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The Conformation of Echinomycin in Solution

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Abstract: We have investigated the ^1H and ^{13}C NMR spectra of echinomycin in deuteriochloroform and dimethyl-d_6 sulfoxide solution. From a consideration of the measured NMR parameters and the results of model building and potential energy calculations we have deduced the conformation of echinomycin in solution.

Introduction

Echinomycin is an antibiotic whose biological effects are attributable to an inhibition of DNA template activity.[2,3] The molecule is a cyclic octapeptide incorporating a thioacetal cross-linkage and two quinoxaline rings[4,5] (structure I). The binding of echinomycin to DNA has been shown[6,7] to occur by the simultaneous intercalation of both quinoxaline rings between the base pairs. In order to explore the structural basis of this novel bifunctional intercalative binding, it is necessary to establish the conformational structure of echinomycin. We report here nuclear magnetic resonance data, model-building studies, and semiempirical potential energy calculations which together allow us to describe the conformation of echinomycin in solution.

Experimental Section

Echinomycin (supplied by Ciba-Geigy, Switzerland) was examined at concentrations between 2 mM and 110 mM in deuteriochloroform or deuteriodimethyl sulfoxide, containing hexamethyldisiloxane (HMS) as a chemical shift reference.

The NMR spectra were obtained using Bruker WH 270 (^1H, 270 MHz), Varian SC300 (^1H, 300 MHz), and Varian XL 100 (^1H, 100 MHz; ^{13}C, 25.2 MHz) spectrometers equipped with Fourier transform facilities. All measurements were made in the Fourier transform mode of operation using acquisition times of up to 4 s. When the highest resolution was required, spectral widths of 3 kHz were digitized over 12K words.

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$La(fod)_3$ and $Eu(fod)_3$ (fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate) were obtained from Digby Chemical Services, London. Aliquots of a concentrated (~25 mM) solution of $La(fod)_3$ or $Eu(fod)_3$ were added to solutions of echinomycin in deuteriochloroform (19 and 9 mM, respectively).

Details of the procedures used for the semiempirical potential energy calculations have been given elsewhere.[8]

Results and Discussion

The thioacetal cross-linkage in echinomycin introduces an asymmetry into the molecule causing the two halves to be nonequivalent. This nonequivalence is manifested in both the ^1H and ^{13}C NMR spectra where multiplets with different chemical shifts are observed for some of the nuclei occupying corresponding positions in the two halves of the molecule. Thus in the ^1H NMR spectrum of echinomycin in $CDCl_3$ at 270 MHz (Figures 1 and 2) we see two different absorptions for the D-Ser NH, Ala NH, Ala CH_3, NCH_3 of NMe Val and NMe Cys, and the Val CH_3 nuclei.

A partial assignment of the ^1H spectrum at 100 MHz has already been reported;[5] the remaining assignments were made by spin decoupling experiments at 270 and 300 MHz.

^1H Chemical Shifts. The ^1H chemical shifts for echinomycin in $CDCl_3$ and Me_2SO-d_6 were measured at different temperatures and the results are summarized in Table I. Most of the nuclei have shifts similar to those observed in simple dipeptides; however, the D-Ser NH and the CysαCH resonances are considerably to low field of where they normally absorb. The low-field shift of the D-Ser NH is easy to explain in terms of ring current shifts from the quinoxaline rings attached to

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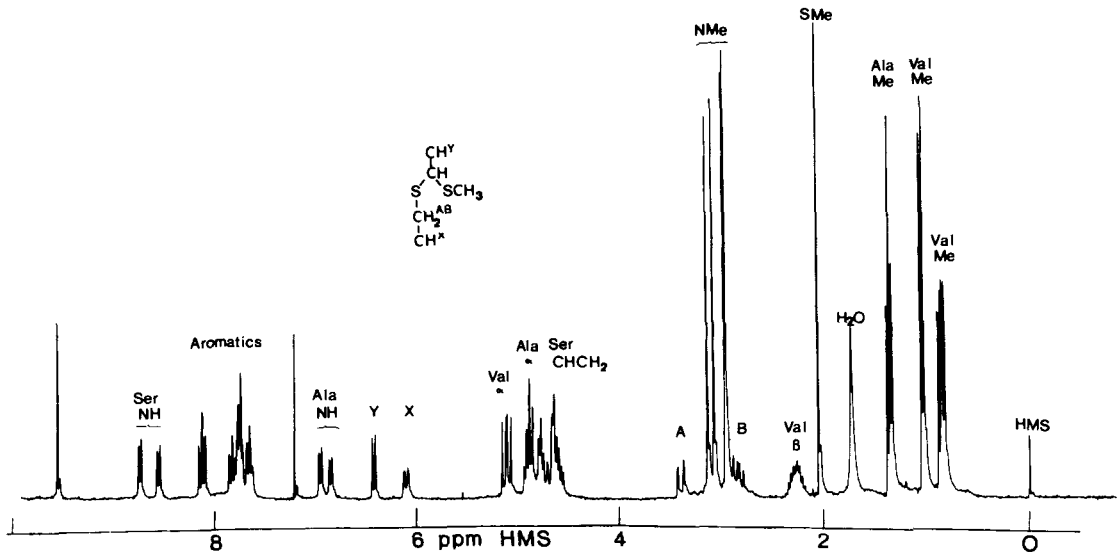


Figure 1. The ^1H 270-MHz resonance spectrum of 40 mM echinomycin in $CDCl_3$ solution.

the serine residues. However, such effects cannot be used to explain the anomalous chemical shifts of the CysαCH as will be seen later.

In the variable temperature study we were particularly interested in the behavior of the D-Ser and Ala NH proton chemical shifts. It is well known that NH protons which are complexes with solvent molecules such as Me_2SO-d_6 have characteristically high values for the temperature coefficients of their chemical shifts.[9] The small values observed for the temperature coefficients of the chemical shifts for the D-Ser NH protons in $CDCl_3$, Me_2SO-d_6, and pyridine-d_5[10] clearly show that they are not accessible to the solvent. Although the temperature coefficients for Ala NH protons are small in $CDCl_3$, appreciable values are measured in pyridine-d_5 solution[10] (8.2 and 8.6 $\times$ 10^3 ppm/°C) reflecting some solvent accessibility of this NH proton. It was not possible to obtain accurate values for the temperature coefficients of these protons in Me_2SO-d_6 solution because of problems of signal overlap in the aromatic region. However, the large downfield shifts (>1 ppm) for the Ala NH protons on changing solvent from $CDCl_3$ to Me_2SO-d_6 further suggest that these protons are solvent accessible.

Several protons in echinomycin show an approximately linear concentration dependence of their ^1H chemical shifts in the range 5–40 mM (see Table II). The D-Ser NH and the aromatic protons are more shielded (shifted to higher field values) at higher concentrations while the Ala CH_3 and NH protons and one of the CysαCH protons (H_Y) are deshielded. The other protons are largely unaffected (to within ±0.02 ppm) by the concentration changes. These data are most

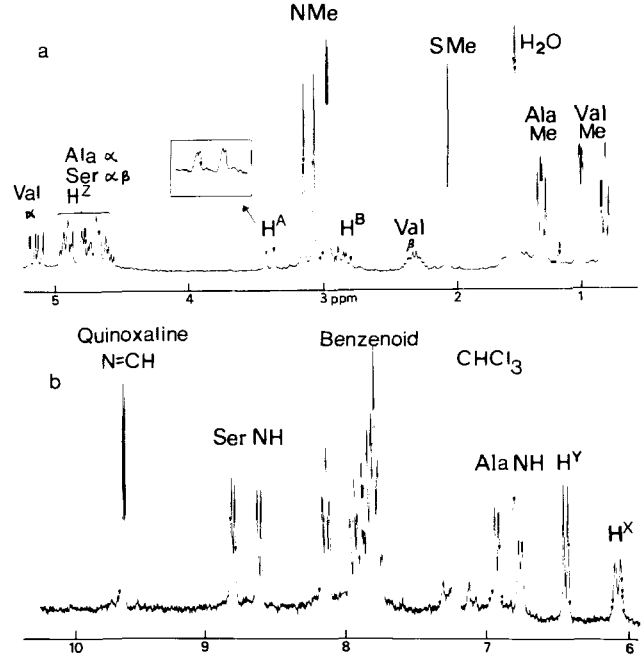
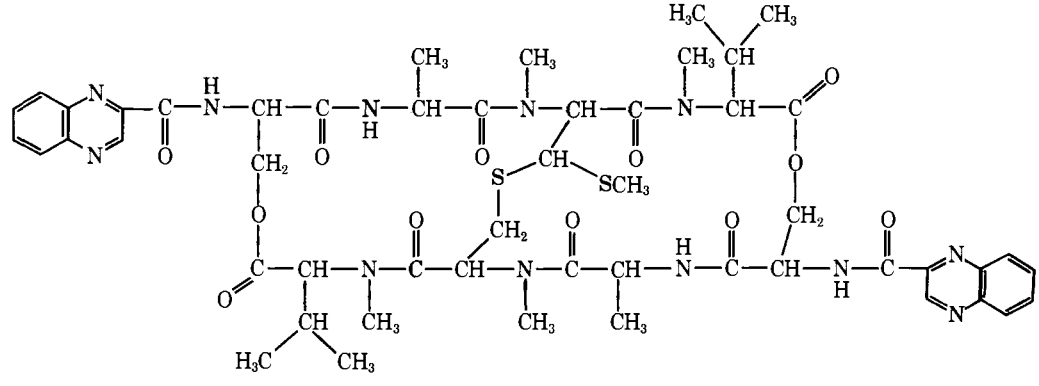


Figure 2. (a) The high-field region and (b) the low-field region of the 270-MHz ^1H spectrum of 3.5 mM echinomycin in $CDCl_3$ solution.

readily interpreted in terms of self-association of echinomycin in the more concentrated solutions. The observed upfield shifts of the aromatic protons could arise from ring current shifts



I

Table I. The ^1H Chemical Shifts and Their Temperature Coefficients for Echinomycin[b]

Residue		δ (ppm) 40 mM $CDCl_3$	δ (ppm) 5 mM $CDCl_3$	10^3 × temp coeff (ppm/°C) for 5 mM $CDCl_3$ soln[b]	δ (ppm) 41 mM Me_2SO	10^3 × temp coeff (ppm/°C) for 5 mM Me_2SO soln[b]
Val I[c]	γ	0.87, 0.83	0.85, 0.82	+0.4, −0.3	0.80, 0.70	
	β	2.24				
	α	5.09	5.09	+0.3	4.77	
Val II[c]	γ	1.04, 1.04	1.02, 1.03	0,0	0.94, 0.87	
	β	2.30				
	α	5.15	5.15	~0	4.85	
NMe		3.14	3.12	~0	3.22	
		3.07	3.05	+0.6	3.18	
		2.96	2.94	~0	2.85	
		2.95	2.94	~0	2.83	
Ala I	β	1.34	1.29	~0	1.32	
	α	4.78			(4.7–5.0)	
	NH	6.88	6.76	+0.3	7.89	
Ala II	β	1.37	1.35	~0	1.32	
	α	4.91			(4.7–5.0)	
	NH	6.98	6.91	−1.1	7.95	
Ser I	β	4.59, 4.65			~4.5	
	α	4.93			(4.7–5.0)	
	NH	8.53	8.61	~0	8.22	+1.5
Ser II	β	4.65, 4.70			~4.5	
	α	4.79			(4.7–5.0)	
	NH	8.75	8.79	−2.0	8.38	+1.0
SMe		2.04	2.03	+0.3	1.99	
$-{}^{\alpha}CH^{X}-$		6.08	6.06	>+1.8	5.98	+1.8
$S-CH_2^{AB}$		3.40[a]	3.39[a]	+1.2[a]		
		2.84			~2.6	
$-{}^{\alpha}CH^{Y}-$		6.45	6.42	−0.5	6.48	−1.6
CH^{Z}		4.87	4.87			
S S						
Quinoxaline	H3	9.55, 9.54	9.59, 9.58	~0	9.34, 9.31	~0
	H8	8.08, 8.13	8.10, 8.15	~0	~8.0	~+3[d]
	H6, H7	7.2–7.3	7.7–7.9	~−0.8	7.1–8.0	~+8[d]
	H5	7.3–7.4	~7.9	~+0.5		

[a] Gauche $H_{X\beta}$. [b] Positive values correspond to downfield shifts. Protons where value of ~0 is given have temperature coefficients less than 0.2×10^{-3}. Chemical shifts measured from HMS internal reference. [c] These assignments could be reversed. [d] Coefficient given refers to temperature range of 30–60 °C.

Table II. The Changes in ^1H Chemical Shifts in Echinomycin on Increasing the Concentration from 5 to 40 mM in $CDCl_3$

Proton	Change in chemical shift ($10^2 \times \Delta\delta$ ppm) Low-field signal	High-field signal
Ala NH	+8	+12
Ser NH	−4	−7
Quinoxaline N=CH	−4	−4
Benzenoid[a]	~(−12)	
Ala CH_3		+5
CH_YCHS_2	+3	

[a] Three of the benzenoid protons show this large concentration shift; the other (the lowest field one) has a smaller shift (−2). All other protons have downfield concentration shifts equal to or less than 2×10^{-2} ppm.

Table III. The ^1H-^1H Spin Coupling Constants (Hz)[a] for Echinomycin (Measured as a 40 mM Solution in $CDCl_3$)

Residue	$J_{\alpha\beta}$	$J_{\alpha\beta'}$	$J_{\beta\beta'}$	$J_{\beta\gamma}$	$J_{\alpha CHNH}$
Val I	10.05			6.8,[c] 7.0[c]	
Val II	10.05			7.4[c]	
Ala I	7.4				7.3
Ala II	7.5				7.05
Ser I	2[b,d]	5.2	11.1		7.7
Ser II	2	5[b,d]	11.6		6.3
$-{}^{\alpha}CH-$ / $S-CH_2$	1.7	11.2	16.1		
$-{}^{\alpha}CH-$ / CH / S S	8.7				

[a] Errors ±0.1 Hz. [b] Error ±0.5 Hz. [c] These assignments could be reversed. [d] Coupling constants measured in $Eu(fod)_3$ shifted spectra.

resulting from the stacking of the aromatic rings in the complex; in such an interaction the planes of the rings would be roughly parallel to each other. The downfield shifts observed for the Ala protons would result if these nuclei are located approximately in the plane of the quinoxaline ring. In all the possible conformational models of echinomycin (see below) the Ala NH and CH_3 protons are on the same side of the molecule as the quinoxaline rings; it is therefore easy to construct models for the aggregate where the quinoxaline rings are all mutually stacked and arranged such that the Ala protons are at the periphery of one of the quinoxaline rings where they would experience the observed deshielding. A less likely explanation for the Ala shifts is that they are caused by association-induced conformational changes; however, since no changes in coupling constants with concentration were observed there is no evidence for a change in conformation with concentration.

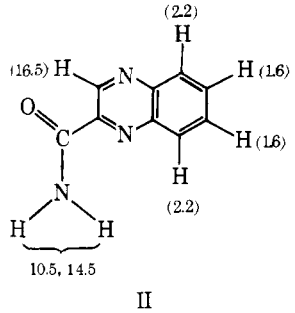
Three-Bond Coupling Constants. Many of the resonances in the echinomycin ^1H spectrum (Figures 1 and 2) have multiplet structures which can be analyzed to give coupling constant information. There are no less than 19 three-bond ^1H-^1H coupling constants in the molecule (see Table III) and potentially these contain a good deal of conformational information. Most of the coupling constants can be obtained from a first-

order analysis of the well-resolved multiplets. However, the D-Ser $\alpha CH\beta CH_2$ protons give rise to two badly overlapped ABC type spectra and it is difficult to fully analyze the spectra even at 300 MHz. Fortunately the addition of the paramagnetic reagent $Eu(fod)_3$ induces shifts which result in simpler nonoverlapping spectra and these can be analyzed readily to give the coupling constants shown in Table III. There was no indication that the coupling constants varied with the $Eu(fod)_3$ concentration indicating that the D-Ser $\alpha CH\beta CH_2$ fragments do not change their conformation when the lanthanide complexes bind to echinomycin. We have also examined echinomycin in the presence of the diamagnetic reagent $La(fod)_3$ to assess the extent of conformational changes caused by the binding of lanthanide complexes: the only coupling constants which were significantly influenced by the binding were the D-Ser J_{NH-CH} values (decreased by 1–2 Hz upon binding to equimolar $La(fod)_3$).

Conformational Structure Determination. Three-bond $^1H-^1H$ coupling constants can be related to their appropriate dihedral angles and thus used to provide partial conformational information for various fragments of the molecule. Using this information together with some assumptions based on results of potential energy calculations on simple peptides one can then begin model building using space-filling models. Such studies indicate that while there are three conformational possibilities for the echinomycin ring structure they all have a similar overall shape and appear to be very rigid. A choice between these remaining conformations can be made on the basis of lanthanide-induced chemical shifts.

Conformation of the Quinoxaline-Ser Fragment. The ^{13}C chemical shifts of echinomycin and quinoxaline-2-carboxamide have been measured in $CDCl_3$ solution and the values for the protonated aromatic carbons are presented in Table IV. The shifts are very similar in the two compounds. Of particular significance are the similar shifts of the C3 carbons which strongly indicate that the configuration of the C2-CO bond is the same in the two compounds. Further support for this is found in the ^1H spectra where the aromatic protons also have very similar shifts in dilute solutions (C3 proton shift is 9.59 ppm in echinomycin; 9.63 ppm in quinoxaline-2-carboxamide). In view of the known large anisotropic shielding effects of the CO group, such similar shifts could only be obtained if it has a similar orientation with respect to the C3 position in the two compounds.

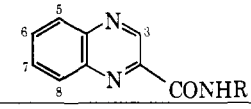
Simple valence bond considerations indicate that the carbonyl group will tend to lie in the plane of the aromatic ring, oriented either toward or away from H3. The shifts observed when $Eu(fod)_3$ binds to quinoxaline-2-carboxamide have also been measured and the values at equimolar concentration are indicated on structure II. The binding is expected to be mainly



II

to the carbonyl oxygen atom and the large lanthanide-induced shift for H3 clearly indicates that the configuration of the quinoxaline carboxamide (C2-CO) bond is as shown in structure II. The ^{13}C and ^1H chemical shift data show this is also true for echinomycin. This configuration also explains the low-field shift noted for the D-Ser NH protons in echinomycin

Table IV. The ^{13}C Chemical Shifts of the Protonated Aromatic Carbons of Echinomycin and Quinoxaline-2-carboxamide in $CDCl_3$ Solution[b]



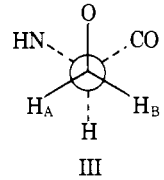
Carbon	Echinomycin, ppm[a]	Quinoxaline-2-carboxamide, ppm
3	143.5	143.8
5	132.1	132.1
6	131.1	131.1
7	129.8	130.0
8	129.1	129.8

[a] Measured from HMS. [b] Concentration of solutions 0.11 M.

since if the peptide bond has the normal trans configuration the D-Ser NH proton will be very near to and in the plane of the quinoxaline aromatic ring system.

The D-Ser J_{NH-CH} values (6.3, 7.7 Hz) characterize the torsion angles for the D-Ser $\alpha CH-NH$ bonds. For molecules with a rigid conformation it is possible to relate J_{NH-CH} coupling constants to the dihedral angle ϕ for the NH-CH bond using the equation proposed by Bystrov.[11] However, in echinomycin, the D-Ser $\alpha CH-NH$ bonds are outside the ring connecting the quinoxaline "tails" to the main ring structure and would thus be expected to have considerable flexibility, as in simple peptides. The observed coupling constants are indeed similar to those observed in small peptides and most likely correspond to a rapidly interconverting mixture of rotamers centered on ϕ values $\sim+80°$ or $+160°$ (see structure IV). Owing to the asymmetry of the thioacetal linkage, the averaged conformations for the two D-Ser $\alpha CH-NH$ bonds are slightly different. Perturbation of the D-Ser $J_{\alpha CH-NH}$ coupling constants by lanthanide binding is further evidence of the considerable flexibility in this region of the molecule.

The D-Ser residues form part of the echinomycin ring structure and the observed three-bond $\alpha CH\beta CH$ coupling constants indicate a well-defined rigid conformation. For both D-Ser residues two small $J_{\alpha CH\beta CH}$ coupling constants (2 and 5 Hz) are observed, clearly showing that these fragments have a conformation where the αCH proton is gauche to both βCH_2 protons. The observed coupling constants agree well with those previously estimated[12] from model compounds for the component coupling constants in this rotamer (III) for amino acids

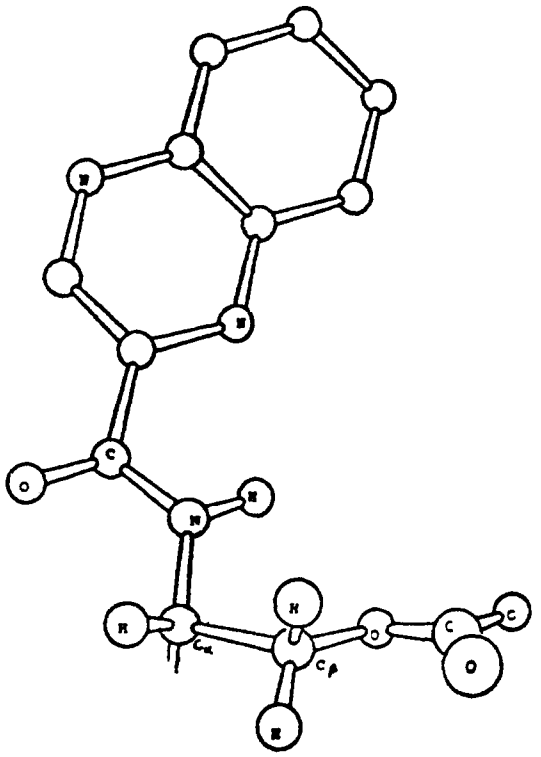


III

(2.9 and 4.7 Hz). These conformational features are summarized in structure IV.

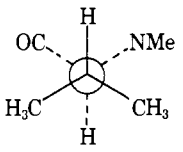
The D-Ser-NMe Val Fragment. It can be assumed that the preferred conformation for the ester linkage between D-Ser and NMe Val residues is the trans (syn-planar) form as found in many crystal studies of esters.[13] Thus the $C_\alpha-C_\beta-O-C(=O)-C_\alpha$ bonds all lie in the same plane as shown in structure IV.

Potential energy calculations on N-methylated peptide residues indicate that both cis and trans peptide bonds have similar energies and both forms must therefore be considered. Manger and co-workers[14] have suggested that the distinction between these forms can be made by considering the shift of the αCH proton (cis, 3.7–3.9; trans, 4.5–4.9 ppm). On this basis, the observed values of the αCH proton shifts for echinomycin indicate trans peptide bonds. Furthermore, it was



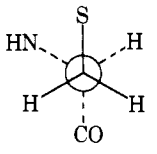
IV

found in the model-building studies that no structure of echinomycin could be built with cis NMe peptide bonds. In the absence of the Val NH proton we are unable to obtain information on the ϕ angle of the Val residue. However, from the observed $J_{\alpha CH\beta CH}$ coupling constant the Val side-chain conformation can be determined unequivocally. Because the observed coupling constant has a very large value (10.05 Hz) it can be ascribed to a rigid trans arrangement of the interacting protons as shown in V.

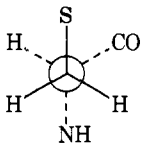


V

Thioacetal Cross-Linkage. In the thioacetal cross-linkage, there are three vicinal coupling constants which can be used to obtain conformational information. For the fragment $-CHCH_2S$ the two couplings (J = 1.7, 11.2 Hz) are exactly those expected for a rigid staggered conformation (either VI or VII; since the two methylene protons cannot be individually

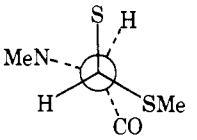


VI



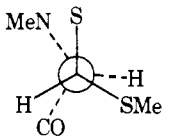
VII

assigned, these two conformations cannot be distinguished). Such extreme values of the coupling constants cannot result from an averaging process involving the different rotamers. In the $-CHCHS_2$ part of the thioacetal linkage only a single coupling constant defines the dihedral angle; if we make the reasonable assumption that this angle is also rigid, then the observed coupling constant (J = 8.7 Hz) corresponds to either structure VIII or IX. The other rotamers which are consistent with this coupling constant have the two protons almost eclipsed (χ = 30°, 335°) and can be eliminated from consideration on the grounds of steric hindrance between the sulfur atoms and the C_α substituents.



$\chi = 150°$

VIII



$\chi = 208°$

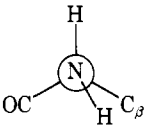
IX

A complicating factor is that the chirality of the carbon bearing the two sulfur atoms is still unknown.

The D-Ser-Ala Fragment. The only remaining information relevant to the conformation of the ring which is available from coupling constants is that for the Ala ϕ dihedral angle. The observed J_{NH-CH} coupling constants (7.3, 7.05 Hz) are slightly different in the two halves of echinomycin but they reflect conformations differing in dihedral angle by less than 5°. Assuming a rigid structure, the Bystrov relationship for an L residue

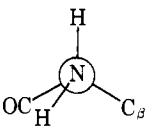
$$J_{NH-CH} = 8.9\cos^2(\phi + 300) - 0.9\cos(\phi + 300) + 0.9\sin^2(\phi + 300) \quad (1)$$

was used to estimate the ϕ dihedral angles (−86, −153, 41, 79°); the most likely values, based on considerations of peptide potential energy calculations,[15] are −86 or −153° (structures X and XI, respectively). When one considers the whole echi-



$\phi = -86°$

X



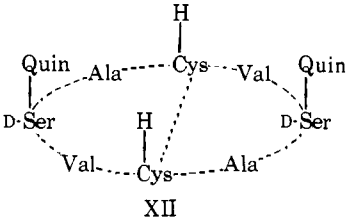
$\phi = -153°$

XI

nomycin molecule including the constraint of cyclization, the potential energy calculations show a clear preference for structure X.

Model-Building Studies. In these studies space-filling (CPK) models were used to build the possible conformational structures for the echinomycin ring. The conformational knowledge obtained from the NMR data and illustrated in structures II–XI was incorporated into the model-building process. The requirement for cyclization of the two halves of the peptide ring structure considerably limits the number of possible conformations. When the requirement for cyclization is combined with the information obtained from the spin-spin coupling constants on the conformation of the D-Ser-NMe-Val fragment and the thioacetal cross-linkage, it is found that all the possible conformations have a number of features in common. In each case, the overall shape of the molecule is that of a rigid disk, with the quinoxaline groups at the extreme ends, and both on the same side of the disk, extending approximately perpendicular to the plane of the disk.

The CysαCH protons are also both on the same side of the disk as the quinoxaline groups (see structure XII). This side

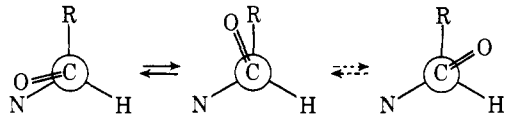


XII

of the ring is designated as the upper side. It was possible to construct models in which the NMe and SMe groups were either on the upper or lower side of the ring without seriously modifying the overall shape of the echinomycin ring.

In building the models we kept in mind the preferred orientations of the various groups in simple peptides,[15-17] amides, and esters. For example, all the NH peptide bonds were as-

sumed to be trans. For NMe peptide bonds both cis and trans forms were considered but no models with cis bonds could be constructed. The possibilities considered for the ψ angles, for which no information is available from coupling constants, were those normally found in peptides (XIII, XIV, XV). For



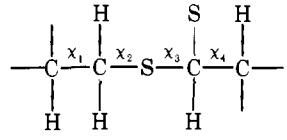
$\varphi = 180$ $\varphi = 90$ $\varphi = 0$

XIII XIV XV

the NMe Val and NMe Cys residues, there is no information available from coupling constants about either the ϕ or ψ angles. It has been shown[16] from semiempirical potential energy calculations (and confirmed with the potential functions used in this work) that the conformational freedom of an NMe-Val residue is very limited, and that the following (ϕ, ψ) combinations correspond to minimum energy conformations: (−119°, 90°), (−124°, −76°), (61°, 86°), and (58°, −91°). We have considered all these possibilities when examining the conformations of the NMe Val fragments of echinomycin. Potential energy calculations based on the whole molecule including the constraint imposed by the cyclization indicate that the (−119°, 90°) combination of angles is favored.

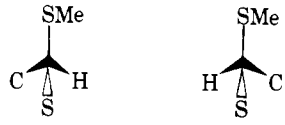
Potential energy calculations on the conformation of an *N,S*-dimethylcysteine residue showed that of the four low-energy conformations available to an NMe-Val residue, only two, (corresponding to (ϕ, ψ) values of (−125°, 90°) and (50°, 80°), are available to *N,S*-dimethylcysteine.[8] These results allow us to eliminate from consideration any conformation in which the carbonyl groups of the Cys residues are above the plane of the echinomycin ring.

The complete thioacetal cross-linkage (XVI) is character-



XVI

ized by four dihedral angles, χ_1, χ_2, χ_3, and χ_4. While χ_1 and χ_4 can be partially defined from studying the three-bond proton coupling constants, no information on the angles χ_2 and χ_3 is available from this source. For each of the chiral forms XVII and XVIII there are essentially two conformational possibil-



XVII XVIII

ities for each of the χ_1 and χ_4 angles which are consistent with the coupling constants. For each of these conformations, cyclization of the main echinomycin ring can be achieved by choosing appropriate values for χ_2 and χ_3. The resulting conformations for the thioacetal cross-linkage have essentially the same separation between the Cys α-carbon atoms. As will be seen later, each of these bridge conformations is consistent with each of the ring conformations to be considered for echinomycin.

Based on the foregoing considerations three basic conformational structures of the echinomycin ring could be constructed.

Model A. The main distinguishing feature of this conformation is that the four NMe groups are all on the upper side of the molecules.

Model B. This model is illustrated in Figures 3 and 4. It is similar to model A except that the NMe and CO of the Ala-

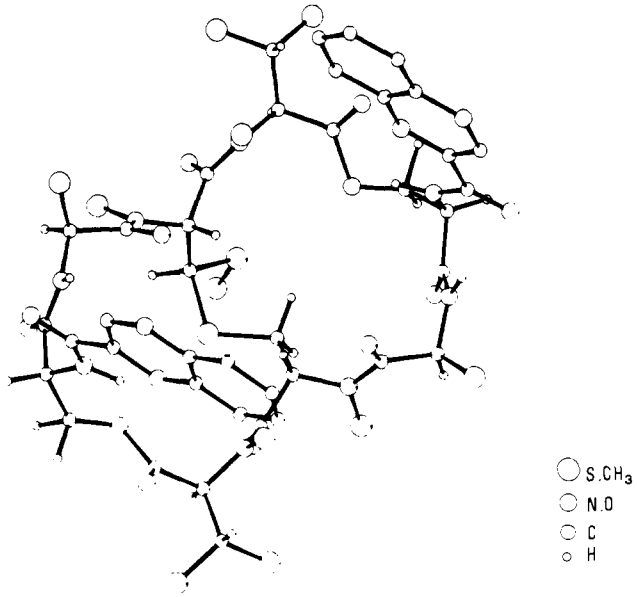
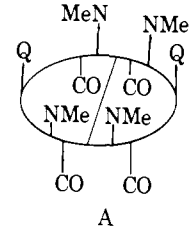


Figure 3. The conformation of echinomycin in model B. The torsion angles used are those obtained from potential energy minimization on model B and their values (given according to the rules of the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970)) are as follows.

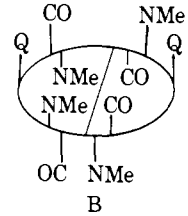
Residue	ω	ψ	ϕ
Val(1)	178.4	74.4	−113.1
Cys(1)	146.6	103.0	−121.8
Ala(1)	−170.3	126.0	−92.6
Ser(1)	170.8	−21.4	86.0
Qui(1)	168.2	−3.3	
Val(2)	174.8	80.6	−115.7
Cys(2)	161.5	97.5	−137.1
Ala(2)	−152.9	113.8	−91.9
Ser(2)	169.5	−25.8	88.0
Qui(2)	162.5	−3.2	

Cys peptide bond have been rotated through 180°. Thus the Cys NMe and CO groups are on the lower side of the ring and the Val NMe and Ala CO groups are on the upper side.

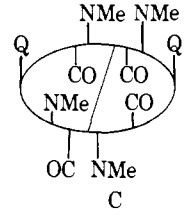
Model C. In this model one-half of the echinomycin ring has a conformation corresponding to model A, and the other half



A



B



C

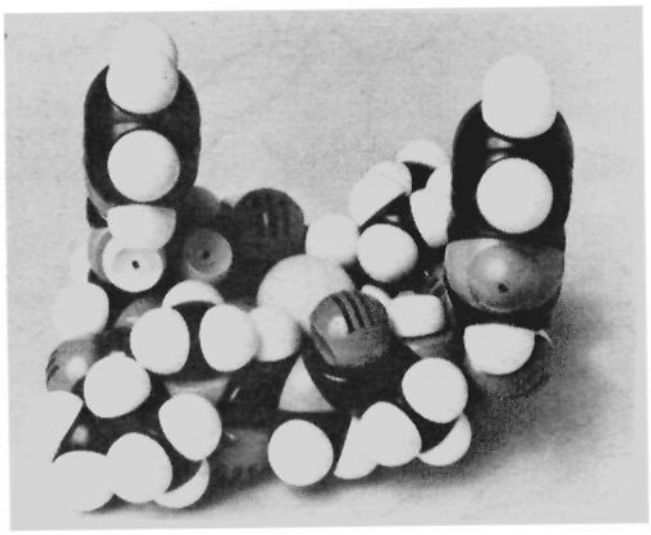


Figure 4. The space-filling model of echinomycin in model B where the Val NMe and Ala CO groups are on the same side of the ring as the quinoxalines and the Cye NMe and CO groups are on the other side of the ring.

Table V. ^{1}H Chemical Shift Changes in Echinomycin (18.8 mM $CDCl_3$) in the Presence of an Equimolar Concentration of La(fod)$_3$

Proton[a]	δΔ, ppm
CH=N	−0.015, −0.035
Ser NH	+0.16, +0.06
Ala NH	+0.135, −0.015
$CH_XCH_ZS_2$	−0.01
CH_XCH_2S	−0.07
Val α	−0.03, −0.06
H_A, H_B	−0.04, −0.06
NMe	−0.04, −0.04
	−0.04, −0.04
SMe	−0.01
Ala Me	−0.03, −0.04

[a] All other protons have shifts of less than 0.01 ppm. Positive shifts are to low field.

one corresponding to model B. Three NMe groups are thus on the upper side of the ring and one below. Because of the asymmetry of the thioacetal cross-linkage, there are two possible versions of this structure; in C1 the SMe group is in the half of the molecule corresponding to model B, while in C2 it is in the half corresponding to model A.

It is interesting to note that none of the models possess intramolecularly hydrogen-bonded NH protons. However, in all the models the D-Ser NH protons are inaccessible to solvent.

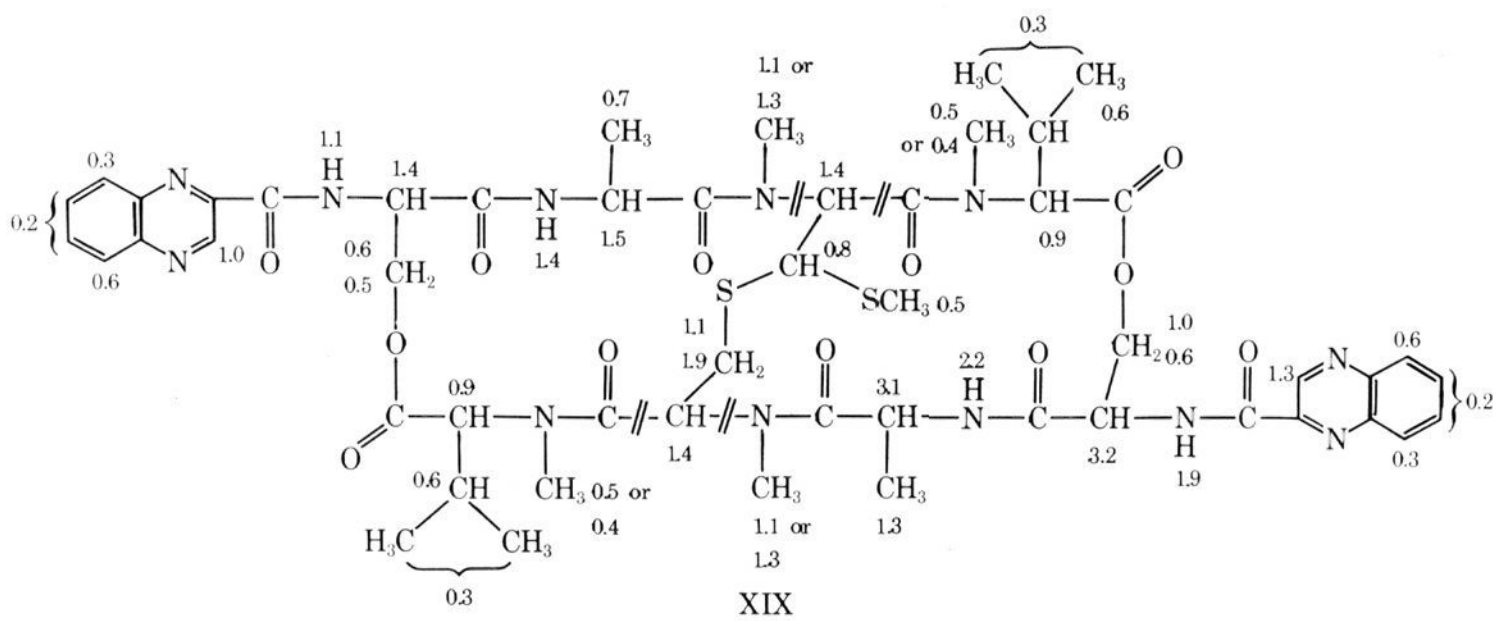
For all three models it was possible to construct conformations of the thioacetal cross-linkage which fitted the coupling constant criteria and yet resulted in the SMe group being either above or below the plane of the ring.

Distinguishing between the Structures. The energy of each of these models has been calculated by the methods described earlier.[8] These relatively crude calculations, which essentially include only torsional and van der Waals potentials, allow us to assess the stereochemical feasibility of the various conformations in more detail than is possible from model building. After optimization of those torsion angles for which no experimental evidence is available, all the models described above had energies which were sufficiently similar to preclude any choice being made between them on the basis of these calculations. We have thus considered ^{1}H chemical shifts and lanthanide-induced shifts in an attempt to make a choice between these models.

Lanthanide-Induced ^{1}H Chemical Shifts. The europium-induced shifts for echinomycin in the presence of Eu(fod)$_3$ have been measured and the values for an equimolar mixture are given in parts per million on structure XIX. It is seen that different lanthanide-induced shifts (LIS) are observed in the different halves of the molecule. In listing the shifts on structure XIX two assumptions have been made: (1) that the larger LIS are found in the same half of the molecule and (2) that the Cys NMe protons have larger LIS than Val NMe protons.

An interesting feature of the data is that for each pair of like protons from the two halves of the molecule the lower field proton almost invariably has the greater europium-induced LIS (the only exceptions are the Val and D-Ser α protons (the former shifting to the same extent) and the lower field NMe pair). This would be consistent with the idea that the less shielded members of pairs of like protons originate from the same half of the molecule and that it is this half of the molecule which tends to bind more strongly to Eu(fod)$_3$ and to show a somewhat greater temperature dependence of its chemical shifts. The more shielded half, on the other hand, is somewhat more affected by intermolecular ring stacking. Because there are several groups in echinomycin capable of binding to lanthanide complexes we have not attempted to reach a quantitative understanding of the lanthanide-induced shifts. Lanthanides are known to coordinate strongly to amides through the carbonyl oxygen.[18-21] Binding to ester groups is much weaker. A comparison of the LIS data for quinoxaline and quinoxaline-2-carboxamide indicates that although there is some binding to the ring nitrogen atoms this is also much weaker than that to the carbonyl oxygen in the carboxamide derivative.

The largest shifts induced by the diamagnetic La(fod)$_3$ complex (see Table V) are around the D-Ser NH-αCH portion of the molecule and reflect conformational changes on binding. It was also noted that the D-Ser $J_{\alpha CHNH}$ coupling constant decreased by 1–2 Hz upon La(fod)$_3$ binding. The conformational change about the D-Ser αCHNH bond (φ) could take place to allow the quinoxaline CO and the D-Ser CO to par-



XIX

ticipate in a more favorable bidentate interaction with the La(fod)$_3$ complex. This is further indicated when the Eu(fod)$_3$ induced shifts in echinomycin (structure XIX) are examined; the largest LIS values are observed for nuclei in the fragments near to the D-Ser and quinoxaline carbonyl groups (Ala αCH 3.1, 1.5), Ala NH (2.2, 1.4), D-Ser αCH (3.2, 1.4), D-Ser NH (1.9, 1.1), and the quinoxaline H3 (1.3, 1.0). The three models A–C can now be examined for their consistency with the ^{1}H chemical shifts and LIS data.

Model A. In this conformation all the CO groups are pointing downward so we would expect the Eu(fod)$_3$ to have little shielding effect on protons above the ring. However, the NMe groups and the CysαCH protons, which are all on the upper side in this structure, have appreciable LIS values which would be difficult to explain if the molecule had this conformation.

This model also offers no explanation for the low-field shift of the CysαCH protons. The quinoxaline rings are too far away from these protons to give substantial low-field ring current shifts.

Model B. This structure has carbonyl groups above and below the ring and one could easily explain the observed LIS data for the NMe and CysαCH protons. Furthermore, the presence of the Ala CO groups adjacent to the CysαCH protons would explain the deshielding of these protons.

Model C. This structure would be difficult to reconcile with the ^{1}H and LIS data. First, only one of the CysαCH protons would have an adjacent carbonyl group yet they both have similar unusual low-field shifts and experience similar LIS effects. Second, one might have expected the Cys NMe group which is pointing down to have shown more characteristically different LIS behavior from the other three NMe groups which are pointing up.

We conclude that the most likely structure for the echinomycin ring in solution is represented by model B with the quinoxaline tails in the conformations shown in structure IV.

Thioacetal Cross-Linkage Conformation. We have already seen that the conformation of this part of the molecule is incompletely defined by the NMR studies. In addition to the ambiguity of the torsion angles as represented in structures VI–IX there are the problems of the unknown chirality of the carbon bearing the two sulfur atoms and the orientation of the SMe group in the overall conformation of echinomycin.

There is some experimental evidence bearing on the question of the SMe group orientation. The ^{1}H chemical shifts for the SMe protons in echinomycin and model compounds such as *S*-methyl-L-cysteine (^{1}H 2.04, 2.05 ppm, respectively) are very similar and this suggests that the SMe group in echinomycin is below the ring. If the SMe were above the ring it is difficult to envisage how it could avoid some anisotropic shielding effects from one of the quinoxaline rings. The absence of self-association effects on the SMe proton shift is further evidence that it is pointing down. In fact potential energy calculations on the whole molecule favor this orientation of the SMe group in a conformation of the thioacetal cross-linkage having structures VII and VIII and the chiral form XVII.

Overall Conformation of Echinomycin. Thus the overall conformation for echinomycin shown in Figures 3 and 4 represents the well-defined ring conformation of model B and also these features of the thioacetal linkage conformation. As noted earlier, the conformation of the latter appears to have little influence on the overall conformation of the echinomycin ring.

It is seen that the overall conformation retains substantial elements of symmetry in spite of the asymmetrical nature of the thioacetal cross-linkage. The downward orientation of the SMe group appears to minimize the conformational difference between the two halves of the molecule.

This model differs from that proposed earlier[8] on the basis of potential energy calculations alone, which corresponds to model C, with the SMe group oriented upward.[23] The more recent potential energy calculations have shown that the energy difference between this conformation and the one shown in Figure 3 is less than 3 kcal/mol, which must be considered small in view of the approximate nature of the calculations.

Relevance of the Echinomycin Conformation to DNA Binding. It is, of course, possible that the conformation of the antibiotic in aqueous solution or in the DNA-bound state may differ from that favored in organic solvents (and investigated here). The evidence that the peptide ring behaves as a substantially rigid unit tends to argue against the suggestion that major conformational changes such as rotation of peptide units through approximately 180° might occur on transfer to an aqueous solvent or accompany complex formation with DNA. Nevertheless, the possibility cannot be eliminated that conformations A and C might be adopted under certain conditions; with this in mind further NMR experiments are in progress, as well as calculations to estimate energy barriers to conformational changes. In any event the flexibility of the serine αCH–NH bonds permits some variation in the mutual disposition of the quinoxaline chromophores. However, it is of interest to note that whatever rotation may occur about these bonds the distance between the points of attachment of the chromophores to the peptide ring remains about 10 Å in all models, effectively setting an upper limit to the separation between them. This distance is practically ideal for the formation of a two-base-pair sandwich in the bifunctional intercalated DNA complex if the average planes of the quinoxaline rings lie more or less orthogonal to a line joining the serine α-carbon atoms, consistent with the essential prediction of the neighbor-exclusion hypothesis for intercalative binding to DNA.[7,21] It is also noteworthy that conformations having the S–CH$_3$ group down (i.e., facing in the opposite direction from the intercalative chromophores) present less steric hindrance to effective penetration of the quinoxaline rings between the DNA base pairs.

To explain the large differences in binding constants for association of echinomycin with different DNA polymers[7,22] we currently favor an important role for hydrogen bonding interactions between narrow-groove substituents on the DNA base pairs and functional groups in the octapeptide ring. Chief among the latter must be the serine and alanine NH groups, and also the alanine CO group(s) in those conformations (B and C) where one or both of them are placed "up". With particular regard to our preference for model B we have looked to see whether both Ala carbonyl groups in the "up" orientation might be able to form stereochemically acceptable hydrogen bonds to the guanine 2-amino functions in a sequence of GC base pairs. It appears difficult to involve both the CO groups in hydrogen bonding in this way without substantial structural distortion of the helix over and above the anticipated unwinding of about 50° (ref 6, 7). This might provide a basis for the suggestion that the base sequence (or sequences) generating the optimum sites in DNA for binding echinomycin may contain all four nucleotides.[7] Conformational studies on triostin A and other structural analogues of echinomycin will help to resolve this problem since it is known that such analogues do display different preferences for DNA binding (ref 22 and our unpublished observations). To this end further NMR experiments and computer-assisted conformational studies are underway.

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(23) In addition, the model proposed in ref 8 has the quinoxaline ring approximately at right angles to the carbonyl group. The anticipated partial double bond character of the C2–CO bond and the LIS results on quinoxaline carboxamide make the conformation shown in structure IV substantially more probable.

Heteronuclear Vicinal Coupling Constants and Site-Specific Isotopic Substitution in the Investigation of Rotational Isomerism in Leucine

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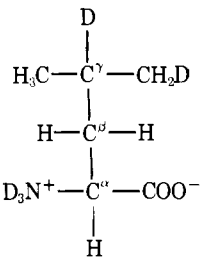
Abstract: Vicinal coupling constants about the C^{α}–C^{β} bond between combinations of ^{1}H, ^{13}C, and ^{15}N nuclei were measured in three isotopic isomers of leucine. The couplings found were used to calculate populations of staggered rotamers. These values, which are overdetermined by the several coupling constants, are found to be self-consistent for both anionic and cationic forms. The necessity for simplification of the spin system by substitution of deuterium for hydrogen is discussed. A statistical method of using the observed couplings to make stereochemical assignments is introduced.

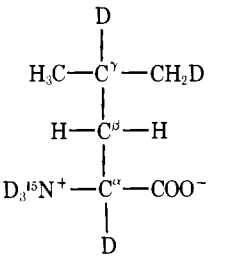
It has been generally accepted that the conformational properties of amino acids, peptides, and proteins in solution can be described by determining the appropriate torsional angles, inasmuch as bond lengths and bond angles remain relatively constant in such molecules.[2] The torsional angles connected with the backbone of a peptide have received major attention, while it has often been assumed that the first torsional angle of the side chains in peptides is averaged by rapid rotation about the C^{α}–C^{β} bond. Vicinal coupling constants have been used to measure the rotamer populations about these bonds[2] in amino acids and a few peptides using standard assumptions.[3] Most studies have centered around the vicinal couplings between protons, but some of the other vicinal couplings have been employed or proposed, such as those between the amide ^{15}N and the β protons[4,5] and those between the carbonyl ^{13}C and the β protons.[6,7]

This report is concerned with NMR studies of a series of isotopic isomers of leucine (**1**–**3**), which were designed to determine as many of the vicinal coupling constants as possible, both homo- and heteronuclear, in a single compound; homonuclear H^{α}–H^{β} couplings for **1** have been previously reported and the equilibria between rotamers derived from these values have been discussed.[8] Syntheses of these isomers were performed by modifications of standard methods.



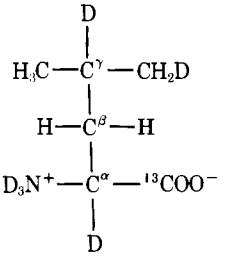
[γ,δ-$^{2}H_2$]leucine

1



[$^{15}N',\alpha,\gamma,\delta$-$^{2}H_3$]leucine

2



[$^{13}C',\alpha,\gamma,\delta$-$^{2}H_3$]leucine

3

Experimental Section

Racemates were used throughout this work. In discussion of identities of rotamers, the L isomer only is considered (see Figure 1). The population states, I, II, III correspond to the torsional angles (−60, 180, +60°) for the L isomer and (+60, 180, −60°) for the D isomer

Amino acid analyses were carried out on a Beckman MS amino acid

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