Analoga denen der entsprechenden offenkettigen Analoga gleichen.

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