Nuclear Magnetic Resonance Studies and Conformations of Bicyclic Inhibitors of Angiotensin-converting Enzyme. Part 1. Octahydropyridazo[1,2-*a*]-pyridizanediones as Models for Alanylproline and Captopril

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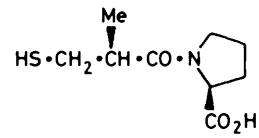
The conformations of several substituted octahydropyridazo[1,2-*a*]pyridazinediones have been determined, using n.m.r. spectroscopy and *X*-ray diffraction, to assess the suitability of the constrained ring system to hold the enzyme binding moieties (pharmacophores) in the optimum three-dimensional configuration. The bicyclic ring system is shown to be rigid in solution, anchored by the axial carboxy group in the reduced pyridazine ring. In all cases a rigid chair–twist boat conformation is found, independent of the substituents in the dione ring. This system is not optimal for binding, as it mimics a relatively high-energy conformation of alanylproline.

Inhibitors of angiotensin-converting enzyme (ACE), in particular captopril[1] (**1**) and enalapril[2] (**2**), have been confirmed as active agents in the clinic for the control of hypertension and congestive heart failure. These inhibitors moderate the process in which the decapeptide angiotensin I is cleaved by the enzyme to liberate the powerful vasoconstrictor angiotensin II, by removal of the *C*-terminal dipeptide (histidyl-leucine). This subject has been reviewed exhaustively.[3,4] Both the agents (**1**) and (**2**) were synthesised as models for the dipeptide alanyl-proline (**3**), which was known to provide the right nucleus for good inhibition from a series of experiments with peptides derived from snake venom.[1,5]

With the aid of computer graphics a number of compounds have been designed and then synthesised with the important functional groups (pharmacophores) constrained in a rigid framework.[6–8] By using such compounds the hypothetical active site of the enzyme could be mapped in three dimensions, using the *in vitro* enzyme assay as a means of estimating binding potency. During the design and early synthetic stages it was vital to know the stereochemistry and conformation of a number of intermediates and final products, to be able to check the spatial orientations of the binding groups.

In the work reported here we describe the unique conformational features of the octahydropyridazo[1,2-*a*]pyridazinediones, determined by n.m.r. spectroscopy and in some cases, by *X*-ray crystallography.† To assign and analyse the complex ^{1}H spectra of these compounds, a combination of high field n.m.r. instruments, selective decoupling experiments, lanthanoid shift reagents, and solvent- and pH-induced shifts was employed. At this stage in the project the more sophisticated use of 2D n.m.r. and n.O.e. was not generally available to us. However these techniques were used with the octahydropyridazo-diazepines to be described in Part 2 of this series.

The syntheses of the compounds examined in this work have been reported previously, together with the detailed computer graphics studies.[6–8] Previous n.m.r. studies on the hexahydropyridazine system were involved primarily with slow synchronous nitrogen inversion in these compounds. Lehn and others[9–11] showed that the *N,N'*-dialkylated derivatives, *e.g.* (**4**)—(**7**), all exhibited this behaviour, with a high barrier to *N*-inversion explained by the two *N*-substituents having to 'pass' each other in the transition state between the two conformational energy minima. Later studies on the analogous carbamate derivatives[12] indicated that although nitrogen inversion was prevented owing to the planarity of the amide bonds, slow rotation about these bonds caused further complexity. In the piperazic acids (**8**)—(**10**) derived from the



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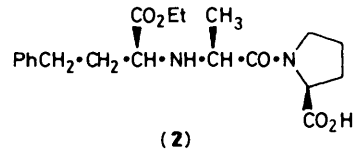

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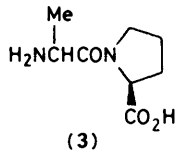

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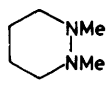

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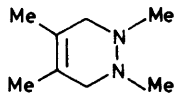

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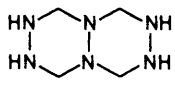

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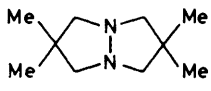

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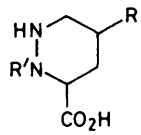


(8) R = H R' = H
(9) R = OH R' = H
(10) R = Cl R' = H
(11) R = H R' = Ac

† We are extremely grateful to Dr. J. J. Daly and Mr. P. Schönholzer, Hoffmann-la Roche Research Laboratories, Basle, for these determinations.

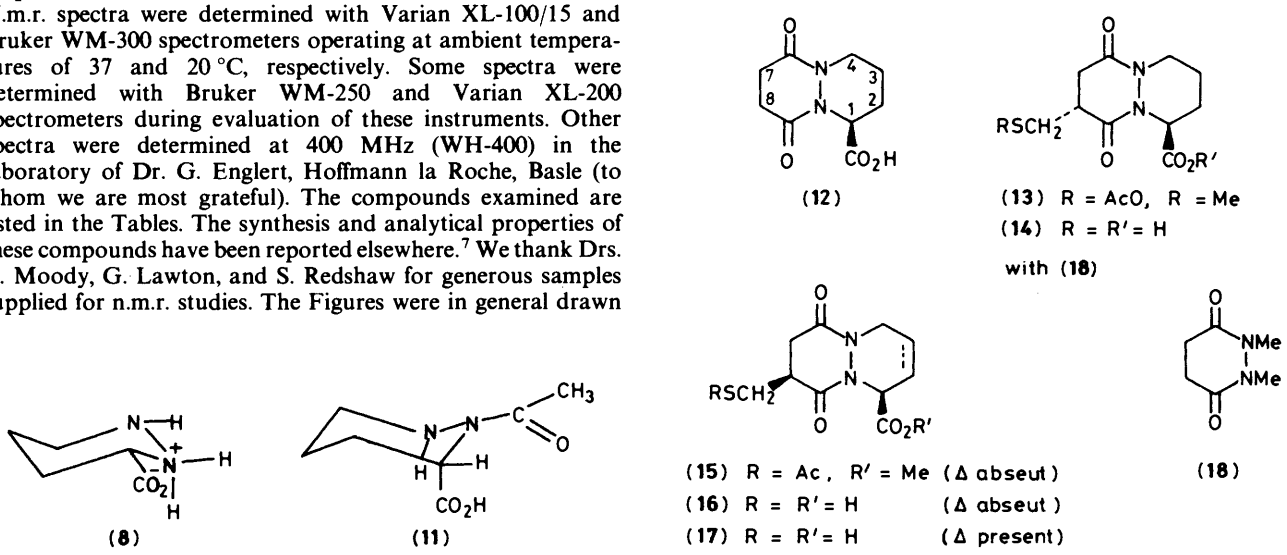
naturally occurring depsipeptide monamycin,[13] it was shown by n.m.r. spectroscopy that although the free imino acids existed in the classical chair conformation, acylation of the adjacent nitrogen atom (as in the natural product) induced a ring inversion due to the so-called allylic strain or $A^{1,3}$ effect,[14] so that the carboxy group is forced into the normally less favourable axial conformation (**11**) to the extent of >90%.[15] Similar effects were observed with 2,6-disubstituted piperidines[16] and in pipecolic acid derivatives.[17,18] This anchoring of the carboxy group in the axial conformation is a recurring feature in the bicyclic compounds described in this work, responsible for the observed rigidity of the ring system.

Experimental

N.m.r. spectra were determined with Varian XL-100/15 and Bruker WM-300 spectrometers operating at ambient temperatures of 37 and 20 °C, respectively. Some spectra were determined with Bruker WM-250 and Varian XL-200 spectrometers during evaluation of these instruments. Other spectra were determined at 400 MHz (WH-400) in the laboratory of Dr. G. Englert, Hoffmann la Roche, Basle (to whom we are most grateful). The compounds examined are listed in the Tables. The synthesis and analytical properties of these compounds have been reported elsewhere.[7] We thank Drs. C. Moody, G. Lawton, and S. Redshaw for generous samples supplied for n.m.r. studies. The Figures were in general drawn by computer graphics techniques with a CALCOMP 81 plotter interfaced to a VAX 11/750 computer and a Megatek 7000 display terminal. We are extremely grateful to Dr. A. Kröhn for providing the computer graphics back-up to this work.

Results

(i) *The '6,6' Thiols and Derivatives.*—The ^{1}H n.m.r. spectra of these compounds showed some common features. In each case the C-1 α-H signal appeared as a narrow doublet of doublets, δ *ca.* 5.3, with splittings normally 2—3 and 6 Hz. The signal for the two C-4 protons was clearly defined in each case as a well separated broad doublet (β-H), and a triplet of doublets (α-H) at



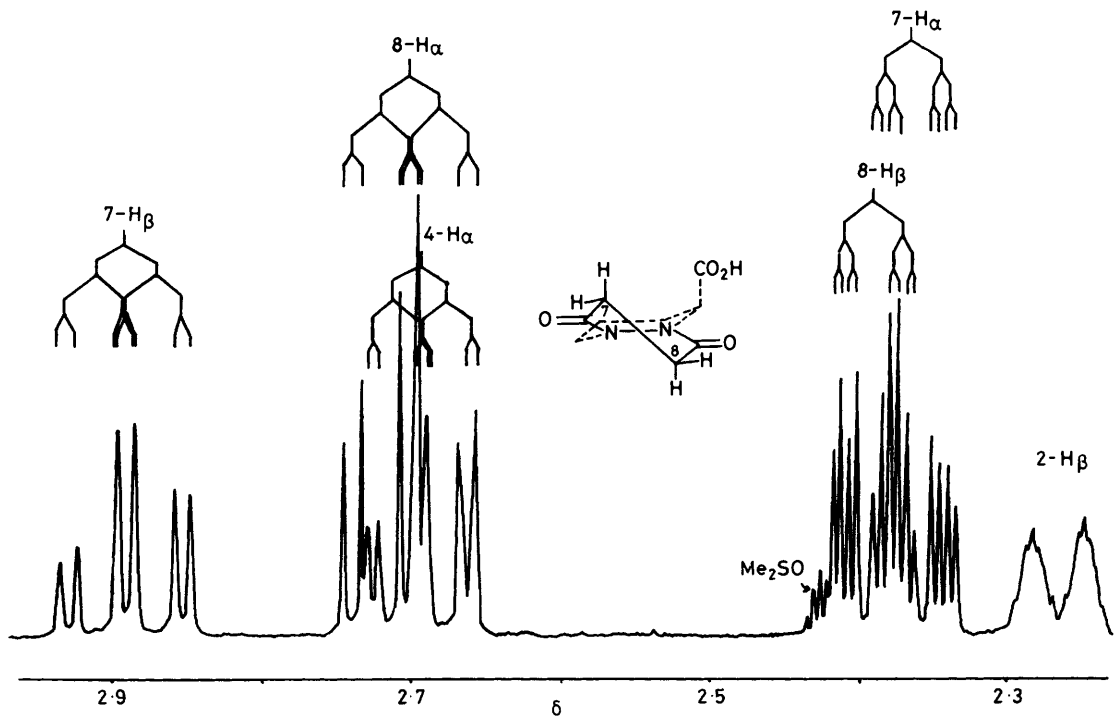
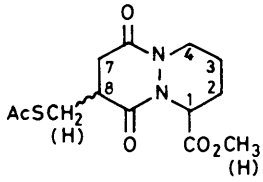
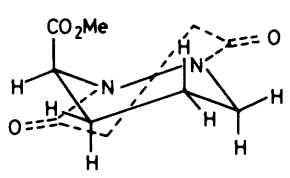
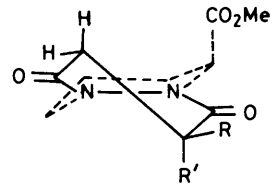


Figure 1. ^{1}H N.m.r. part spectrum of (**12**) [400 MHz; $(CD_3)_2SO$]; assignment confirmed by spin simulation and spin decoupling experiments

Table 1. N.m.r. analyses for the bicyclic compounds (**12**), (**13**), and (**15**)[a]







Chemical shifts[b] (δ)

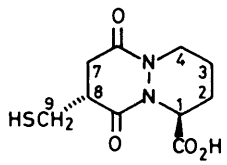
	(12)	(13)	(15)		(12)	(13)	(15)		(12)	(13)	(15)
1α	5.25	5.31	5.32	4β	4.56	4.67	4.69	8α	2.68		2.45
2α	1.67	1.87	1.85	4α	2.67	3.00	2.78	CHS		2.91	3.32
2β	2.26	2.41	2.42	7β	2.87	3.13	2.82	CH'S		3.26	3.05
3α	1.51	1.77	1.74	7α	2.35	2.56	2.67	OMe		3.74	3.75
3β	1.32	1.51	1.48	8α	2.38	2.86		SAc		2.36	2.35

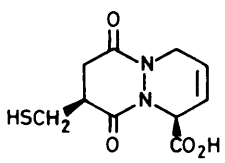
Coupling constants (*J*/Hz)[c]

	(12)	(13)	(15)		(12)	(13)	(15)		(12)	(13)	(15)
1α,2α	5.7	5.9	5.6	4α,7β	0.4	0.5		7β,8α	15.0		14.2
1α,2β	2.0	1.7	2.0	3α,3β	−13.0	−13.6	−13.9	7α,8β	2.0	2.2	
1α,3α	1.0	0.6		3α,4β	2.0	2.4	*ca.* 2.0	7α,8α	4.5		4.1
2α,2β	−13.5	−13.9	−13.8	3α,4α	3.0	3.3	3.0	8α,8β	−15.0		
2α,3α	4.0	4.2	4.0	3β,4β	4.0	4.2	4.0	8β,C*H*S		7.3	
2α,3β	13.3	13.6	13.7	3β,4α	13.0	13.1	13.0	8α,C*H*S			5.7
2β,3α	2.0	2.9	*ca.* 2.0	4α,4β	−12.9	−12.9	−13.0	8β,C*H'*S		8.9	
2β,3β	3.9	4.1	4.0	7α,7β	−15.0	−16.2	−15.0	8α,C*H'*S			6.0
2β,4β	2.0	2.0	*ca.* 2.0	7β,8β	4.2	5.0		C*H,H'*S		−13.8	−13.7

[a] (**12**) unsubstituted on left hand ring, CO_2H on right hand ring, R = R' = H; (**13**) side chain *trans* to CO_2Me, R = H, R' = CH_1SAc; (**15**) side chain *cis* to CO_2Me, R = CH_2SAc, R' = H. [b] Solvent $CDCl_3$, referred to tetramethylsilane; [c] ±0.1 Hz, confirmed by computer simulation.

Table 2. ^1H N.m.r. data for free thiols





	(14) ($CDCl_3$) δ	*J*/Hz		(17) [$(CD_3)_2SO$] δ	*J*/Hz	
1α	5.30 (m)	2.0, 5.8	1α	5.33	4α,4β	*ca.* 18
4β	4.76 (dm)		2 }	5.99	7α,7β	15.5
7α	3.18 (dd)	5.0, 15.6	3 }		7α,8α	4.4
7β	2.72 (dd)	2.0, 15.6	4α	3.71	7β,8α	15.5
2β	2.45 (dm)		4β	4.67	8α,9α	5.3
4α, 8α, 9a,b	2.9—2.5		7α	2.54	8α,9b	6.0
			7β	2.81	9a,9b	13.7
			8α	3.12	9a,SH	8.8
2α, 3α, 3β, SH	1.5—1.9[a]		9α	2.79	9b,SH	8.4
			9β	2.61		
			SH	2.38		
			CO_2H	3.36		

[a] Spectral overlap too much for detailed analysis.

Table 3. ^{1}H N.m.r. of '6,6' amino acids and derivatives

	(19)[a]		(20)[b]		(21)[b]		(22)[c]	
	δ[d]	*J*/Hz	δ	*J*/Hz	δ	*J*/Hz	δ	*J*/Hz
1α	4.97	3, 6[e]	5.20		5.35		5.60	2, 6.6
4α	*ca.* 3.0		2.88	3, 12	3.25		2.72	4.0 12.5
4β	4.52		4.75	*ca.* 12	4.47	*ca.* 12	4.82	*ca.* 13
7α	3.29	*ca.* 15, 15	2.72	5, 15	3.32	6, 17	3.23	5.0, 15.2
7β	2.93	5.8, 15	4.26	15, 15	2.88	3, 17	3.54	14.8
8α	4.53	5.8, 14	5.23	5, 15			4.30	4.9, 14.1
8β					5.02	3, 6		
Aryl H			7.7—8.0		7.7—7.9		7.3—7.4	
Bu^t			1.53		1.50			
PhCH_2							3.08	
2β							2.51	
2α							1.87	
C*H*NH							4.12	5.6—7.1

[a] D_2O; 100 MHz. [b] $CDCl_3$; 100 MHz. [c] [$^2H_5$]Pyridine; 250 MHz. [d] Overlapping complex multiplets not assigned. [e] Approximate couplings.

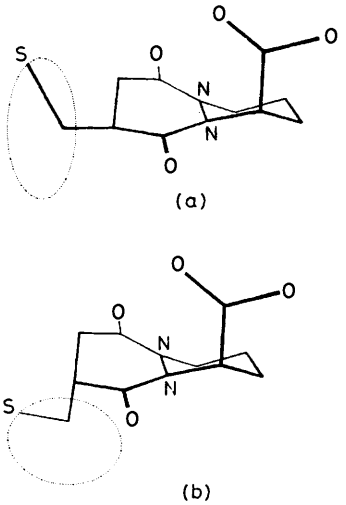


Figure 2. Comparisons by computer graphics (Megatek 7000 display processor interfaced to a VAX 11/750 computer) of the *cis* (a) and *trans* (b) thiols obtained by deprotection of compounds (**15**) and (**13**), respectively. The hoops signify the space available to the thiol groups by free rotation about the CH–CH_2SH bond

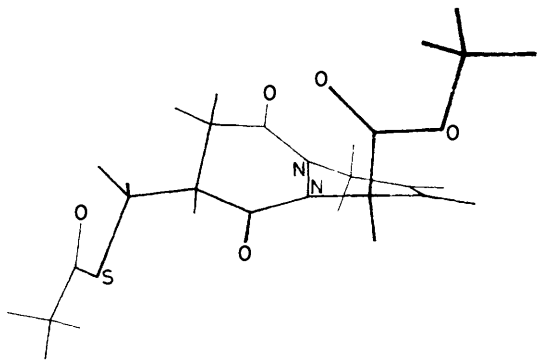
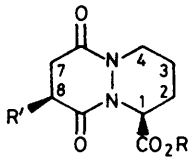


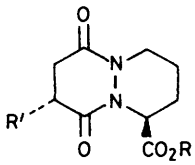
Figure 3. Crystal structure representation of the *S*-acetyl t-butyl ester derivative of the unsaturated compound (**17**)

δ *ca.* 4.6 and 2.8, respectively. This immediately confirmed that the $A^{1,3}$ strain mentioned earlier is operating in these compounds, which therefore probably exist in chair conformations with the carboxy group *axially* oriented. Sequentially selective decoupling experiments together with the use of $Eu(Fod)_3$, which was found to bind primarily to the 'top' amide carbonyl, allowed the full analyses of the spectra of compounds (**12**), (**13**), and (**15**) to be accomplished; the results are given in Table 1. In compound (**12**), the clear non-equivalence of the four protons in the left hand ring as shown in Figure 1, together with one very large vicinal coupling constant (*ca.* 15 Hz) and three small ones (2.0, 4.0, and 4.2 Hz), clearly indicated that the left-hand rings are similarly rigid. This is a consequence of conformational transmission through the two planar amide bonds, allowing the left-hand ring to adopt a twist-boat conformation, with the CH_2–CHR atoms perfectly staggered as shown in Figure 1. A similar conformation was found for the crystal structure of 1,2-dimethylhexahydropyridazine-3,6-dione (**18**) at −165 °C.[19] Only one conformation is allowed by the anchored carboxy group; without the carboxy group the two rings would invert about the N–N bond axis. Since very similar spectra were obtained for the free thiol acids in aqueous solution [see for instance the deprotected form (**14**), Table 2], it is safe to conclude that the only variable is the conformation of the CH_2SH side chain by virtue of rotation about the C–CH_2S bond. The vicinal coupling constants for the SCH_2–CH fragment do not indicate any particular preferred rotational isomer, as expected. The two compounds (**14**) and (**16**) therefore provide an excellent test of the required relationships for binding to ACE between the carboxylic acid group, the amide carbonyl, and the thiol group, the first two of which are firmly fixed in relation to each other, with the sulphur atom in each isomer able to describe a hoop in space by rotation about the SCH_2–CH bond (Figure 2). The enzyme assay clearly shows that the *trans* arrangement (**14**) (IC_{50} 0.04 μM) is preferred to the *cis* form (**16**) (IC_{50} 0.1 μM).[7]

In a few cases, bicyclic thiols were synthesised with a degree of unsaturation in the piperazic acid ring, as this apparently led to an increase in the *in vitro* potency of these compounds against the enzyme. Incorporation of the double bond as shown was expected to change the conformation of the right-hand ring to a half chair, with the carboxy group pseudoaxially oriented. In the unsaturated *cis* thiol (**17**) the narrow multiplet for H-1 and the large chemical shift difference for the two C-4 protons (0.96 p.p.m.) (Table 3) confirmed this conformation. Moreover the left hand ring again occurs in the twist boat conformation with the CH_2SH group equatorial as expected. A crystal structure



(19) R = H, R′ = NH_2
(20) R = Bu^t, R′ = phthalimido
(23) R = H, R′ = $NHCH(CO_2H)[CH_2]_2Ph$



(21) R = Bu^t, R′ = phthalimido

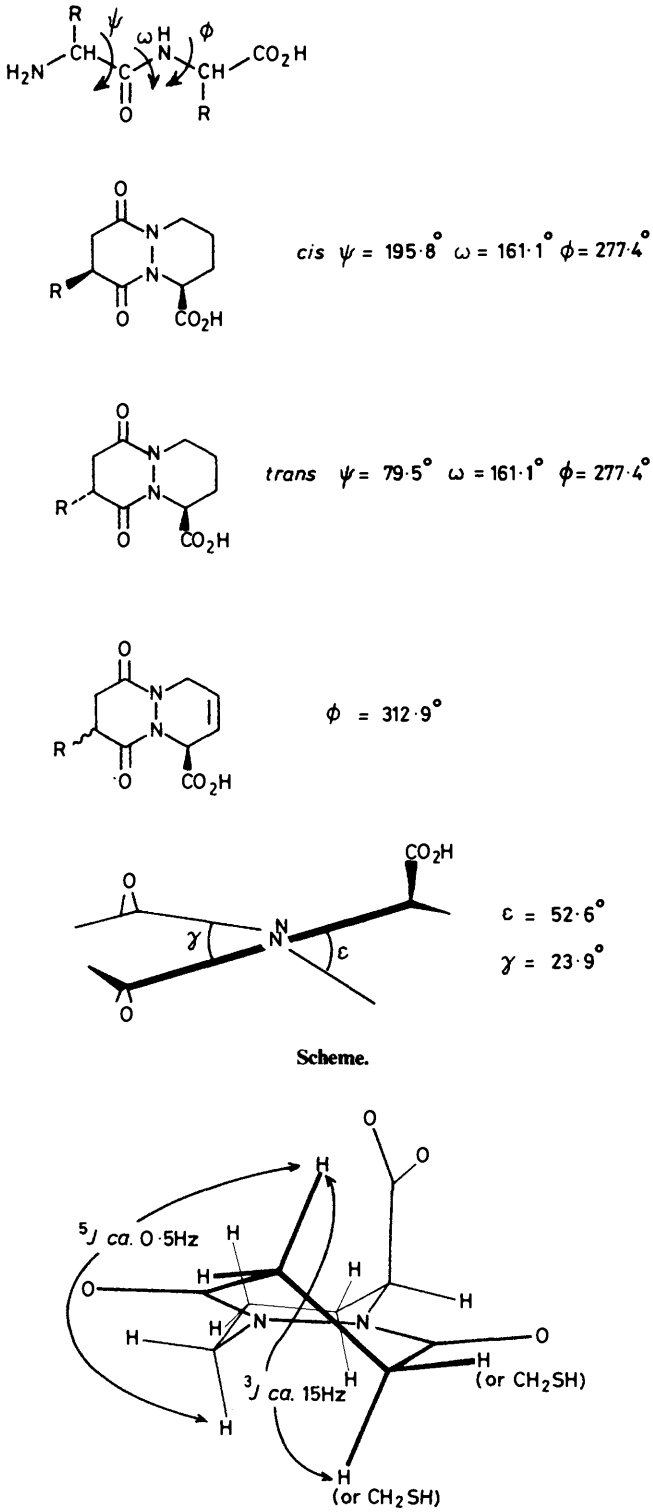
determination of a protected analogue was in exact agreement with this rigid conformation (Figure 3).

(ii) '6,6' *Amino Acids and Derivatives.*—With the tactical move in the design of ACE inhibitors away from thiol-containing compounds which may have associated side-effects, a number of compounds were synthesised with nitrogen-bearing side chains, *e.g.* compounds (**19**)—(**22**), where it was necessary to determine the relative stereochemistry of the two ring substituents in each case. The ^1H n.m.r. spectral data of the four compounds (**19**)—(**22**) are recorded in Table 3. In each case the narrow multiplet for H-1 of the piperazic acid ring together with the typical well separated multiplets for the two 4-protons, one a broad doublet (H-β) and the other a doublet of triplets (H-α) (*J* 4 and 12 Hz) was indicative of a chair conformation with the carboxy group axial, as in the thiols previously examined. Again the piperazinedione ring appeared to adopt a twist boat conformation with the *N*-substituent R′ axially or equatorially oriented, depending on whether the substituent is *trans* or *cis* to the carboxy group, respectively. Thus in the zwitterionic amino acid (**19**), the vicinal couplings $J_{7\alpha,8\alpha}$ = 5.8 Hz and $J_{7\beta,8\alpha}$ = 15 Hz are as expected for equatorial–axial and axial–axial couplings, respectively, confirming the equatorial conformation for the amino group, which is therefore *cis* to the carboxy. In contrast, the phthaloyl derivative (**21**) shows corresponding vicinal couplings of 6 and 3 Hz, indicating an axial *trans* orientation for the phthaloyl group, remarkable for a bulky group of this type, and confirming that owing to the structure of the ring system, 1,3-diaxial interactions are absent.

Several other compounds in this series were investigated by n.m.r. spectroscopy and in all cases the same conformational features appeared. In all the '6,6' bicyclic compounds examined, the dominance of the axial requirement for the carboxy group ensured the conformational rigidity of the whole ring system.

Discussion

From the results described above it is clear that the 1-carboxyoctahydropyridazo[1,2-*a*]pyridazinediones provide an excellent 'template' as a dipeptide mimetic of dependable rigidity. For comparison with the classic ψ, ω, φ angles of a dipeptide, the corresponding angles of the *cis* and *trans* substituted compounds in the bicyclic series are as shown in the Scheme (from *X*-ray diffraction co-ordinates). The ω value of *ca.* 160° suggests some degree of non-planarity of the amide bonds,



Scheme.

Figure 4. End-on view of the octahydropyridazopyridazine ring system, indicating the more unusual coupling constants observed

confirmed by examining the torsion angles about the N–N bond. The value for ε is close to that expected for a perfect chair conformation of the piperazic acid ring. The value of γ is somewhat lower than expected for a true twist-boat conformation of the left-hand ring. However the coupling constants reported for these compounds suggest and confirm

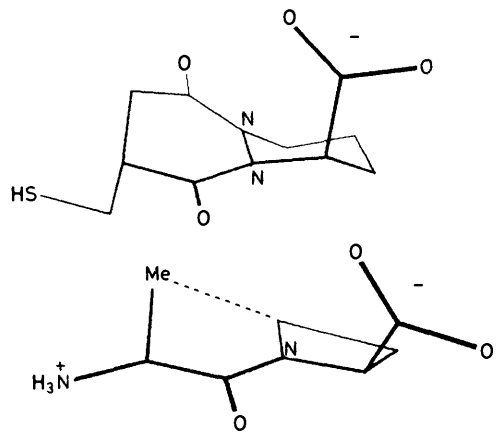


Figure 5. Comparison between the conformations of the bicyclic compound (**14**) and the corresponding 'matched' conformation of alanylproline (**3**), indicating the unfavourable van der Waals contact between the alanine methyl group and the proline δ-CH_2 group (2.65 Å)

the conformation found in the crystal, with the vicinal protons perfectly staggered in both rings.

Coupling Constants.—Several features emerge from the detailed analyses reported in this study. According to the Karplus relationship[20] ($^3J\alpha \cos^2\varphi$, where φ is the torsion angle between the coupling protons) and subsequent modified equations, the maximum value for ^3J should be in the range 10—12 Hz.

In the twist-boat conformation found in these molecules, we see values for φ − 180° of 15.0—15.5 Hz, by far the largest vicinal couplings ever recorded for a CH_2–CH fragment (Figure 4). This unexpected increase in vicinal coupling is clearly due to the adjacent carbonyl groups, which act in some way to enhance the positive coupling between the two diaxial protons in an *anti* relationship. Normally electron-withdrawing groups adjacent to coupling protons reduce the vicinal coupling in linear proportion to their electronegativity. In this case it seems the π electrons of the two carbonyl groups are acting in tandem to enhance the coupling.

An interesting long-range coupling of *ca.* 0.5 Hz is observed in several compounds between 7-H_β and 4-H_α, but not between any other protons separated by five bonds. This is analogous to the homoallylic coupling seen across the olefinic double bond, and between the α-protons of peptides, *i.e.* RC*H*–CO–NH–C*H*R^1, which is reported to be angle-dependent, in the range 0—1.5 Hz.[21]

Conformational Effects and ACE Inhibition.—Although the '6,6' bicyclic framework apparently leaves the carboxy group and the amide carbonyl in mutually favourable positions for binding to angiotensin-converting enzyme, a comparison of the conformation with that of alanylproline indicates that the bicyclic system is mimicking a relatively high-energy conformation of alanyl-proline, with the alanyl methyl group too close to the proline δ carbon atom (Figure 5). The biological activities of the compounds examined[6–8] confirm that the octahydropyridazo[1,2-*a*]pyridazinedione system is not the optimal constrained form for binding. In Part 2, modifications to the structure leading to more potent ACE inhibitors will be reported.

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