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A NUCLEAR MAGNETIC RESONANCE STUDY OF THE STRUCTURE AND INTERACTION OF ACTINOMYCIN D: ANALYSIS AND CONFORMATIONAL PROPERTIES[1]

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Actinomycin D (ACD), Figure 1, is the most important member of a family of naturally occurring actinomycins whose primary biological function is the inhibition of DNA dependent RNA synthesis (5). If mechanisms proposed for the biological activity of ACD (6) are to have a plausible structural basis, it is essential to have detailed knowledge of the conformational properties of ACD. Such information can be obtained from an analysis of the proton magnetic resonance spectrum of ACD, and we report the results of such a study in this communication.

The proton resonance spectrum of a 0.011 M solution of ACD in C_6D_6, recorded at 60 Mcps and 80°C, is illustrated in Figure 1. Spectra were also recorded in a variety of aqueous and nonaqueous solvents over a wide temperature range. However, the best resolution of signals in the high-field region of the spectrum was obtained in C_6D_6. As is apparent from Figure 1, the spectrum is quite complicated, and a detailed assignment required the use of deuterium exchange, solvent perturbation, and spin decoupling techniques.

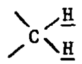
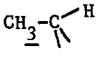
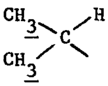
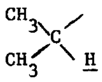
Deuterium exchange measurements, carried out in dioxane-d_8 containing small amounts of D_2O, permit the assignment of the four N-H and two amino protons (7). These are the only exchangeable protons in ACD under normal solvent and temperature conditions. Decoupling experiments, on the other hand, enable the assignment of the following coupled sets of protons: $NH\text{-}C^{\alpha}H\text{-}C^{\beta}H\text{-}CH_3$ (thr and val); $\text{-}C^{\alpha}H\text{-}C^{\beta}H\text{-}CH_3$ (meval); H(A)C=CH(B) and $H(B)C\text{=}C\text{-}CH_3(A)$ (actinocin ring); H(A)-C-H(X) (sar); and $\text{-}C^{\alpha}H\text{-}C^{\beta}H$ (pro). Since the protons of the four $N\text{-}CH_3$ groups (sar and meval) and the $CH_3(B)$ of actinocin are not coupled to other protons an assignment of their signals is based upon results of solvent perturbation studies in a variety of aqueous and nonaqueous binary solvent mixtures (7). A summary of the chemical shifts and coupling constants derived from an analysis of the spectrum in C_6D_6 is given in Table I.

A number of points regarding the analysis and relating to the conformation can be deduced from the data. All of the N-H signals are split into doublets in benzene and in other solvents containing non-exchangeable hydrogens. The splittings are clearly due to coupling with the adjacent $C^{\alpha}H$ proton. If it is assumed that the vicinal $HNC^{\alpha}H$ couplings follow a Karplus type dependence (8) upon ϕ_{vic}, as proposed recently by Bystrov and coworkers for dipeptides (9), then the $J_{HNC^{\alpha}H}$ values suggest a trans conformation for all four valyl* and threonyl residues in ACD.

An estimate can also be made of the vicinal angles between the $HC^{\alpha}\text{-}C^{\beta}H$ protons of the threonyl residues. Here, free rotation about the $C^{\alpha}\text{-}C^{\beta}$ bond is prohibited by the lactone linkage at C^{β}. Based on the Karplus relationship (8), the vicinal angles are estimated to have values in the range of ∿70° and/or 100°, indicating a predominantly gauche conformation. It is interesting to note that the magnitude of $J_{HC^{\alpha}C^{\beta}H}$ in the free threonine amino acid (10) is roughly twice as large (4.8 cps) as the corresponding couplings in ACD. Since vicinal couplings fall in the ranges 0-3 and 8-12 cps for predominantly gauche and trans conformations (10) respectively, it can be concluded that formation of the lactone ring results in a more gauche conformation for the vicinal methine protons of the threonyl residues.

No estimate of vicinal angle can be made for the $HC^{\alpha}C^{\beta}H$ protons of the valyl and methyl valyl residues because of the possibility of rotation about these bonds. However, the respective couplings of <0.5 cps and 9.0 cps differ markedly from the values generally observed, ∿6-7 cps where free rotation is occurring (10). Moreover, both the valyl and methylvalyl couplings differ significantly from the corresponding couplings of 4.4 and 7.3 cps respectively in the free amino acids (10). These results are consistent with gauche and trans conformations for the $HC^{\alpha}C^{\beta}H$ protons of the valyl and methylvalyl residues respectively, and further indicate that these conformations are more favored in the cyclic peptide rings than in the monomers.

Table I. CHEMICAL SHIFTS AND COUPLING CONSTANTS FOR ACTINOMYCIN D*

Group	Residue	Chemical Shift†	Coupling Constant†		Group	Residue	Chemical Shift†		Coupling Constant†
			Type					Type	
$-NH_2$		7.26	-	-	$-CH_2-$	sar (A)	4.50 4.54		17.3
$-N\underline{H}$	thr	7.62	$\underline{H}N-C^{\alpha}\underline{H}$	6.3		sar (X)	3.02	"	17.3
	thr	8.17	$\underline{H}N-C^{\alpha}\underline{H}$	5.7		pro	1.8 -	-	-
	val	8.50	$\underline{H}N-C^{\alpha}\underline{H}$	5.7			4.25		
	val	8.70	$\underline{H}N-C^{\alpha}\underline{H}$	5.7					
$=C\underline{H}$	ring B	6.84	$\underline{H}C=C\underline{H}$	7.6	$-CH_{\underline{3}}$	ring A	2.26	$CH_{\underline{3}}-C=C\underline{H}$	<0.3
	A	7.68	$\underline{H}C=C\underline{H}$	7.6		B	1.96		-
>CH	thr α	5.04	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	2.2	$N-CH_{\underline{3}}$	sar	2.79	-	-
	thr α	5.08	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	2.2		sar	2.79	-	-
	thr β	5.71	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	2.2		meval	2.09	-	-
	thr β	5.71	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	2.2		meval	2.21	-	-
	val α	3.97	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	<0.5		thr	1.58	$CH_{\underline{3}}-C\underline{H}$	6.1
	val α	4.12	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	<0.5		thr	1.61	"	6.1
	val β	2.48	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	<0.5					
	val β	2.48	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	<0.5					
	meval α	3.99	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	9.0		val	1.25		6.7
	meval α	3.99	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	9.0		val	1.44 1.48		6.6
	meval β	2.85	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	9.0					
	meval β	2.85	$\underline{H}C^{\alpha}-C^{\beta}\underline{H}$	9.0					
	pro	6.32	-	-		meval	0.54	"	6.2
						meval	0.92 0.94	"	6.1

*0.02 M solution in C_6D_6; T = 80°C. †Chemical shifts are in ppm to low-field from internal TMS and are accurate to 0.01 ppm; coupling constants are in cps and are accurate to 0.10 cps.

Most of the chemical shifts for groups in the same residues in different penta-peptide rings are non-equivalent in C_6D_6, with the main exceptions being the methylvalyl (α and β), threonyl (β) and sarcosyl methyl protons. The shifts for the latter group are particularly interesting in that the equivalence is maintained in a wide variety of solvents and over a wide temperature range. This unusual behavior may arise from a screening effect exerted by the proline rings located in very close proximity to the methyl group. A favorable conformational relationship of this type can be shown with Dreiding molecular models of cyclic penta-peptide rings.

An appreciable temperature and solvent dependence was noted for the exchangeable hydrogens of ACD. Both the valyl N-H and NH_2 signals shift up-field with increasing temperature, as would be expected for protons involved in hydrogen bonding interactions (7). The threonyl N-H signals, in contrast, shift to low-field with increasing temperature. Such a low-field shift would occur if increased thermal motion leads to a more in-plane orientation of the threonyl residues relative to the phenoxazinone ring. Accordingly, the HNCO group of the threonyls must be located out-of-the-plane of the phenoxazinone ring at room temperature.

Full details regarding the analysis and the influence of solvent and temperature upon the ACD spectrum, along with the relationship of these measurements to the conformational properties of ACD, will be published shortly.

*Note that rotation about the C-N bond of the val residues is not possible in the cyclic peptide ring form.

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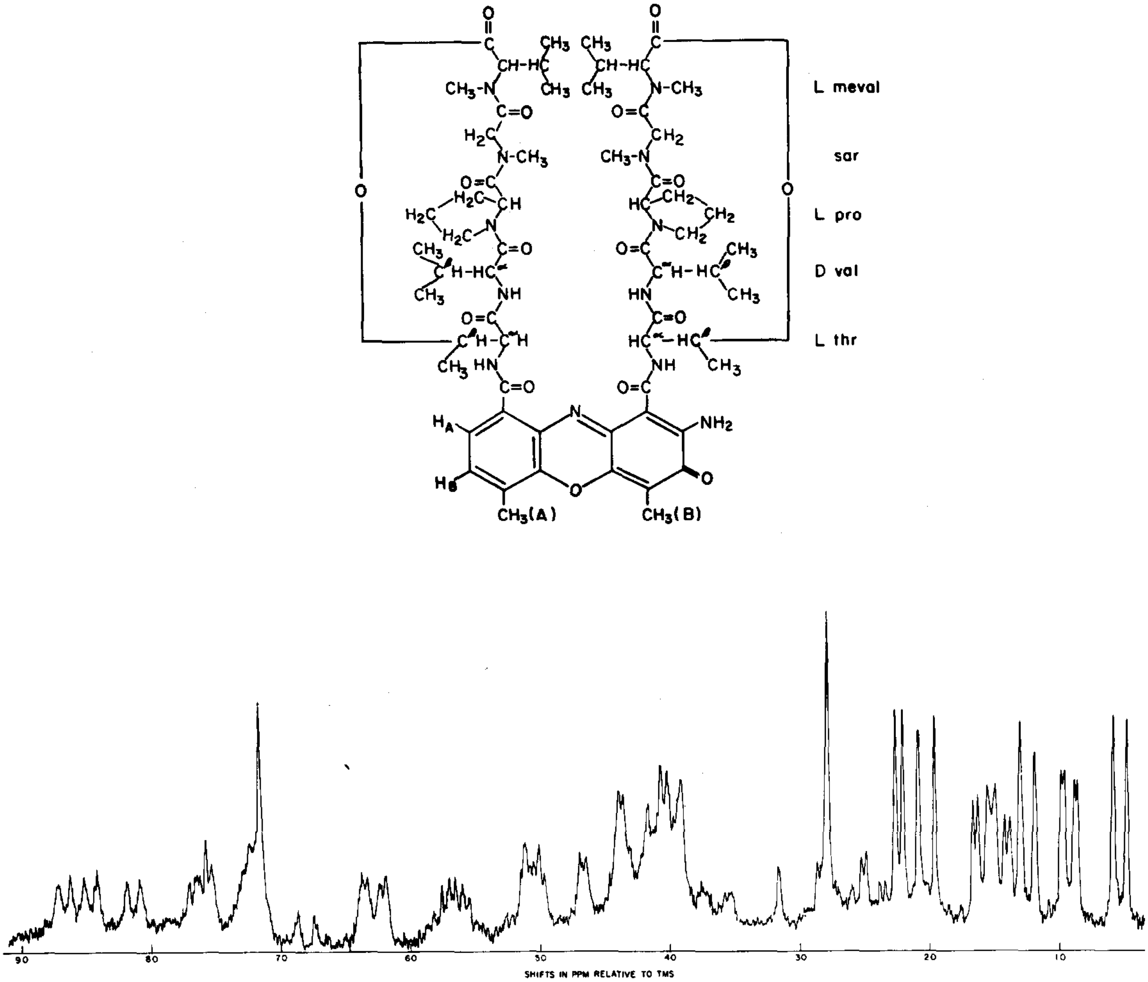

Figure 1 (upper). Structure of actinomycin D in the penta-peptide form. Abbreviations: meval, methyl valine; sar, sarcosine; pro, proline; val, valine; thr, threonine. (lower) A single scan proton resonance spectrum of actinomycin D in C_6D_6 at 80°C. Shifts are in P.P.M. to low-field from internal TMS.