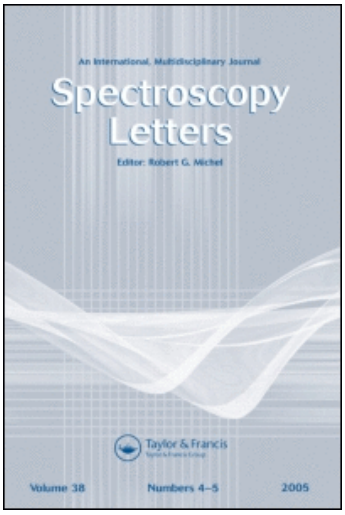

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Conformational Features of Penicillin G in Solution as Revealed by ^{13}C-{^{1}H} Selective Nuclear Overhauser Effects

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CONFORMATIONAL FEATURES OF PENICILLIN G IN SOLUTION AS REVEALED BY ^{13}C-{^1H} SELECTIVE NUCLEAR OVERHAUSER EFFECTS

KEY WORDS ^{13}C-{^1H} nuclear Overhauser effect; penicillin G

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ABSTRACT

^{13}C-{^1H} nuclear Overhauser effects (n.O.e.) were measured for penicillin G in $^2H_6$ -DMSO upon selective presaturation of proton resonances and interpreted on the basis of effective correlation times as calculated by analysis of ^{13}C and ^1H spin-lattice relaxation rates. Preferential occurrence of a compact C_2 puckered conformation with folding of the benzoylamino side chain towards the methyl at C_2 was given evidence. A Dreiding model of such conformation was built.

INTRODUCTION

Penicillin G contains a fused β-lactam-thiazolidine nucleus and a benzoylamino side chain (figure 1). The thiazolidine ring is flexible and it can exist in two principal conformations that are interconvertible via the pseudorotation associated with S_1-C_2-C_3-N_4 torsion (figure 2) which causes the C_2-methyl substituents and the C_{15}-carboxyl group to move synchronously. In one conformation (C_2 puckered) the C_2 atom of the ring deviates significantly from the mean plane defined by the other four atoms, while in the other (C_3 puckered) a similar deviation is shown by C_3. Both conformations

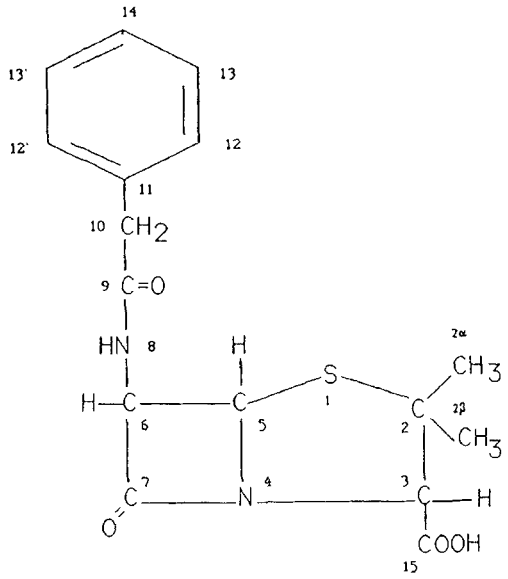


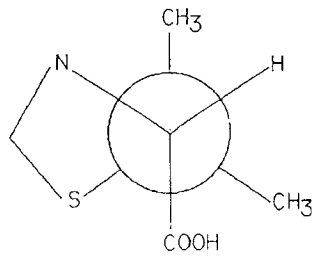
Figure 1 Molecular formula of penicillin G

have been encountered in solid state studies;[1] whereas only the C_2 puckered conformation has been observed in aqueous solution with the aid of lanthanide induced shifts.[2] Since lanthanides have been shown to bind to the carboxyl group,[3] the conclusion that the same conformation is the preferred one for the free antibiotic is not straightforward.

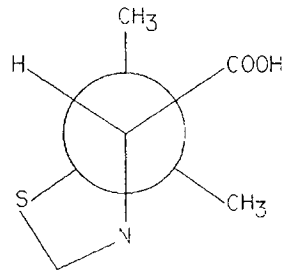
Here we present our analysis of ^{13}C-{^1H} nuclear Overhauser effects (n.O.e.) for penicillin G in | $^2H_6$ |-DMSO that, together with an interpretation of ^{13}C and ^1H spin-lattice relaxation rates allows to obtain some conformational dynamics parameters, that may be relevant for characterizing the biological activity of the antibiotic.

MATERIALS AND METHODS

Penicillin G was supplied by Sigma Chemical Co. and was used without further purification. Solutions were made in | $^2H_6$ |-DMSO



(a)



(b)

Figure 2 (a) C_2 puckered and (b) C_3 puckered conformations of the thiazolidine ring of penicillin G

99.96 % (Merck) and were carefully deoxygenated by bubbling nitrogen gas.

NMR experiments were performed on a Varian VXR-200 spectrome-ter at the constant temperature of 298 $\pm$ 1 °K. Chemical shifts were referenced to internal | $^2H_4$ |-TSP 2 mM.

^{13}C and ^1H spin-lattice relaxation rates were measured with inversion recovery pulse sequences. Selective ^1H spin-lattice relaxation rates were measured with inversion recovery pulse sequences where selective inversion was achieved by gating on the proton decoupler at low power for relatively long times (ca. 20 ms).

All proton relaxation rates were calculated in the initial rate approximation. Errors were generally evaluated at ± 4%.

Nonselective $^{13}C-\{^1H\}$ nuclear Overhauser effects were measured with gated broadband high power decoupling sequences, whereas the selective $^{13}C-\{^1H\}$ nuclear Overhauser effects were measured after selective presaturation of the desired proton resonance by gating on the proton decoupler at low power for long times (ca. 10 s).

RESULTS AND DISCUSSION

^{13}C-NMR relaxation rates and $^{13}C-\{^1H\}$ nuclear Overhauser effects (measured upon broadband gated saturation of all proton resonances) are reported in Table 1.

As expected, all protonated carbons exhibit the maximum n.O.e., thus indicating that the dipolar interaction with the directly bonded hydrogen(s) provides the main relaxation mechanism. In such a case some information on molecular tumbling motions in solution can be obtained. The three protonated carbons of the fused β-lactam-thiazolidine nucleus display relatively fast relaxation rates quite similar to each other. Consideration of the one-bond $^{13}C-^1H$ dipolar interaction[4] with r_{C-H} = 1.09 Å[5] leads to calculate the correlation time for modulation of such interaction at τ_c = 0.17 ± 0.02 ns at 298 °K. Since the motion is very likely to be anisotropic, this τ_c must be considered as an 'effective' correlation time that arises from combination of at least two motional correlation times. The evaluation of τ_c of ring carbons immediately allows to gain information on internal reorientational motions of the two methyls at C_2, that do not behave as free rotors (one should find $R_1(CH) << 3R_1(CH_3)$)[6] nor are they completely locked to the motion of the thiazolidine ring (one should find $R_1(CH) >> 1/3\ R_1\ (CH_3)$).[6]

When going to the aromatic side chain, it is apparent that both C_{10} and aromatic carbons experience some degrees of internal flexibility as manifested by slower normalized relaxation rates. Moreover, the fact that ortho and meta carbons display slower relaxation rates than C_{14} can be taken as an indication that the $C_{11}-C_{14}$ molecular axis is the expected axis for internal librational

Table 1

50.3 MHz ^{13}C-NMR and 200 MHz ^{1}H-NMR parameters of penicillin G 10 mM in | $^{2}H_6$|-DMSO. T = 298 °K

Peak	δ (ppm)	^{13}C-R_1 (s^{-1})	n.O.e. (η unit)	Peak	δ (ppm)	R^{nsel} (s^{-1})	R^{sel} (s^{-1})	$\Sigma \sigma_{ij}$ (s^{-1})
C_{15}	173.11	0.20	0.39	H_8	8.64	2.07	1.84	0.23
C_7	170.32	0.43	0.83					
C_9	169.57	0.28	0.60					
C_{11}	135.94	0.32	0.80					
$C_{13,13'}$	128.97	1.12	1.86	arom	7.35	0.42		
$C_{12,12'}$	128.07	1.07	1.91					
C_{14}	126.29	1.52	1.90					
C_3	74.07	4.24	1.81	H_3	4.21	0.59	0.54	0.05
C_5	66.78	4.45	1.84	H_5,H_6	5.45	0.80	0.78	0.02
C_2	64.25	0.32	1.38					
C_6	57.69	4.12	1.87					
C_{10}	41.37	4.44	1.92	H_{10}	3.68	2.31	2.17	0.14
$C_{2\alpha}$	31.47	4.02	1.97	$H_{2\alpha}$	1.57	3.65	3.50	0.15
$C_{2\beta}$	27.49	4.90	1.95	$H_{2\beta}$	1.48	3.76	3.58	0.18

motions and rotations within the phenyl ring. The relative range of such internal motions can be inferred by considering the 'effective' motional correlation time of 87 ± 4 ps at 298 °K for modulation of the C_{11}-C_{14} relaxation vector, as calculated with the formula of Allerhand et al.[4] This correlation time is of course only indicative of the range of motions but, anyway, one can use the methods of

Table 2

Observed selective ^{13}C-{^{1}H} n.O.e. upon presaturation of the H_8 resonance of penicillin G 10 mM in | $^{2}H_6$|-DMSO. T = 298 °K

Peak	n.O.e. (η units)
C_9	0.41
C_6	0.02
C_7	0.09
$C_{2\alpha}$	0.02
C_{10}	0.01

Solomon,[7] Woessner,[8] and Wallach[9] to calculate the internal correlation time at 24 $\pm$ 5 ps at 298 °K by considering that the angle between the axis of internal libration and that of phenyl ring motion is 60°.

Conformational features in solution were obtained by interpreting the nuclear Overhauser effects generated on specific carbons upon selective presaturation of the NH resonance (table 2). The different ^{13}C-{^{1}H} nuclear Overhauser effects are accounted for by the following general equation:

$$f_{Ci}\{Hj\} R_{Ci} = f(\tau_c/r^6_{CiHj}) \qquad (1)$$

where R_{Ci} is the ^{13}C spin-lattice relaxation rate, r_{CiHj} is the involved carbon-to-proton distance and τ_c is the correlation time for motional modulation of the corresponding relaxation vector.

The equation describing such n.O.e. is given by:[10,11]

$$f_{Ci}\{H_j\}R_{Ci} = 0.1\,\frac{h^2\gamma_H^3\gamma_C}{r_{CiHj}^6}\left\{\frac{6\tau_c}{1+(\omega_H+\omega_C)^2\tau_c^2} - \frac{\tau_c}{1+(\omega_H-\omega_C)^2\tau_c^2}\right\} \qquad (2)$$

It follows that when presaturation of Hj generates n.O.e. on any two carbons, e.g. Ci and Ck, the following equation can be used, provided the same τ_c describes reorientation of both Ci-Hj and Ck-Hj vectors:

$$\frac{f_{Ci}\{H_j\}}{f_{Ck}\{H_j\}} = \frac{R_{Ck}r_{Ck\text{-}Hj}^6}{R_{Ci}r_{Ci\text{-}Hj}^6} \qquad (3)$$

It is obvious that, within the limits of validity of eq.(3), any one of the two distances can be calculated if the other is known. In the case of penicillin the distance between H_8 and C_9, which is independent of conformation, can be calculated with the aid of molecular models (r = 2.12 Å) and leads to calculate the corresponding motional correlation time at 0.10 ± 0.04 ns at 298 °K by using eq.(2) and the experimental value of $f_{C9}\{H_8\}$ (table 2). The agreement with the range of correlation times calculated by interpreting nuclear relaxation rates is noteworthy. By the same way, almost the same correlation time (τ_c = 0.11 ± 0.03 ns at 298 °K) was calculated to modulate the reorientation of the C_6-H_8 vector which is characterized by the same fixed distance of r = 2.12 Å. The much lower n.O.e. for C_6 is due to the much faster relaxation rate.

Besides nuclear Overhauser effects on C_6 and C_9, presaturation of H_8 generates effects on C_7, C_{10} and on the low-field methyl at C_2. Among the involved relaxation vectors, H_8 and C_{10} are, again, at a fixed distance, due to the perfect planarity of the amide bond.[12] As a consequence the n.O.e. can be used to evaluate τ_c at 0.13 ± 0.04 ns at 298 °K, again in very good agreement with relaxation rate analysis. The other two n.O.e. are, on the contrary, suitable for evaluating distances by using eq.(3) and the $f_{C9}\{H_8\}$ n.O.e. for calibration. It came out that C_7 and H_8 are at a distance of 2.55 Å whereas H_8 is 2.28 apart from $C_{2\alpha}$. Even though these distances are not likely to be very accurate, especially the

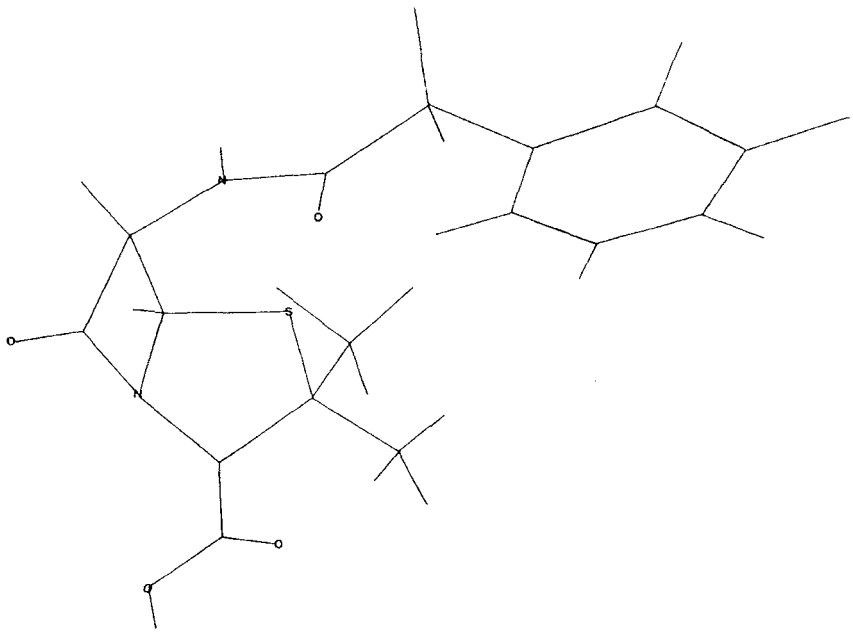


Figure 3 Projection of the Dreiding model of the 'preferred' conformation of penicillin G in $[^2H_6]$ DMSO solution

second one, since they were calculated under the assumption of equal correlation times as that for modulation of the C_8-H_9 vector, they allow some relevant insight on conformation in solution, also at the light of the missing nuclear Overhauser effects.

Some more information was obtained by interpreting the ^1H spin-lattice relaxation rates, also reported in Table 1. In fact the difference between R^{nsel} (eq.(4)) and R^{sel} (eq.(5)) for any proton resonance provides the sum of pairwise dipolar cross relaxation rates contributing the relaxation pathway of that particular proton:[13,14]

$$R_i^{nsel} - R_i^{sel} = \sum_{j \neq i} \sigma_{ij} \qquad (4)$$

The possibility of obtaining distances relies on an approximate knowledge of the reorientational correlation time and it depends also on the capacity of separating the different contributions of different protons nearby. Nevertheless, the following features can anyway be delineated:

i) the small difference between R^{nsel} and R^{sel} of H_5,H_6 is very likely to arise from a dipolar interaction between H_6 and H_8 that can be located at a distance of 2.92 Å by assuming the reorientational correlation time measured for protonated carbons within the β-lactam-thiazolidine nucleus. Such distance well agrees with the distance between H_8 and C_7 as it was calculated by selective $^{13}C-\{^1H\}$ Overhauser effects.

ii) The difference (R^{nsel} - R^{sel}) of H_3 can be accounted for by a dipolar interaction with protons of one of the methyls at an average distance of 2.64 Å.

These last findings, together with those previously observed, strongly suggest the occurrence of a 'preferred' conformation in solution very similar to that observed with lanthanide induced shifts.[2] The formation of a $^{13}C-\{^1H\}$ n.O.e. on $C_{2\alpha}$ upon presaturation of H_8 and the location of H_8 relative to C_7 and H_6 yield evidence of a preferred C_2 puckered conformation with folding of the side chain towards the $C_{2\alpha}$ methyl, thus providing what has been called a compact structure in solution.[15,16] This 'preferred' conformation is shown in figure 3 as a projection of the Dreiding model. Unfortunately, the poor resolution of aromatic protons does not allow to carefully delineate the conformation of this moiety relative to the side chain.

REFERENCES

1. Dexter D.D., Van der Veen J.M., Conformations of Penicillin G: Crystal Structure of Procaine Penicillin G Monohydrate and a Refiniment of the Structure of Potassium Penicillin G. J.Chem.Soc., Perkin I 1978, 185.

2. Dobson C.M., Ford L.O., Summers S.E. Summers, Williams R.J.P., Nuclear Magnetic Resonance Study of the Conformations of

Penicillins in Solution Using Lanthanide Ion Probes. J.Chem.Soc., Faraday Trans.II 1975, 71:1145.

3. Levine B.A., Williams R.J.P., Determination of the Conformations of Small Molecules in Solution by means of Paramagnetic Shift and Relaxation Perturbations of NMR Spectra. Proc.R.Soc.London A 1975, 345: 5.

4. Allerhand A., Doddrell D., Komoroski R., Natural Abundance Carbon-13 Partially Relaxed Fourier Transform Nuclear Magnetic Resonance Spectra of Complex Molecules. J.Chem.Phys. 1971, 55: 189.

5. Dill K., Allerhand A., Small Errors in C-H Bond Lengths May Cause Large Errors in Rotational Correlation Times Determined from Carbon-13 Spin-Lattice Relaxation Measurements. J.Amer.Chem.Soc. 1979, 101: 4376.

6. Wehrli F.W., Wirthlin T., *Interpretation of Carbon-13 NMR Spectra* , London: Heyden & Son Ltd., 1976.

7. Solomon I., Relaxation Processes in a System of Two Spins. Phys.Rev. 1955, 99: 559.

8. Woessner D.E., Spin Relaxation Processes in a Two-Proton System Undergoing Anisotropic Reorientation. J.Chem.Phys. 1962, 36: 1.

9. Shimizu H, Effect of Molecular Shape on Nuclear Magnetic Relaxation. J.Chem.Phys. 1967, 47: 5258.

10. Niccolai N., Rossi C., Brizzi V., Gibbons W.A., Proton-Carbon NOE Difference Spectroscopy Studies of Carbon Microenvironments, Internuclear Distances, and Hydrogen Bonding in Rifamycin S. J.Amer.Chem.Soc. 1984, 106: 5732.

11. Niccolai N., Rossi C., Mascagni P., Brizzi V., Gibbons W.A., The Derivation of Carbon-Proton Internuclear Distances in Organic Natural Products from ^{13}C Relaxation Rates and Nuclear Overhauser Effects. J.Chem.Soc. PerkinTrans. I 1985, 239.

12. Paschal J.W., Dorman D.E., Srinivasan P.R., Lichter R.L., Nuclear Magnetic Resonance Spectroscopy. Carbon-13 and Nitrogen-15 Spectra of the Penicillins and Cephalosporins. J.Org.Chem. 1978, 43 : 2013.

13. Freeman R., Hill H.D.W., Tomlinson B.L., Hall L.D., Dipolar Contribution to NMR Spin-Lattice Relaxation of Protons. J.Chem.Phys. 1974, 61: 4466.

14. Valensin G., Kushnir T., Navon G., Selective and Nonselective Proton Spin-Lattice Relaxation Studies of Enzyme-Substrate Interactions. J.Magn.Reson. 1972, 46: 23.

15. Rao V.S.R., Vasudevan T.K., Conformation and Activity of β-Lactam Antibiotics. CRC Crit.Rev.Biochem. 1983, 14 : 173.

16. Wolfe S., Khalil M., Weaver D.F., MMPEN: Development and Evaluation of Penicillin Parameters for Allinger's MMP2(85) Programme. Can.J.Chem. 1988, 66: 2715.

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