Vol. 81, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1978 Pages 481-485

# SHIELDING EFFECTS OF THE D-PHE AROMATIC RING IN THE $^1$H NMR SPECTRUM OF GRAMICIDIN S

I. D. Rae and H. A. Scheraga
Department of Chemistry, Cornell University, Ithaca, New York 14853

Received December 22,1977

SUMMARY: Analysis of the side-chain coupling constants for the D-Phe residue of gramicidin S shows that the rotamer predicted for the minimum-energy structure is the predominant one. The aromatic ring of D-Phe shields the Pro $C^{\delta}$ hydrogens, and the calculated shielding closely matches that found in the $^1$H NMR spectrum. As the temperature is raised, the rotamer population changes and the shielding is reduced accordingly. The shielding is absent in the saturated analogue of gramicidin S in which the aromatic rings were hydrogenated without changing the conformation of the rest of the molecule.

The cyclic decapeptide antibiotic gramicidin S, cyclo(Pro-Val-Orn-Leu-D-Phe)$_2$, has $C_2$ symmetry and gives rise to relatively simple hydrogen (1) and carbon (2) NMR spectra. A calculated minimum-energy structure (3) incorporating this symmetry condition is in close agreement with that deduced from NMR studies (1), particularly in that it predicts the D-Phe-Pro β-turn and the Val-Orn-Leu β-sheet structures in the molecule. The calculated structure also predicts preferred conformations for the amino acid side chains in gramicidin S, and we report here some NMR studies of the side chain of the D-Phe residue and its relationship to the Pro residue which follows it.

## MATERIALS AND METHODS

Pure gramicidin S dihydrochloride (15.4 mg) was dissolved in 100% DMSO-$d_6$ (Aldrich) (0.35 ml), and tetramethylsilane was added (∿0.1%). $^1$H NMR spectra were recorded at 10° intervals, from 23° to 83°C, in the pulse-FFT mode on a Brűker HX-270 instrument at the National Magnet Laboratory, Cambridge, Mass. The instrument parameters were such that the precision of measurement was 0.005 ppm for chemical shifts. Coupling constants were measured from expanded traces and are believed to be accurate to ±0.1 Hz.

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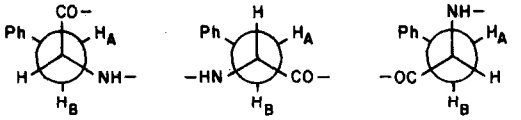
Abbreviations: NMR, nuclear magnetic resonance; DMSO-$d_6$, deuterated dimethyl sulfoxide.

0006-291X/78/0812-0481$01,00/0

Gramicidin S dihydrochloride (118 mg) was hydrogenated in glacial acetic acid (50 ml) using platinum oxide catalyst (1.06 g) and hydrogen gas at 750 p.s.i. The filtered solution was evaporated and all acetic acid was removed with a vacuum oil pump. The product was a glass which gave a single spot on thin layer chromatograms and showed no aromatic hydrogen signal in its NMR spectrum. The NH resonances closely resembled those reported for this compound by other workers (4). Hydrolysis in 6 N HCl for 96 hours, followed by amino acid analysis, gave the following result: Pro, 0.87; Val, 0.93; Orn, 0.73; Leu, 1.00; Phe, 0.00; β-cyclohexyl-Ala, 0.94. A sample of 7.4 mg in 0.35 ml of 100% DMSO-$d_6$ (0.1% tetramethylsilane) was used for the NMR experiment.

## RESULTS AND DISCUSSION

The vicinal couplings in the side chain of the D-Phe residue in gramicidin S, given in Table I, are close to those (10.5 and 4.5 Hz) reported by Stern et al. (5) for this compound in $CD_3OD$ at 19°. Using the treatment of Pachler (6), we calculated the relative populations of the three side-chain rotamers (Fig. 1) to be as shown in Table I. The dominance of rotamer I, with $\chi^1 = 180°$, is more pronounced than in other Phe peptides (7,8), and this is also the rotamer ($\chi^1 = 179°$) predicted (3) by the calculation (Fig. 2). In this conformation, the D-Phe side chain is close to the proline ring and the Pro $C^\delta$ hydrogens are expected to be strongly shielded by the aromatic ring. Using the atomic coordinates of the calculated structure (3) and the ring-current model of Johnson and Bovey (9), we calculated that, in rotamer I, the nearer of the Pro $C^\delta$ hydrogens ($H_R$), which is 2.93 Å from the center of the aromatic ring, should experience shielding of 1.50 ppm, and the farther Pro $C^\delta$ hydrogen ($H_S$), 4.63 Å from the ring, 0.33 ppm. Since this shielding occurs only in rotamer I, i.e., in 74% of the population, the resultant shielding effects are 1.11 and 0.24 ppm. The Pro $C^\delta$ hydrogens have chemical shifts of 3.626 and 3.682 ppm, respectively, in trans-N-acetylprolinamide (10); thus, their chemical shifts in gramicidin S would be expected to be reduced by this shielding to 2.52 and 3.44 ppm. At 23° (Table II), we observe these signals at 2.486 and 3.580, in confirmation of the assignments of Wyssbrod and Gibbons (1), and quite close to the positions predicted by consideration of the calculated structure.




I II III

Fig. 1. Conformations about the $C^{\alpha}$-$C^{\beta}$ bond for D-Phe.

Table I. $^1H$ NMR Parameters for D-Phe Side Chains in Gramicidin S and Derived Rotamer Populations[a,b]

| Temp. (°C) | Chemical Shifts (ppm) | | Coupling Constants (Hz) | | | Rotamer Populations | | |
|---|---|---|---|---|---|---|---|---|
| | $C^{\beta}H_A$ | $C^{\beta}H_B$ | $J_{AB}$ | $J_{AX}$ | $J_{BX}$[c] | I | II | III |
| 23 | 2.863 | 2.969 | 12.9 | 10.7 | 5.4 | 0.74 | 0.26 | 0.00 |
| 63 | 2.908 | 2.993 | 13.0 | 9.9 | 5.9 | 0.67 | 0.30 | 0.03 |

a. Solvent is DMSO-$d_6$.
b. See rotamer structures and definitions of $H_A$ and $H_B$ in Fig. 1.
c. Hydrogen X is the D-Phe $C^{\alpha}H$.

As the temperature is raised, the side-chain coupling constants (Table I) show that the population of rotamer I falls to 0.67 at 63°. This would lessen the calculated shielding of the Pro $C^{\delta}$ hydrogens by 0.11 and 0.02 ppm, respectively, i.e., result in downfield shifts of this magnitude. The Pro $C^{\delta}$ hydrogen resonances are observed to shift downfield linearly with increasing temperature, and by 63° the chemical shifts have changed by 0.137 and 0.014 ppm (Table II). We attribute these shifts to the changes in rotamer populations about the $C^{\alpha}$-$C^{\beta}$ bond for the D-Phe residue, with consequent changes in the aromatic shielding of these two Pro $C^{\delta}$ hydrogens.

Finally, we observed the complete removal of these shielding effects when the aromatic rings are hydrogenated, to produce 4,4'-di-β-cyclohexylalanyl gramicidin S. This compound has been prepared before (4,11) and characterized by NMR methods (4,11) as having a similar backbone conformation to that of gramicidin S itself. We observed a signal (corresponding in area to two hydro-

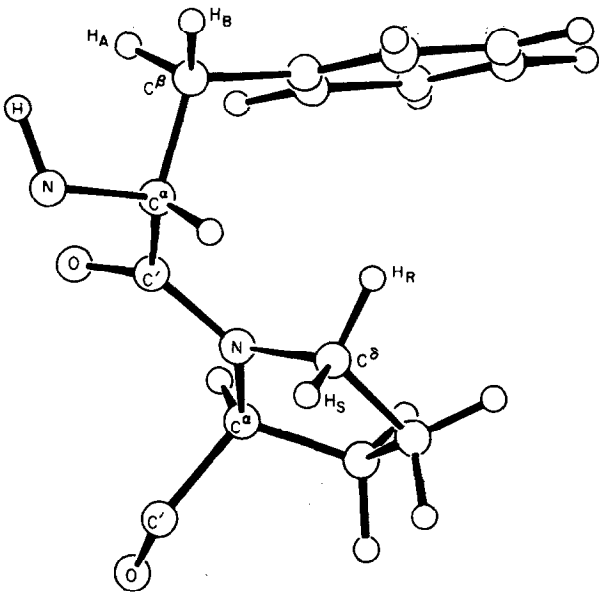



Fig. 2. A segment of the calculated structure (3), showing D-Phe and Pro residues.

Table II. Chemical Shifts of Proline $C^\delta$ Hydrogens in Gramicidin S[a]

| Temp. (°C) | Chemical Shift (ppm) | |
|---|---|---|
| | $C^\delta H_R$ | $C^\delta H_S$ |
| 23 | 2.486 | 3.580 |
| 33 | 2.534 | 3.596 |
| 43 | 2.556 | 3.591 |
| 53 | 2.584 | 3.604 |
| 63 | 2.623 | 3.594 |
| 73 | 2.660 | 3.598 |
| 83 | 2.690 | 3.602 |

a. Solvent is DMSO-$d_6$.

gens) in the spectrum of this compound at 3.46 ppm, which we assigned to the two $C^\delta$ hydrogens of proline. This relatively normal position, in the absence of aromatic shielding, confirms the involvement of the aromatic ring in such shielding in gramicidin S.

The proximity of the D-Phe aromatic ring and the Pro hydrogens in gramicidin S has also been demonstrated by nuclear Overhauser effects on the D-Phe aromatic ring hydrogens (12). The Pro $C^{\alpha}$ hydrogen is so far from the aromatic ring (almost 5 Å) that little through-space shielding of this hydrogen is observed but, because of spin relaxation within the "island" of adjacent spins, irradiation of this hydrogen also gives rise to nuclear Overhauser effects on the D-Phe hydrogens (12).

## ACKNOWLEDGMENTS

This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases (AM-13743) and from the National Science Foundation (PCM-08691). We thank Mr. T. Thannhauser for carrying out the amino acid analyses, and Dr. Leo Neuringer for providing access to facilities at the National Magnet Laboratory, Cambridge, Mass.

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