# THE CONFORMATION OF GRAMICIDIN S AND ITS N,N'-DIACETYL DERIVATIVE IN SOLUTIONS

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Summary. Conformational study of gramicidin S and N,N'-diacetyl gramicidin S has been carried out by a number of techniques. Optical rotatory dispersion measurements showed both cyclodecapeptides to possess similar conformations in different solvents. Quantitative infra red studies in dilute chloroform solutions lead to the inference that there are ca. six intramolecular hydrogen bonds in the N,N'-diacetyl derivative. Of these deuterium exchange rates by proton magnetic resonance measurements indicate four strong H-bonds due to the Val and Leu NH groups and two weaker bonds due to the Orn $\alpha$-NH groups. The NH-$C^{\alpha}H$ proton coupling constants show the protons of this fragment to be trans in Val, Orn and Leu residues and gauche in D-Phe residues. The data obtained provide unequivocal proof of the Hodchkin-Oughton-Schwyzer model of gramicidin S, for which $\Phi$ and $\Psi$ coordinates are given.

A large number of papers has appeared in recent years on the conformation of gramicidin S (GrS) (Fig 1)[1], a cyclodecapeptide antibiotic whose relative simplicity and high biological activity has made it especially attractive as a probe for evaluating approaches to the secondary structure of peptides.

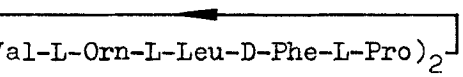


Fig. 1. Gramicidin S

However, despite of the work done insufficiency of experimental data has not permitted unequivocal choice between the over a dozen of different structures that have been proposed for the antibiotic[2-26].

Hodgkin and Oughton[5] have proposed β-pleated sheet model of GrS as one of the most probable structures being in accord with X-ray diffraction data[3]. This structure was supported by Schwyzer on the basis of chemical[6,7] and indirect NMR[21,22] data. However, neither of these studies defined it in terms of atomic coordinates or rotation about $N-C^{\alpha}$ and $C^{\alpha}-C'$ bonds. Other models subsequently proposed for GrS whilst also of the β-pleated sheet type differed essentially from each other[15,24]. The β-pleated sheet model of GrS usually involves four strong intramolecular hydrogen bonds (IMHB) formed by the NH and CO of the Val and Leu residues. Indeed, it was demonstrated that Val and Leu protons are less prone to undergo exchange with deuterium[24] or tritium[26] than other NH protons. However, Momany et al.[15] noted that slow deuterium exchange is not necessarily due to hydrogen bonding and could also be the result of the screening of free NH groups buried in the interior of the GrS molecule. This could also explain the comparatively weak temperature dependence of the Val and Leu NH chemical shifts found in GrS by Ohnishi and Urry[27]. Moreover, the $\Phi$ and $\Psi$ values proposed for the β-model of GrS by Stern et al.[24] in their valuable paper were largely based on their interpretation of stereochemical dependence of the $^{3}J_{NH-CH}$ constant that soon after[28,29] was shown to be inaccurate so that the conformation proposed by the American authors required critical re-examination.

Another conformation of GrS containing two α-helical segments was deduced by Liquori et al.[10] from analysis of the conformational maps. However the similarity of ORD and CD parameters of GrS and α-helical polypeptides noted by several authors [16-20] could not be regarded as strong evidence in favour of the α-helical conformation of the former because similar curves

have been displayed also by non-helical peptides and amides[30, 31]; the NMR data which had been forwarded as consistent with the α-helical conformation[23] have been found to contain several errors[24,25]. Other models of GrS are either purely speculative [8,9] or have been deduced theoretically[12-15] and have as yet received no experimental backing.

The present paper describes the conformation of GrS established by the approach used earlier for structural study of the membrane active cyclodepsipeptide antibiotics valinomycin[32] and enniatin B[33], based on the joint use of several independent physicochemical methods. In order to facilitate interpretation of the spectral data and the physicochemical measurements in non polar solvents N,N'-diacetyl gramicidin S (AGrS) was studied as well as GrS. AGrS was prepared in 81% yield by acetylation of GrS with N-hydroxysuccinimide acetate,

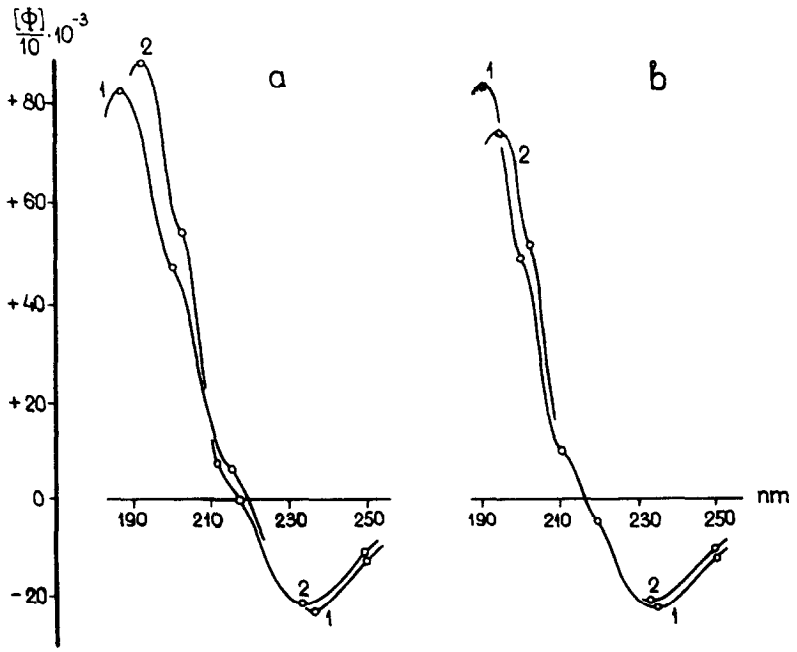


Fig. 2. ORD curves of gramicidin S (a) and N,N'-diacetyl gramicidin S (b) in 12:1 heptane-ethanol (1) and 2:3 ethanol-0.1 N HCl (2) mixtures

m. p. 305-6°, $[\alpha]_D^{25}$ -302° (c 1.5, 70% EtOH) (cf.[4]).

Attention is first directed to the close similarity and comparative rigidity of the interacting amide chromophoric system which follows from the similar and very little solvent dependent ORD curves of GrS and AGrS (Fig. 2). The IR spectra of AGrS measured in $CHCl_3$ (25°, $1.06 \times 10^{-4}$ mole/l) display a strong band at 3314 $cm^{-1}$ (hydrogen-bonded NH) and a weaker band at 3426 $cm^{-1}$ (free NH). The H-bonding is intramolecular as follows from dilution experiments and molecular weight measurements (at concentrations $17.4 \times 10^{-3}$, $6.3 \times 10^{-3}$, $3.2 \times 10^{-3}$ and $1.4 \times 10^{-3}$ mole/l experimental molecular weights are correspondingly 5504, 2592, 1852 and 1247; molecular weight of AGrS monomer is 1224). Calculation of integral stretching band intensities according to Ramsay[35] and correlation of the values obtained with the IR spectra of compounds containing only free or only H-bonded groups NH reveal ca. six H-bonded NH groups (Table 1).

NMR studies of AGrS in different solvents revealed a striking differentiation of NH groups according to their deuterium exchange rates (Table 2), indicating that of the six IMHB

Table 1. Integral NH stretching band intensities (A, $M^{-1} \cdot l \cdot cm^{-1}$) in AGrS and model compounds

| Compounds | Free NH groups | | | | H-Bonded NH groups | | | |
|---|---|---|---|---|---|---|---|---|
| | $\nu_{N-H}$, $cm^{-1}$ | $\Delta\nu_{1/2}$, $cm^{-1}$ | $A \cdot 10^{-4}$ | Number of NH groups* | $\nu_{N-H}$, $cm^{-1}$ | $\Delta\nu_{1/2}$, $cm^{-1}$ | $A \cdot 10^{-4}$ | Number of NH groups* |
| MeCO-NHCHCO-$NMe_2$ (Me) | 3418 | 37.5 | 1.42 | 1.00 | No bonded NH groups | | | |
| Valinomycin + $C_{12}H_{25}SO_3K$ | No free NH groups[35] | | | | 3309 | 66 | 31.82 | 6.00 |
| AGrS | 3426 | 31.0 | 4.46 | 3.14 | 3314 | 107 | 27.63 | 5.29 |

*Relative to the model compound

Table 2. Half life times of NH groups at 25°

| Amino acid residue | Solvent | |
|---|---|---|
| | $CDCl_3$-$CD_3OD$ (2:1) | $CD_3OD$ |
| L-Val | 25 days | 15 days |
| L-Orn ($\alpha$) | 48 hrs | 30 min |
| L-Orn ($\delta$) | 2.5 hrs | 30 min |
| L-Leu | 25 days | 15 days |
| D-Phe | 2.5 hrs | 5-10 min |

found by IR four strong H-bonds are formed by Val and Leu NH groups and two weaker H-bonds, unstable in $CD_3OD$, by Orn $\alpha$-NH (analogous results in $CD_3OD$ were obtained for GrS by Stern et al.[24]). The NMR-$^1H$ spectra of the peptide groups of AGrS display non-overlapping doublets with the following $^3J_{NH-CH}$ values: Val 10.0 hz, Orn 9.6 hz, Leu 10.0 hz*. As was recently shown[28, 29] large $^3J_{NH-CH}$ values may correspond to both cis and trans arrangement of protons in NH-$C^{\alpha}H$ fragments. However, maximum $^3J_{NH-CH}$ values found in the literature for compounds with predominant cis conformation do not exceed 8.0-8.9 hz[28,29,32,36] thus leading to the inference that NH-$C^{\alpha}H$ protons in the valine, ornithine and leucine residues are trans ($\Phi$=60±15 for L-Val and L-Leu and 60±20 for L-Orn). The value of 4.1 hz* for $^3J_{NH-CH}$ constant of the D-Phe residues assuming conformational rigidity of AGrS (see ORD curves) corresponds to the gauche conformation, i. e. to four possible $\Phi$ coordinates: ca. 70, 170, 245 or 355.

The results obtained in this work and by other authors allow determination of the following parameters of GrS and AGrS:

*Corrected for electronegativity of the substituents (+0.6 hz[28]). Solvent $CH_3OH$, temperature 25-43°, concentration ~0.07 mole/l. More detailed account of the NMR work will be given elsewhere.

a) the presence of a dyad axis[5];

b) trans-configuration of the secondary amide groups (from relative intensities of amide I and amide II bands in the IR spectra of GrS[11]);

c) simultanious participation of Val and Leu NH groups in IMHB;

d) trans-orientation of the NH-$C^{\alpha}H$ protons in the Val, Orn and Leu residues;

e) gauche-orientation of the NH-$C^{\alpha}H$ protons in D-Phe residues.

Theoretical analysis and examination of molecular models with account of possible cis and trans tertiary amide bonds formed by the Phe and Pro residues and of all plausible combinations of the H-bonds showed that only one conformational type (Fig. 3) of the cyclodecapeptide chain of the antibiotic satisfies the above requirements. This structure has the following $\phi$ and $\psi$ values*:

| | L-Val | L-Orn | L-Leu | D-Phe $\omega=0$ | L-Pro |
|---|---|---|---|---|---|
| $\phi$ | 60 | 70 | 60 | 235 | 120 |
| $\psi$ | 300 | 290 | 290 | 70 | 140 |

It is of the $\beta$-pleated sheet type proposed by Hodgkin, Oughton and Schwyzer and excludes all other models proposed for GrS; the $\phi$ and $\psi$ values given by Stern et al.[24] also differ markedly from ours. The characteristic features of the established conformation are rigidity, location of the ornithine side chains on one side of the mean plane of the ring; such separation of usually protonated $\delta$-amino groups from the polypeptide backbone is probably essential for manifestation of biological activity. The facile preparation of N,N'-carbonyl GrS (by

*Possible deviations from the listed mean angles in some cases reaching 15-20° do not lead to any considerable distorsion of the proposed structure.

treating GrS with N,N'-carbonyldiimidazol, yield 79%, m. p. 303-4°; $[\alpha]_D^{25}$ -305°, c 0.03, EtOH), a bycyclic derivative of GrS with Orn side chains bridged by carbamide linkage is in accord with the proposed conformation; the spectral characteristics of this compound (ORD curves, IR spectra, $^3J_{NH-CH}$ values, deuterium exchange rates) are similar to those of GrS and AGrS.

Contrary to valinomycin, enniatin B and the related cyclodecapeptide antamanide[37,38], GrS and AGrS do not possess an internal cavity capable of accomodating metal cations, which accounts for their non-ability to form complexes with the latter.

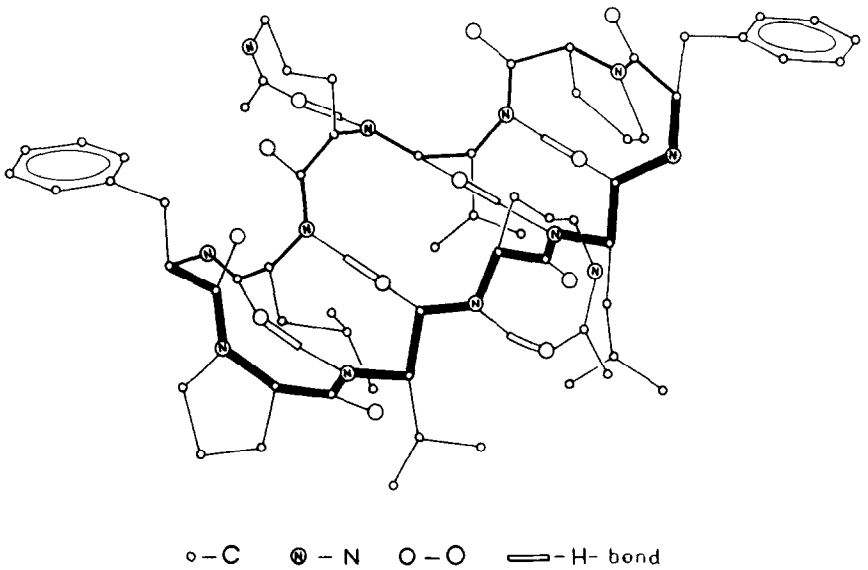


Fig. 3. Conformation of N,N'-diacetyl gramicidin S in non polar solvents

Comparatively unstable IMHB of Orn α-NH groups of AGrS in non-polar solvents are due to their interaction with the CO groups of the acetyl ornithine side chains; as seen on Fig. 3 their formation is accompanied by stabilization of two nine-membered rings.

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