Nuclear Magnetic Resonance Studies and Conformational Analysis of Bicyclic Inhibitors of Angiotensin-converting Enzyme. Part 2.[1] The Octahydro-6*H*-pyridazo[1,2-*a*][1,2]diazepines

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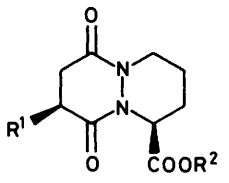
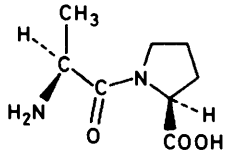
The conformations are determined of a number of bicyclic heterocycles synthesised as part of a programme related to the design of inhibitors of angiotensin-converting enzyme (ACE). In these compounds, a six-membered ring is fused to a seven-membered ring *via* a diaza bridge. By detailed ^{1}H n.m.r. spectroscopy, the 6-oxo series is shown to be rigid with the axial 1-carboxy anchoring the system. In the 6-deoxy series some flexibility in the seven-membered ring is apparent, though nitrogen inversion is prevented. *X*-Ray diffraction, molecular graphics, and MNDO calculations are employed to assist in the conformational analyses. The three-dimensional arrangement of the three enzyme-binding groups in these compounds provides very potent inhibitors (I_{50} 1.6—3nM).

In Part 1[1] the conformations of bicyclic compounds involving two fused six-membered rings (**I**) were determined. Compounds of type (**I**) were generally less active against angiotensin-converting enzyme (ACE) than at first expected.[2–4] However, in detailed computer graphics studies it became clear that the 6,6-bicyclic compounds were mimicking a high-energy conformation of the peptide alanylproline (**II**), where the methyl group of the alanine was close to the proline δ-methylene group.[4] Since this was unlikely to be the 'active' conformation, ways of relieving this strain were investigated. It was reasoned that replacing the left-hand pyridazine ring by a diazepine ring might well relieve the strain, and produce compounds which would match a more realistic lower energy conformation of alanylproline. Molecular graphics investigations confirmed that this idea was reasonable, and a series of octahydro-6*H*-pyridazo [1,2-*a*][1,2]diazepines (**III**)—(**IX**) was thus designed and eventually synthesised.

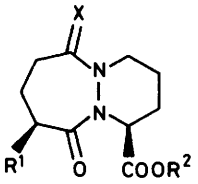
It was then necessary to determine whether the conformational predictions made by molecular graphics were borne out in practice. Detailed high-field n.m.r. studies were carried out on compounds (**III**)—(**IX**) and the corresponding intermediates in the syntheses. The complexity of some of the spectra, with up to 27 non-equivalent protons present, necessitated the use of fairly sophisticated assignment techniques. Homonuclear shift-correlated two-dimensional spectra methods were used in some cases where complete assignment was not possible by conventional selective decoupling experiments. The conformations were determined by application of the well established dependence of vicinal coupling constants on the dihedral angle φ between the coupling protons ($J \propto \cos^2\varphi$).[5] NOE Experiments were also attempted to assist with assignments and with conformational predictions. However, in this particular series of compounds, some of which were found to be conformationally mobile, ambiguous results were sometimes obtained.



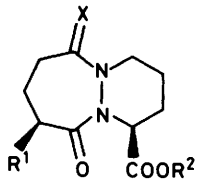
(**I**) R^1 = $PhCH_2CH_2CH(COOH)NH$, R^2 = H



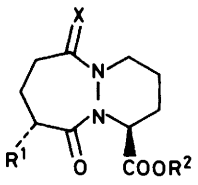
(**II**)



(**III**) $R^1 = NH_2$, R^2 = H, X = O
(**IV**) R^1 = $PhCH_2CH_2CH(COOEt)NH$, R^2 = H, X = O
(**V**) $R^1 = NH_2$, R^2 = H, X = H_2
(**VI**) R^1 = $PhCH_2CH_2CH(COOEt)NH$, R^2 = H, X = H_2



(**VII**) R^1 = Phthalimido, R^2 = Bu^t, X = O
(**VIII**) R^1 = Phthalimido, R^2 = Bu^t, X = H_2



(**IX**) R^1 = Phthalimido, R^2 = Bu^t, X = O

Experimental

The compounds examined in this work were synthesised by Drs. Attwood, Redshaw, and Lawton to whom we are grateful for supplying generous samples. Their synthesis has been described briefly,[4] and will be reported in full elsewhere.[6] N.m.r. spectra were normally obtained on a Bruker WM-300 spectrometer. Some spectra were obtained at 400 MHz (Bruker WH-400 and AM-400 instruments) and a few of the most complex spectra were obtained on Bruker AM-500 instruments. Computer graphics studies, run in parallel with the n.m.r. investigations, were carried out using a Megatek 7000 display processor interfaced to a VAX 11/750 computer, with software largely developed in-house. The CALCOMP plotter attached to this system was used to produce some of the Figures shown here. MNDO Calculations, which will be reported in full elsewhere,[7] were also carried out on the VAX 11/750 computer.

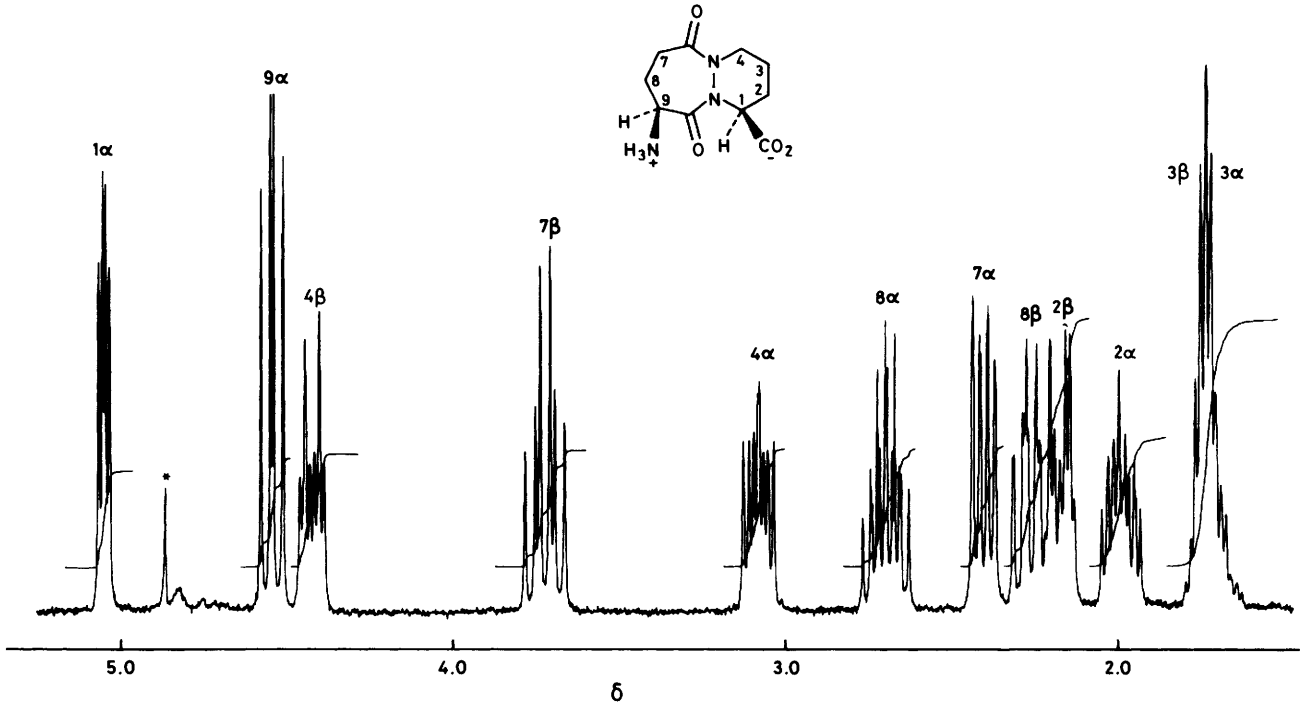


Figure 1. 300 MHz ^{1}H N.m.r. spectrum of the bicyclic amino acid (**III**) in deuterium oxide solution. Assignments are shown. • Residual water peak suppressed by gated decoupling

Results

(A) *The 6-Oxo Series* (**III**), (**IV**), (**VII**), *and* (**IX**).—(1) *The conformation of the* 7,6-*amino acid* (**III**). The first compound to be examined in detail was the 7,6-amino acid (**III**). At the time of synthesis and by the methods used, the relative stereochemistry of the two chiral centres was not known. However, by the synthetic route chosen only one stereoisomer was isolated in a pure state, and this was examined in detail. The ^{1}H n.m.r. spectrum in D_2O is shown in Figure 1. The clear pH dependence of the two low-field resonances allowed the assignment of these two multiplets to 1- and 9-H, respectively. The narrow multiplet for 1-H (splittings 6.1 and 3.8 Hz) suggest that as in the previous octahydropyridazopyridazines the carboxy group is axially oriented in a chair conformation.[1] Spin-decoupling experiments and further comparison with the spectra of the 6,6-bicyclic compounds allow the assignment of the protons on C-2, -3, and -4. As seen previously the two protons on C-4 are widely separated, at δ 3.07 (axial) and 4.42 (equatorial) due probably to the equatorial proton (4β) lying in the plane of the amide group and experiencing a strong deshielding effect. On the other hand, in neutral aqueous solution, the two protons on C-3 have almost degenerate chemical shifts. Protonation of the carboxy group, in DCl–D_2O removed this degeneracy.

For the seven-membered ring, spin decoupling of the 9-H multiplet allowed the assignment of 8-H_2 at δ 2.25 and 2.68, and hence 7-H_2 at δ 2.39 and 3.71. At this stage without consideration of the conformation of the molecule it was not possible to assign the relative stereochemistry, nor to assign α or β status to the individual methylene protons on C-7 and -8. However, the individual coupling constants and chemical shifts were easily determined.

The large chemical shift differences for the protons on C-4 and those on C-7, together with the wide range of vicinal coupling constants (1.3—13.0 Hz), strongly indicate a *rigid* conformation for the bicyclic system. Any conformational averaging would result in closely similar chemical shifts for the methylene pairs and consequent averaging of the coupling constants.

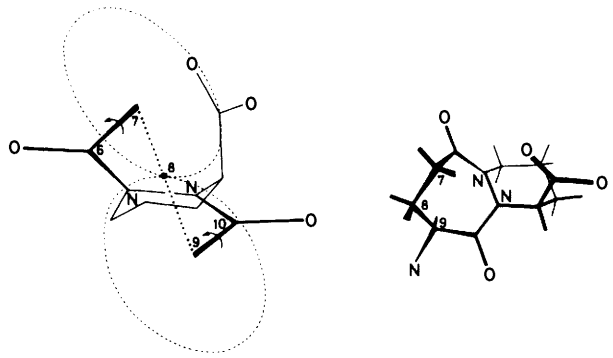


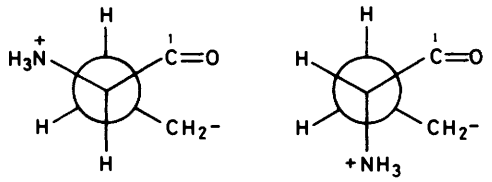
Figure 2. Computer graphics representation of how the seven-membered ring in the 6-oxo series can have only one conformation. The enforced axial carboxy group in the six-membered ring, which adopts a chair conformation, requires that C-7 and -9 are fixed in space. The position of C-8 is found uniquely by drawing two circles of radius 1.54 Å by rotation about the C-6–C-7 bond and the C-9–C-10 bond. The point of interaction of the circles defines C-8 and hence the whole ring conformation shown on the right

Making the reasonable assumption that the amide groups are planar with a torsion angle of *ca.* 60° between the two planes because of the requirements of the chair conformation for the six-membered ring, with its carboxy group fixed in an axial conformation, the relative positions of C-7 and -9 are therefore fixed in space as shown in Figure 2. This leaves only one possible position for the carbon linking these two, *i.e.* C-8, and hence the total ring conformation can be predicted as shown. Molecular graphics was used to confirm these predictions. It was of interest to note that the proposed conformation of the seven-membered ring is identical to that observed for the seven-membered ring of

Table 1. ^{1}H Chemical shifts for the bicyclic compounds (III)—(IX)

	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)
1α	5.04[a]	5.35[b]	4.64[c]	4.80[b]	5.30[b]	4.96[b]	4.30[b]
2α	1.97	2.03	~1.8	1.76	1.6—1.9	~1.75	1.7—2.1
2β	2.16	~2.3	2.32	2.41	2.26	2.35	1.7—2.1
3α	1.72[c]	1.72	1.42	1.35	1.6—1.9	1.42	1.7—2.1
3β	1.72[c]	1.62	~1.7	1.75	1.6—1.9	~1.75	1.7—2.1
4α	3.07	3.04	3.11	3.08	2.93	3.22	4.65
4β	4.42	~4.5	3.04	2.98	4.71	3.02	~3.3
6α			3.51	3.59		6.47	
6β			2.63	2.54		2.51	
7α	2.39	2.38	~2.03	2.09	2.47	2.29	3.06
7β	3.71	3.63	~1.73	1.72	3.68	1.87	2.52
8α	2.68	2.67	~2.08	2.04	2.38	2.43	3.32
8β	2.25	~2.25	~1.83	1.65	3.42	2.10	2.40
9α	4.54	4.49	4.90	4.50	5.29	5.70	
9β							5.33
C*H*NH		4.17		3.68			
CH_2CHNH		~2.3		~2.12			
PhCH_2		2.82		~2.75			
Ph		~7.2		7.15—7.30			
CH_3		1.34		1.30			
CH_3CH_2		4.31		4.22			
Bu^t					1.49	1.50	1.46
Phthalimido					7.75	7.70	7.74
					7.88	7.85	7.87
Solvent	D_2O	CD_3OD	D_2O	CD_3OD	$CDCl_3$	$CDCl_3$	$CDCl_3$

[a] Referenced to internal sodium trimethylsilylpropionate. [b] Referenced to internal tetramethylsilane. [c] In DCl–D_2O $\Delta\delta_{3\alpha 3\beta}$ = 23 Hz (0.08 p.p.m.).



(Xa) (Xb)

isocolchicine by n.m.r. spectroscopy[8] and *X*-ray crystallography.[9]

The large coupling constants for the 9-H (8.5, 11.5 Hz) are much more in agreement with the staggered arrangement (**Xa**) rather than (**Xb**), which would require much smaller vicinal couplings. This means that the $\overset{+}{N}H_3$ group is *cis* to the COO^- about the mean molecular plane, and 9-H is α in the enantiomer (**III**) (the *SS*-enantiomer). The larger coupling from 9α-H to 8-H_2 (11.5 Hz) is clearly to 8β-H which is *anti* to 9α-H. Similarly 8α-H in our predicted conformation is *anti* to 7β-H which will also have the larger coupling constant in this case, 13.0 Hz. Again, the smallest coupling constant (1.3 Hz) is expected to be between the two pseudoequatorial 7α- and 8β-H. The low-field shift of 7β-H is due to the fact that in the proposed twisted conformation of the seven-membered ring, this proton is close to the amide deshielding zone (see Figure 2). In Tables 1 and 2 the ^{1}H chemical shifts and coupling constants are recorded. Simulation of the spectra using the spin simulation program SIMEQ[10] confirmed the analysis.

(2) *The angiotensin-converting enzyme inhibitor* (**IV**). By a process of reductive alkylation, the appropriate side-chain was added to the amino acid (**III**) converting it into the prodrug of a highly potent ACE inhibitor (**IV**) (I_{50} = 3nM).[4] The ^{1}H and ^{13}C n.m.r. spectra of the hydrochloride were determined for solutions in deuteriomethanol rather than deuteriochloroform in which line broadening occurred apparently due to slow NH exchange. Assignments of the ring protons were very similar to those of compound (**III**) and those of the side-chain protons were trivial except for the four non-equivalent PhCH_2CH_2 protons which appeared as a complex ABMN pattern. Spin decoupling experiments again confirmed the assignments. The ^{1}H chemical shifts and coupling constants for this compound are recorded in Tables 1 and 2 and ^{13}C chemical shifts are recorded in Table 3. Full assignment was not attempted, as the information would add nothing to the conformational arguments.

It is clear from the coupling constants in Table 2 that the ring conformation is the same as that for the amino acid (**III**), *i.e.* it is not perturbed by the presence of the side-chain. No information could be obtained concerning the conformation of the side-chain itself; it is probably an average of several possible rotational isomers. Our predictions from graphics and experimental proof by n.m.r. spectroscopy were borne out by eventually having a crystal structure determination* of the *p*-methoxy derivative of (**IV**). The ring conformation in the crystal is precisely the same as that predicted from n.m.r. data within experimental error.

(3) *The phthalimido t-butyl esters* (**VII**) *and* (**IX**). An intermediate in the preparation of (**IV**) is the substituted compound (**VII**). Also isolated in the synthesis of this compound was the *trans*-isomer (**IX**). It was of interest to examine the n.m.r. spectra of these two compounds, to see if the *cis*-compound (**VII**) was in a similar conformation to that of (**III**) and (**IV**), and also to investigate the conflict of conformational preferences in the *trans*-compound (**IX**). In the latter the carboxy group has a strong preference for the axial position in a chair conformation, whereas from the studies outlined previously in this report it is clear that the phthalimido

* Detailed co-ordinates of the crystal structures reported in this manuscript are held by Dr J. J. Daly, Central Research Laboratories, Hoffman La Roche, Basle, Switzerland.

Table 2. Coupling constants for the bicyclic compounds (III)—(IX)

J/Hz	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)
1α2α	6.1	6.0	6.2	(Sum =6.1)	(Sum =7.9)	(Sum =6.25)	(Sum =10.0)
1α2β	3.8	2.8	2.0				
2α2β	14.0	13.0	13.0	13.6	13.9	*	*
2α3α	4.0	4.3	Small	3.0	*		
2α3β	11.5	13.0		~12.0			
2β3α	~2		Small	Small	Small		
2β3β	4.0			Small	Small		
3α3β	14.0	13.5	13.5	13.8		12.5	
3α4α	5.0		3.0	3.0	4.2	2.5	
3α4β	4.3		Small	3.0	Small	Small	
3β4α	10.5	12.0	~11	13.4	11.3	~12	
3β4β	4.3	3.8	Small	~2.0	Small	Small	
4α4β	12.9	12.0	14.0	13.8	12.9	12.5	
6α6β			12.2	11.8		10.8	
6α7α			4.2	4.3		4.1	
6α7β			6.3	4.3		4.1	
6β7α			7.0	8.4		9.3	
6β7β			5.1	5.4		5.5	
7α7β	13.0	13.5	~12		13.0	12.5	12.7
7α8α	6.9	7.0	3.0	~4.0	6.3	3.75	8.5
7α8β	1.3	<2.0	10.0	~10.3	1.3	11.2	12.7
7β8α	13.0	13.2	8.3	7.9	12.9	6.5	1.6
7β8β	9.0	8.8	3.0	~3	8.7	2.5	6.5
8α8β	13.0	12.8	12.0	12.3	13.0	12.5	13.3
8α9α	8.5	8.5	6.0	8.0	8.7	6.8	
8α9β							11.4
8β9α	11.5	10.8	8.1	8.0	11.7	8.8	
8β9β							8.9

* Full analysis prevented by overlap of multiplets and second-order effects.

Table 3. ^{13}C Chemical shifts for ACE inhibitors (IV) and (VI)[a]

(IV)		(VI)	
CH_3	14.40	CH_3	14.55
CH_2	21.04	CH_2	17.33
CH_2	26.05	CH_2	26.29
CH_2	29.50	CH_2	26.29
CH_2	30.66	CH_2	29.12
CH_2	31.97	CH_2	32.64
CH_2	33.00	CH_2	34.77
NCH_2	42.36	NCH_2	52.11
CH	54.43	CH	52.61
CH	57.66	NCH	53.03
CH	60.32	CH	58.96
OCH_2	64.17	CH	60.88
Ph	127.71	OCH_2	62.48
Ph	129.50	Ph	127.10
Ph	129.77	Ph	129.35
Ph	140.98	Ph	129.41
CO	167.47	Ph	142.07
CO	169.94	CO	173.42
CO	172.06	CO	173.56
CO	173.15	CO	175.09

[a] Solvent [$^2H_4$]methanol. [b] Referenced to internal tetramethylsilane.

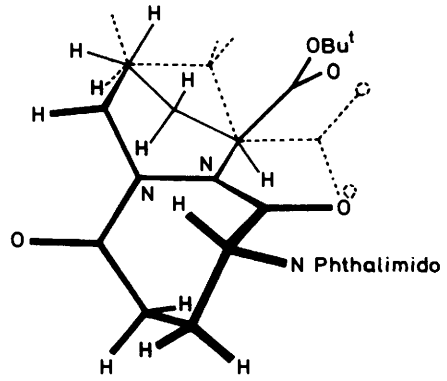


Figure 3. Predicted conformation of the *trans*-phthalimido butyl ester (IX) showing the seven-membered ring in the usual twisted conformation (in a mirror image sense) and the six-membered ring in a boat conformation. The dotted line indicates the alternative chair conformation with the carboxy group in the unfavourable equatorial position

group strongly prefers a pseudoequatorial conformation. With planar amide groups one of these preferences has to give way to the other.

Details of the ^1H n.m.r. spectra of (VII) and (IX) are given in Tables 1 and 2. The *cis*-compound (VII) is clearly in an identical bicyclic conformation to those of compounds (III) and (IV), the only difference being the low-field shift of the 8β-H, lying in the deshielding zone of the phthalimido group.[11] In the *trans*-isomer (IX), the protons of the seven-membered ring have coupling constants and shifts very similar to those in (VII) but in a mirror image sense, *i.e.* the seven-membered ring adopts the same conformation as in (VII) with the 9α-substituent pseudoequatorial. Since the chair conformation of the six-membered ring with the ester group equatorial is not allowed, a boat or twist boat conformation is adopted, reflected by the appearance of 1α-H as a triplet (*J* 5.0 Hz) at δ 4.31, and unusual shifts and couplings for the 2-, 3-, and 4-H. The preferred conformation of the *trans*-compound is shown in Figure 3.

(B) *The 6-Methylene Series* (V), (VI), *and* (VIII).—From knowledge gained with the analogous 6,6-bicyclic series it was evident that the 6-carbonyl group was unnecessary for binding to the enzyme.[4] Moreover reduction of CO to CH_2 might lead

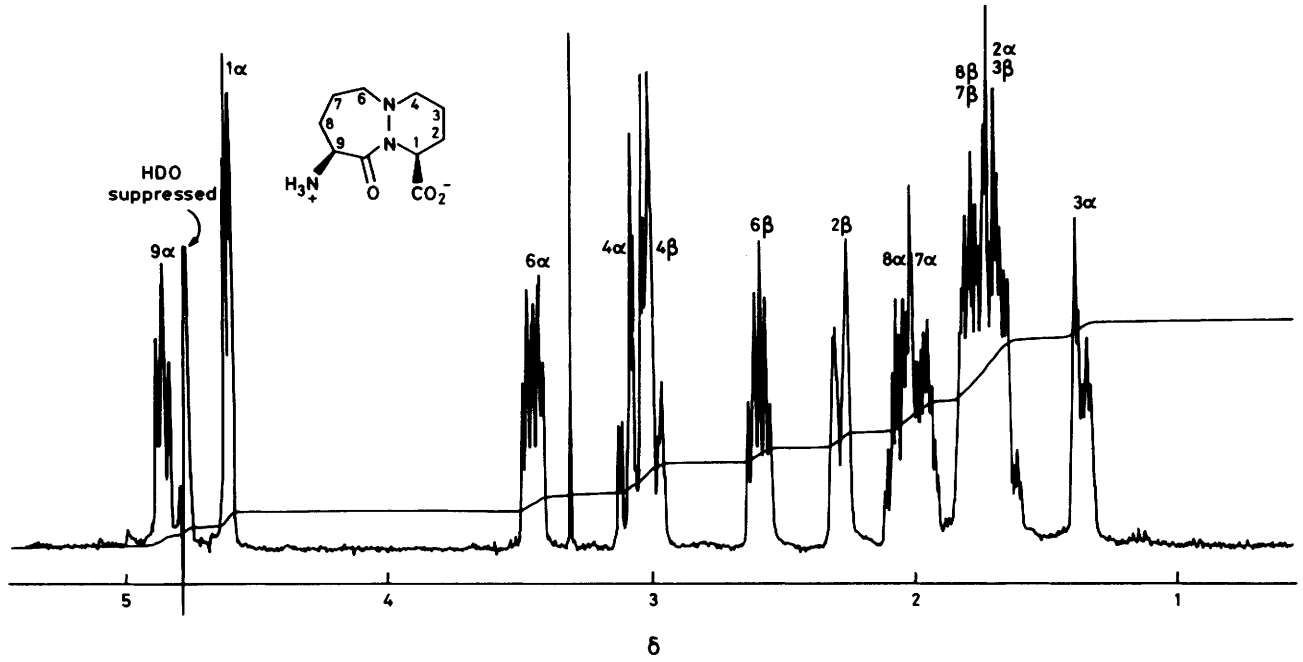


Figure 4. The 300 MHz ^{1}H n.m.r. spectrum of the amino acid (V) in deuterium oxide solution. Assignments are as shown. The water peak is suppressed by gated decoupling

to more lipophilic compounds with better absorption characteristics.[4]

(1) *The amino acid* (V). The complex ^{1}H n.m.r. spectrum of the amino acid (V) in D_2O is shown in Figure 4. Using spin decoupling and pH shifts (two-dimensional experiments were not used in this case) sufficient data were obtained to quote chemical shifts and some coupling constants with confidence (Tables 1 and 2), although individual assignment of the methylene pairs required conformational arguments as discussed later.

(2) *The phthalimido t-butyl ester* (VIII). Similar ^{1}H spectra were obtained for this compound, assigned by similar experiments plus COSY-45 2D n.m.r. Variable-temperature experiments are reported later. Data are given in Tables 1 and 2.

(3) *The* ACE *inhibitor* (VI) *cilazapril*. Complete analysis of the n.m.r. spectrum of this compound, which has 27 ^{1}H multiplets, required COSY-45 2D at 500 MHz (Figure 5). A full analysis was necessary for this compound since this is the prodrug ester form of a highly active inhibitor of ACE (I_{50} 1.6nM).[4] Coupling constants and chemical shifts are recorded in Tables 1 and 2.

Ring Conformations in the 6-Methylene Bicyclic Compounds.—The evidence from the chemical shifts and coupling constants for for the protons in the six-membered ring strongly indicates the characteristic chair conformation, with the 1-carboxy group axial. It seems probable that the lone pair of electrons on N-5 is α-oriented, *i.e. trans* to the carboxy group, for the following reasons. (i) Due to the *A*(1,3) effect[12,13] the C-6–N-5 bond prefers to be axial like the 1-carboxy group. (ii) In quinolizidine (X), the protons on the carbon adjacent to nitrogen are separated by *ca.* 0.9 p.p.m. with the higher field axial proton *anti* to the lone pair as shown.[14] Similarly, in *N*-alkylpiperidines (XI), the axial protons on C-2 or -6 are well to high field of the equatorial protons.[15] If the same followed in our 6,7-bicyclic series and the N-5 lone pair is α-oriented, the large shift difference between 6α- and 6β-H in all three compounds (0.9—1.1 p.p.m.) allows 6β-H to be assigned to the high-field proton of the pair. (iii) If the lone pair is α-oriented as suggested, it bisects the two C-4–H bonds and therefore should have an equal influence on both. The value for $\Delta\delta_{4\alpha4\beta}$ of only 0.1—0.2 p.p.m. supports this hypothesis.

It seems likely therefore that not only is the lone pair on N-5 α-oriented but that nitrogen inversion is prevented, so that the C-6–N-5 bond stays axial [(XIV)]. This seems at first surprising, but it is in reality just another manifestation of the *A*(1,3) effect observed for instance with acylation of *cis*-2,6-dimethylpiperidine [(XII)→(XIII)] which forces the flanking methyl groups into energetically disfavoured axial conformations.[13]

Conformation of the Seven-membered Ring.—The diazepinone ring in these compounds can be compared favourably with caprolactam (XV), which adopts a chair conformation in the crystal.[16] Theoretical calculations on cycloheptene, on the other hand, indicate that while the chair conformation (XVIa) is the most stable, the twist boat form (XVIc) is only 0.6 kcal mol^{-1} higher in energy, whereas the boat form (XVIb) is of considerably higher energy.[17] In solution therefore it would be expected that the diazepinone would adopt the chair conformation (XV) with a contribution also perhaps from one or more of the possible twist-boat conformations.

MNDO calculations[6,7,18] on all possible conformations of the amino acid (V) reveal a ranking file where the chair–chair form (Va) is the most stable, followed by two twist-boat–chair forms (Vb) and (c), all three having the C-6–N-5 bond and the COOH group axially oriented in the six-membered ring. The alternative chair–chair form (Vd) where the N-5–C-6 bond is equatorial is much higher in energy.

During these deliberations, the crystal structure of the hydrate form of compound (VI) was determined, a representation of which is shown in Figure 6. This confirms that the bicyclic system adopts the favoured chair–chair conformation in the crystal, with the carboxy groups on C-1 axial and the side-chain on C-9 equatorial. As predicted, the C-6–N-5 bond is axial, though somewhat distorted, presumably through 1,3-interaction with the 1-carboxy group. The amide bond is almost

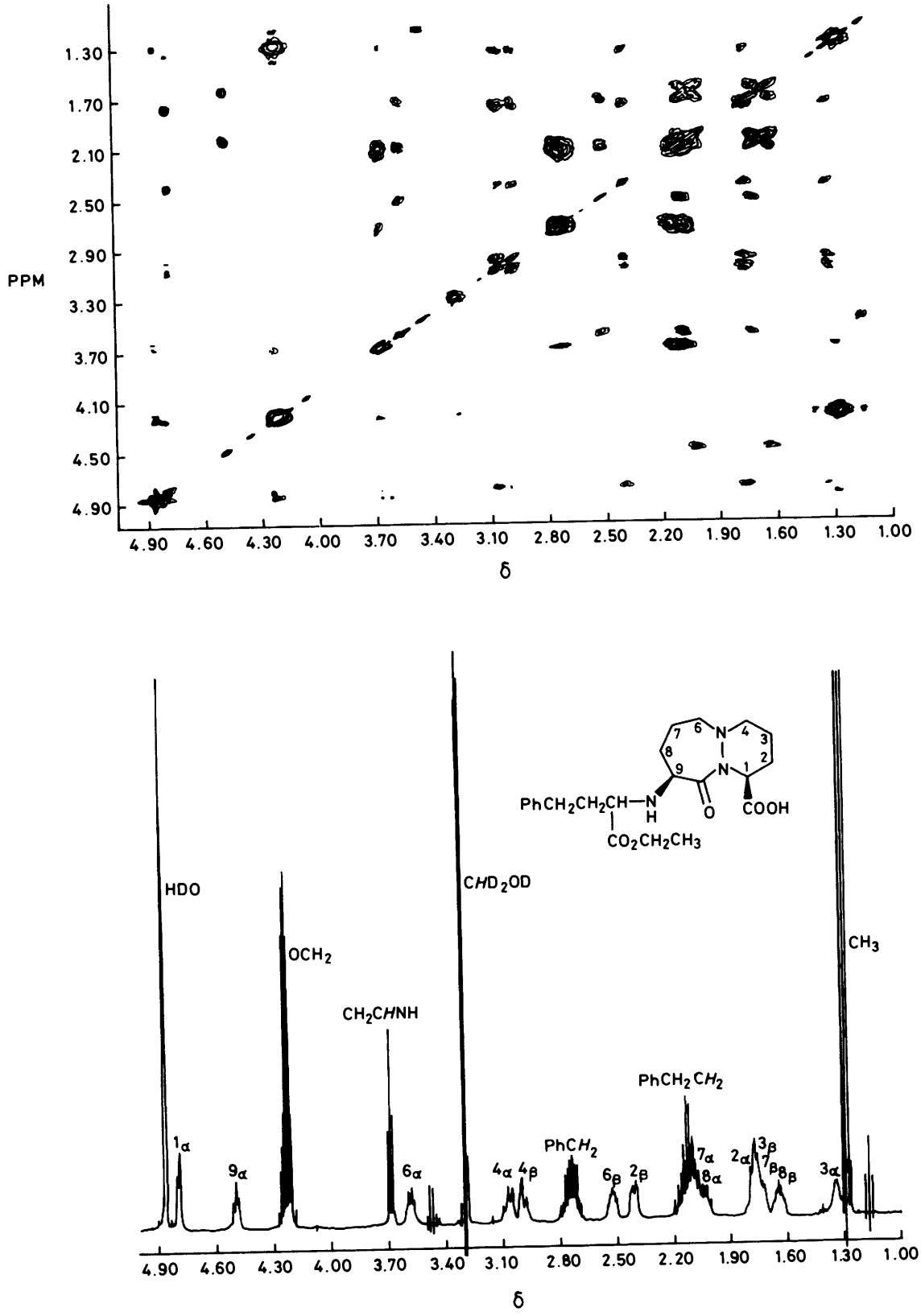
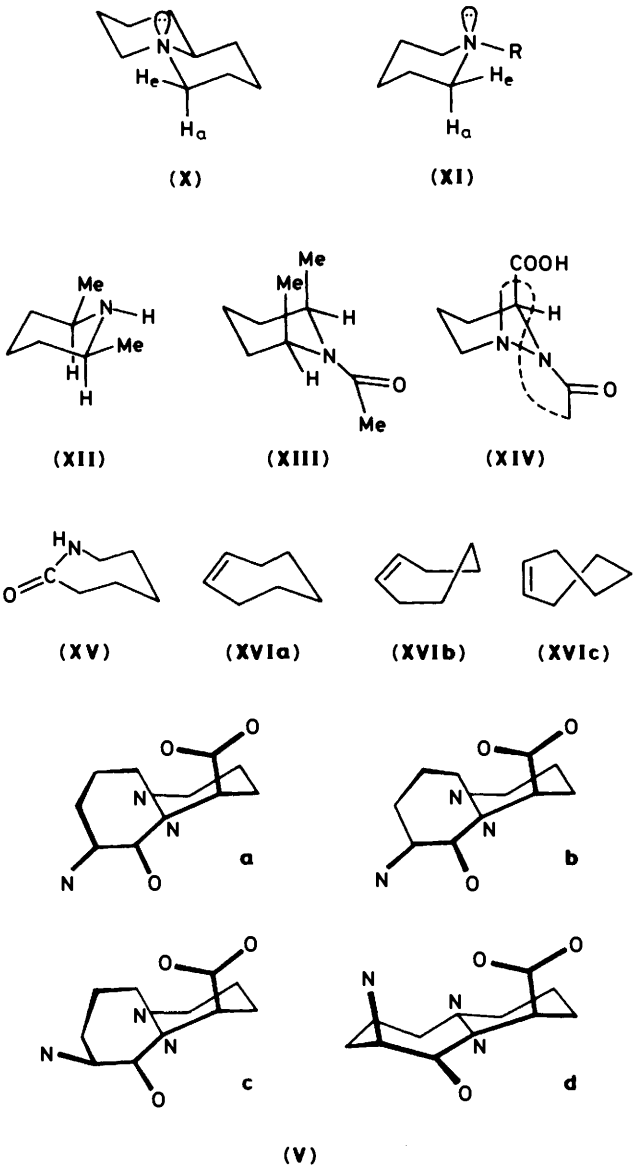


Figure 5. A COSY-45 2D spectrum at 500 MHz of the ACE inhibitor (VI) in CD_3OD, with consequent assignments



planar, and the side-chain is in an extended conformation. Crystals of the phthalimido butyl ester (**VIII**) were also examined by *X*-ray diffraction. The same ring conformation was observed in the crystal structure of this compound as with the previous compound (**VI**).

Examining the torsion angles for the diazepinone ring from these crystal structures, it is possible to predict the probable vicinal coupling constants expected for the ring protons, using the Karplus $\cos^2\varphi$ relationship[5] together with previous information on the rigid 6-oxo derivatives. As shown in Table 4 the coupling constants are not those expected from the crystal conformation alone, suggesting that another conformation is present causing weighted averaging of the *J* values observed. Low-temperature n.m.r. experiments (Figure 7) on the phthalimido butyl ester (**VIII**), both in ^1H and ^{13}C-indicate that two rate processes are occurring. Slow rotation about the bond from the phthalimido nitrogen to the ring is observed to 'freeze' out on the n.m.r. time-scale. In the ^{13}C spectrum at -70 °C the aromatic carbons and carbonyl groups of the phthalimido group split into doublets, which coalesce again at ambient temperature. Some carbon resonances are still broad at this temperature, indicating that a second rate process is being

Table 4. Torsion angles (φ) and coupling constants in the 6,7-deoxy series

	^3J actual[a]			φa[b]	$\cos^2\varphi a$	φb[c]	$\cos^2\varphi b$
Protons	(V)	(VIII)	(VI)				
9α8β	8.1	8.75	8.0	167	0.95	144	0.65
9α8α	6.0	6.75	8.0	76	0.06	254	0.82
8α7α	3.0	3.75	4.0	49	0.43	55	0.33
8α7β	8.3	6.5	7.9	68	0.14	173	0.99
8β7α	10.0	11.2	10.3	169	0.96	−63	0.21
8β7β	3.0	2.5	3.0	52	0.38	55	0.33
7α6α	4.2	4.1	4.3	46	0.48	−58	0.28
7α6β	7.0	9.3	8.4	165	0.93	62	0.22
7β6α	6.3	4.1	4.3	71	0.10	174	0.99
7β6β	5.1	5.5	5.4	48	0.45	55	0.33

[a] Coupling constants for three compounds (**V**), (**VIII**), and (**VI**). [b] Torsion angles for 'MNDO relaxed' crystal structure, *i.e.* (**Va**). [c] Torsion angles for most stable twist-boat form, *e.g.* (**Vb**).

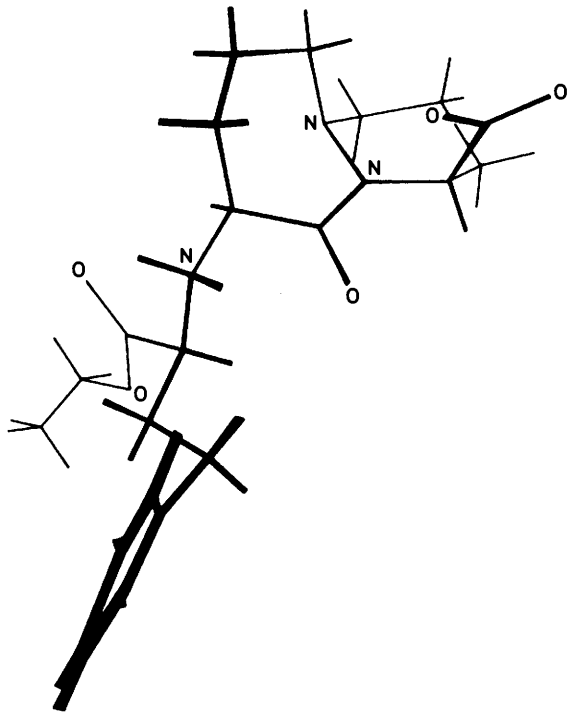


Figure 6. *X*-Ray structure of the ACE inhibitor (**VI**) as the hydrate

slowed down. At the same temperature in the ^1H spectrum severe line broadening occurs, confirming this suggestion, *i.e.* that ring inversion is occurring.

The chair–boat energy barrier in caprolactam derivatives is *ca.* 10 kcal mol^{-1};[19] if the same is true for the 7,6-bicyclic compounds, the ring inversion should have frozen out at *ca.* −70 °C. Since this is not the case, the energy barrier may be considerably lower.

On examination of the torsion angles in the alternative twist-coat–chair conformation (**Vb**) compared with those in the higher energy forms, the expected coupling constants can be predicted, albeit without much accuracy. It is clear that the observed couplings lie in the range between those expected for the crystal conformation and the lowest energy twist-boat–chair. Because many of the coupling constants are for angles of *ca.* 60 or 120°, the calculated values are not predicted accurately since the slope of the Karplus curve is steepest at this point.

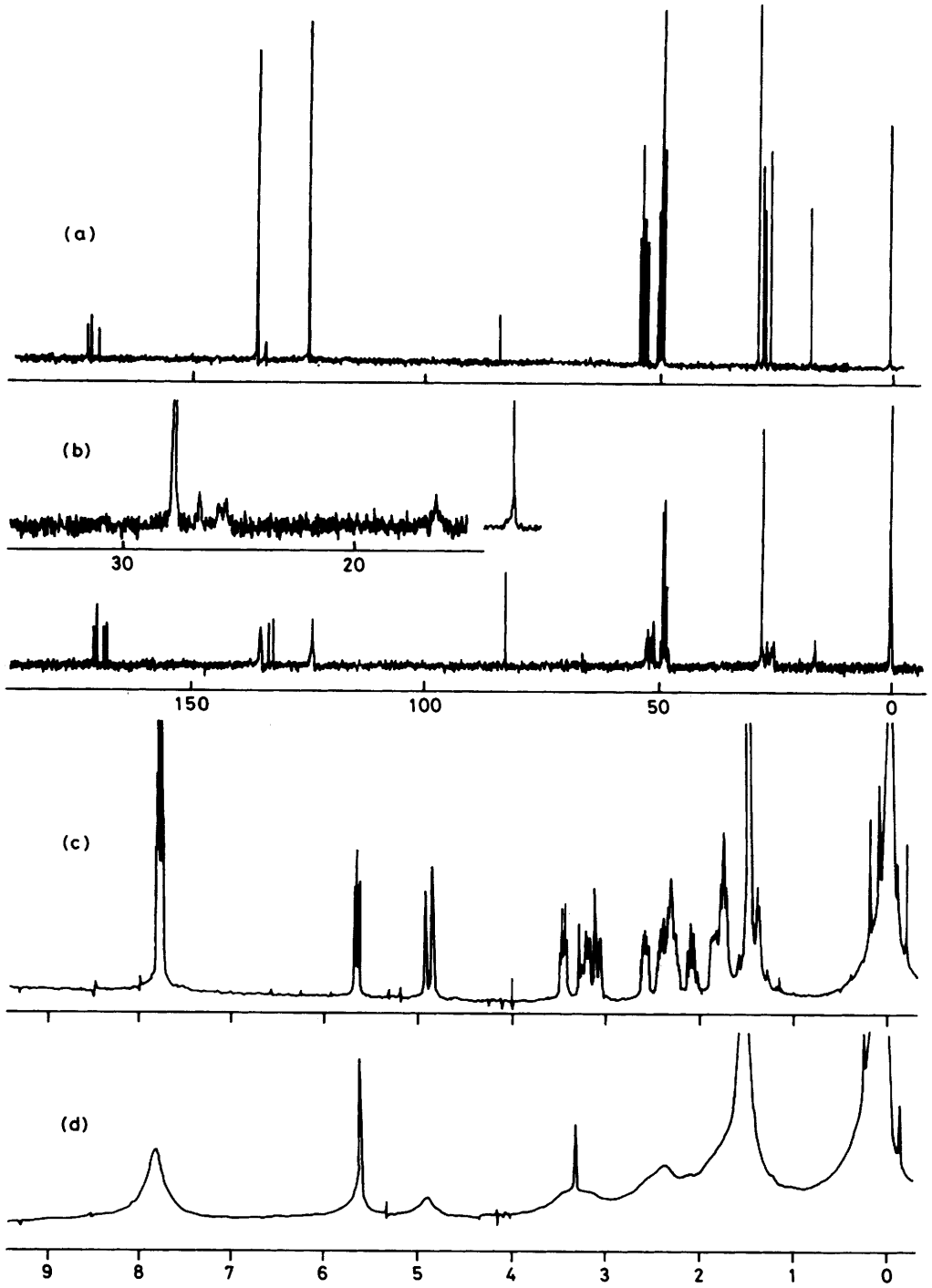


Figure 7. Variable-temperature experiments on the phthalimido butyl ester (**VIII**) in $CDCl_3$: (a) normal ^{13}C spectrum at ambient temperature; (b) ^{13}C spectrum at -80 °C, expansion shows line broadening of some carbons at this temperature; (c) normal ^1H spectrum at ambient temperature; (d) ^1H n.m.r. spectrum at -70 °C

However, it is probable that the ratio is *ca.* 3:1 in favour of the crystal form, judging by the magnitudes of the averaged coupling constants observed. It should be noted that the two conformations are almost identical as far as the three-dimensional array of the pharmacophoric groups in the compound is concerned, *i.e.* either form is correct for binding to the enzyme, judging by what is known about receptor requirements.

In summary, therefore, the 6-oxo bicyclic ring compounds here are entirely rigid in solution due to the anchoring effect of the axial 1-carboxy group conformationally transmitted through planar amide groups to the seven-membered ring. In the 6-deoxy series, though the $A(1,3)$ effect apparently prevents inversion of the bridgehead sp^3-hydridised nitrogen, some flexibility is apparent for the seven-membered ring in solution. As far as the groups important for binding are concerned

however, in the deoxy series the three pharmacophores are not affected by the chair-twist boat flipping of the seven-membered ring. Judging by the high potency of inhibitors containing this ring structure, the φ,ψ angles for the peptide backbone, induced by the conformational restraint of the 7,6 ring system, are close to optimum for presenting the binding groups to the enzyme in the correct three-dimensional array.

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