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SELECTIVE N-METHYLATION OF PEPTIDE BOND. PREPARATION AND PROPERTIES OF [MeOrn$^{2,2'}$,D-MePhe$^{4,4'}$]GRAMICIDIN S

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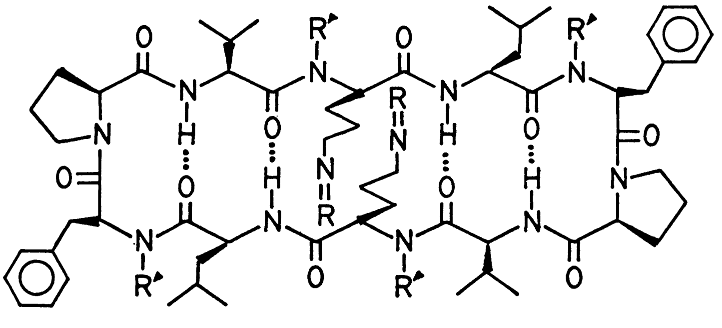
Diphthaloyl derivative of gramicidin S was subjected to selective N-methylation using CH_3I–Ag_2O in DMF. Methylation occurred exclusively at ornithine and D-phenylalanine residues affording tetra-N-methylated product in high yield. Removal of the phthaloyl groups furnished the title compound, which showed essentially the same antimicrobial activity as gramicidin S itself.

N-Methyl analogs of biologically active peptides often exhibit enhanced potencies or prolonged activities.$^{1)}$ Such analogs are usually prepared by peptide synthesis using protected N-methylamino acids as building blocks. In this communication an attempt is described to prepare N-methylated peptide analogs by modifying parent peptide molecules.

We have already reported selective methylation of peptide NH's with CH_3I–Ag_2O in DMF, by which conformational analysis of submicromolar amount of peptides can be achieved.$^{2a)}$ Application of this method to some derivatives of gramicidin S (**1**) and its analogs were performed successfully, where rate of N-methylation of the NH's which were exposed to solvent were much higher than that of intramolecularly H-bonded NH's.$^{2)}$ This selective methylation seems useful for synthesizing new peptide analogs in preparative scale. Thus it was attempted to prepare an analog of **1** in which α-NH's of Orn and D-Phe residues are methylated keeping intramolecularly H-bonded NH's of Val and Leu intact.

To the solution of diphthaloylgramicidin S (**2**, mp 308–310 °C; 0.03 mmol) in DMF (1 ml) were added CH_3I (40 mmol) and Ag_2O (1 mmol) and the mixture was stirred for 4 h at room temperature. Methanol was added to the reaction mixture and insoluble



	R	R'
1	H_2	H
2	Pht	H
3	Pht	Me
4	H_2	Me

(Pht = phthaloyl)

material was filtered off. After evaporation of the solvent the residue was taken to $CHCl_3$ and was subjected to SiO_2 column chromatography ($CHCl_3$–MeOH) to afford diphthaloyltetramethylgramicidin S (3, mp 171–173 °C, yield 88%). From experiments under various conditions it was shown that the tetramethyl product 3 was obtained in 80–90% yield when 0.03 mmol of 2 in 1 ml of DMF was treated with 16–40 mmol of CH_3I and 1–2 mmol of Ag_2O for 4–20 h at room temperature. ^1H NMR spectrum of 3 showed presence of two kinds of N-methyl groups ($\delta_{\mathrm{DMSO\text{-}d6}}^{270\ \mathrm{MHz}}$ = 3.02 and 3.28 at 80 °C) and amino acid analysis of the acid hydrolyzate indicated exclusive N-methylation of the Orn and D-Phe residues (Val:Orn:Leu:Phe:Pro=1.00:0.00:0.95:0.00:1.00). Thus the structure of 3 was established as cyclo(-Val-MeOrn(Pht)-Leu-D-MePhe-Pro-)$_2$. Anal: C, 64.93; H, 7.09; N, 11.56% ($C_{80}H_{104}N_{12}O_{14}\cdot H_2O$).

Removal of the phthaloyl groups of 3 with $H_2NNH_2\cdot H_2O$ afforded the desired tetramethyl derivative of gramicidin S, cyclo(-Val-MeOrn-Leu-D-MePhe-Pro-)$_2$ (4). Though ^1H NMR spectrum of 4·2HCl (mp 200.5–203 °C) recorded in DMSO-d_6 solution at 25 °C showed broad peaks suggesting equilibrium of multiple conformers, measurement at elevated temperature gave a spectrum assignable to a single conformer with C_2-symmetry. The spectrum showed presence of two kinds of N-methyl groups as well as absence of NH groups belonging to Orn and D-Phe residues. From decoupling and NOE experiments at 80 °C the conformation was assumed to be similar to that of 1[3)] having four intramolecularly H-bonded NH's of Val and Leu residues.

The tetramethyl analog 4 showed essentially the same antimicrobial activity as parent 1 (Table 1), as expected from conformational similarity of the two compounds. This result indicated that amide NH groups of Orn and D-Phe residues do not play any important role in binding of 1 to receptor molecules for manifesting its activity. Syntheses and antimicrobial activities of analogs of 1 having N-methylvaline and N-methylleucine residues were reported by Izumiya and coworkers.[4)] Preparation of other analogs of 1 and also application of this methylation to the preparation of analogs of linear peptides are in progress.

Table 1. Antimicrobial Activity of Tetramethylgramicidin S (4) and Gramicidin S (1)

	Minimum inhibitory concentration / ug·ml^{-1}	
Microorganism	4·2HCl	1·2HCl
Staphylococcus aureus FDA 209 PJC-1	1.56	1.56
Staphylococcus aureus MS 353	3.1	3.1
Bacillus subtilis ATCC 6633	1.56	1.56
Escherichia coli NIHJ-JC-2	100	50
Escherichia coli K 12 C 600	50	50

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