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CONFORMATIONAL ANALYSIS OF ENALAPRIL (MK-421) IN SOLUTION BY ^{1}H AND ^{13}C NMR

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ABSTRACT

The conformations of enalapril (MK-421, (−)-(*N*-((*S*)-1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl-L-proline), in CD_3OD, were investigated in order to account for their biological activity as inhibitors of angiotensin converting enzyme (ACE) The ratio of *trans* to *cis* conformation around the amide bond is 3 2 The preferred optimum structures of the *trans* and *cis* forms are postulated With reference to the proline ring the *N*-type *trans* isomer was more prevalent than the *S*-type *trans* isomer

INTRODUCTION

Recently, a number of drugs controlling blood pressure have been developed and clinically widely utilized [1–3]. One of them, enalapril (MK-421) is an inhibitor of angiotensin converting enzyme (ACE). The structural formula of MK-421 is shown in Fig 1 with the numbering scheme MK-421 has been studied by potential energy [4], and X-ray analysis [5,6], showing only a *trans* form around the amide bond However both *trans* and *cis* conformations are expected in solution Accordingly, we measured several ^{1}H and ^{13}C NMR spectra to ascertain the conformations of MK-421 in solution, our purpose being to investigate the whole structure of the molecule in solution in more detail, in

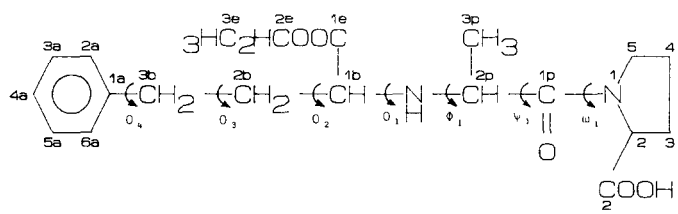

Fig 1 Molecular structure of MK-421

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order to postulate the optimum conformation of MK-421 for biological activity in vivo

To obtain the main dihedral angles values of vicinal coupling constants of ^{1}H–^{1}H and ^{13}C–^{1}H were measured The optimum conformations of *trans* and *cis* forms in solution were examined The ratio of the *S*-type and the *N*-type isomers with reference to the proline ring in the solution was also investigated

EXPERIMENTAL

MK-421, donated by Banyu Pharmaceutical Co Ltd., was used without further purification

^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX-400 spectrometer, operating at 400.0 MHz and 100.4 MHz respectively at 25 °C using a deuterium lock system The concentration of the sample was 20% (w/v) Chemical shifts were measured relative to tetramethylsilane The experimental conditions were as follows: number of accumulations, 128–2048, data points, 64k; spectral width, 6000 Hz or 24 000 Hz; flip angle, 45° or 30°; interval of pulse to pulse, 8 s or 2 s

We carried out two-dimensional ^{1}H–^{1}H *J* resolved spectroscopy, low power selective proton decoupling, complete proton noise decoupling and selective ^{13}C–^{1}H long range *J* resolved two dimensional spectroscopy to determine long range coupling constants for particular protons The program DAPH written by T Ashida at the Computer Center of the University of Tokyo was used

RESULTS AND DISCUSSION

Optimum conformation of MK-421 in solution

D_2O is the best solvent in which to study in vivo conformation by NMR However, in this work, CD_3OD was used since MK-421 was much more soluble in this solvent than in D_2O The ratio of *trans* and *cis* conformations in CD_3OD was 3 2, as was also the case in D_2O; this value was obtained by integration of spectra Detailed assignments of ^{1}H and ^{13}C for the *trans* and *cis* conformations are listed in Table 1

For NMR study, the dihedral angles between vicinal hydrogens and vicinal carbon protons are required Values of $^{3}J_{H\text{-}H}$ and $^{3}J_{C\text{-}H}$ in each conformation have been estimated by using Karplus equations (1) and (2) [7,8]

$$^{3}J_{HH} = 7\ 00 - 1\ 00\cos\theta + 5\ 00\cos 2\theta \quad (1)$$

$$^{3}J_{CH} = 4\ 26 - 1\ 00\cos\theta + 3\ 56\cos 2\theta \quad (2)$$

The values of coupling constants observed by ^{1}H and ^{13}C NMR were substituted in eqns (1) and (2) The main dihedral angles (ϕ_1, θ_1, θ_2, θ_3 and θ_4) are

TABLE 1

^{1}H and ^{13}C chemical shifts for the *trans* and *cis* conformers of enalapril

	trans	*cis*		*trans*	*cis*
H2	4 49	4 56	C2	60 65	60 54
H3,3′	2 01	2 27	C2′	174 76	174 68
	2 27	2 34	C3	29 97	31 91
H4,4′	2 01	1 75	C4	25 84	23 11
		1 96	C5	48 04	
H5,5′	3 57	3 46	C1p	169 10	168 90
	3 64	3 64	C2p	56 04	56 70
H2p	4 29	4 12	C3p	15 84	16 40
H3p	1 58	1 56	C1b	60 12	60 05
H1b	3 94	3 96	C2b	33 48	33 30
H2b	2 27		C3b	32 04	31 95
H3b	2 73	2 84	C1e	169 55	170 01
H2e	4 29		C2e	63 91	63 80
H3e	1 32		C3e	14 41	14 35
H2,6a	7 25		C1a	141 17	141 21
H3,5a	7 25		C2,6a	129 48	
H4a	7 25		C3,5a	129 64	
			C4a	127 53	

TABLE 2

Coupling constants (Hz)

	trans	*cis*
$^{3}J_{H1b\ H2b}$	6 0	5 7
$^{3}J_{H2b\ H3b}$	7 7	
$^{3}J_{H2p\ H3p}$	6 8	7 0
$^{3}J_{H2\ H3}$	5 1	4 7
$^{3}J_{H2\ H3}$	8 8	6 3
$^{3}J_{H2\ C1p}$	1>	1>
$^{3}J_{H2p\ C1b}$	1 8	1>
$^{3}J_{H1b\ C2p}$	2 6	2 3
$^{3}J_{H3b\ C2\ 6a}$	6 1	

needed to obtain the optimum conformation of *trans* and *cis* forms in solution The values of the main dihedral angles (ϕ_1, θ_1, θ_2, θ_3 and θ_4) were determined using the experimental values of $^{3}J_{H2p,C1b}$, $^{3}J_{H1b,C2p}$, $^{3}J_{H1b,H2b}$, $^{3}J_{H2b,H3b}$ and $^{3}J_{H3b\ C2,6a}$, respectively

The values of $^{3}J_{H\text{-}H}$ and $^{3}J_{C\text{-}H}$ for *trans* and *cis* conformations are shown in Table 2 Their selected C–C–C–C dihedral angles are listed in Table 3, in which

TABLE 3

Main dihedral angles (deg)

	This work		Potential energy data [4]	X-ray data [6]
	trans	*cis*		
ϕ_1	175	164	180-220	175
ψ_1	160	160	160-170	156
ω_1	180	0	180	−178
θ_1	63	60	70-130	57
θ_2	52	52	180-240	68
θ_3	180		180	179
θ_4	89		90	84

the values of potential energy [4] and X-ray [6] are also given for comparison For example, the values of dihedral angle C2b–C1b–N–C2p (θ_1) were determined from the value of $^3J_{H1b,C2p}$ as follows The experimental value of $^3J_{H1b,C2p}$ of the *trans* form was 2 6 Hz, from the low power selective proton decoupling by irradiation of H3p as shown in Fig. 2(a2). This value of $^3J_{H1b,C2p}$ was substituted in eqn (2) and consequently, the four values of the dihedral angle H1b–C1b–N–C2p (54°, 117°, −117° and −54°) were obtained. These four values of the dihedral angle H1b–C1b–N–C2p were then applied to Newman projections Consequently, four values of the C–C–N–C dihedral angle (θ_1, 171°, −126°, 0° and 63°) were obtained. Though the best value of the four possible dihedral angles was not determined exactly from the NMR coupling constants, we chose the best value (63°) of the dihedral angle (θ_1) from a comparison with potential energy [4] and X-ray [6] data (Table 3) The value corresponded with that at optimum biological activity. The best values of other dihedral angles were determined in the same way.

The possible values of the dihedral angle C1b–N–C2p–C1p (ϕ_1) were determined from the value of $^3J_{H2p,C1b}$ which was 1 8 Hz for the *trans* form, from low power selective proton decoupling with the triple irradiation of H2b and H3b, as shown in Fig. 2(b2) The values of dihedral angles C3b–C2b–C1b–N (θ_2) and C1a–C3b–C2b–C1b (θ_3) were determined from the values of $^3J_{H1b,H2b}$ and $^3J_{H2b,H3b}$ which were determined by two dimensional ^{1}H–^{1}H J resolved spectroscopy From the results of these cross sections, the value of $^3J_{H2b,H3b}$ of 7 7 Hz was obtained for H3b at both δ2 73 and δ2 84 Also the value of $^3J_{H1b,H2b}$ for *trans* was 6 0 Hz at δ3.94. The values of dihedral angles C2,6a–C1a–C3b–C2b (θ_4) were determined from the value of $^3J_{H3b,C2,6a}$. The value of 6 1 Hz for $^3J_{H3b,C2,6a}$ was obtained by selective ^{13}C–^{1}H long range J resolution two-dimensional spectroscopy for the purpose of observing C2a,6a by irradiation of 3b protons

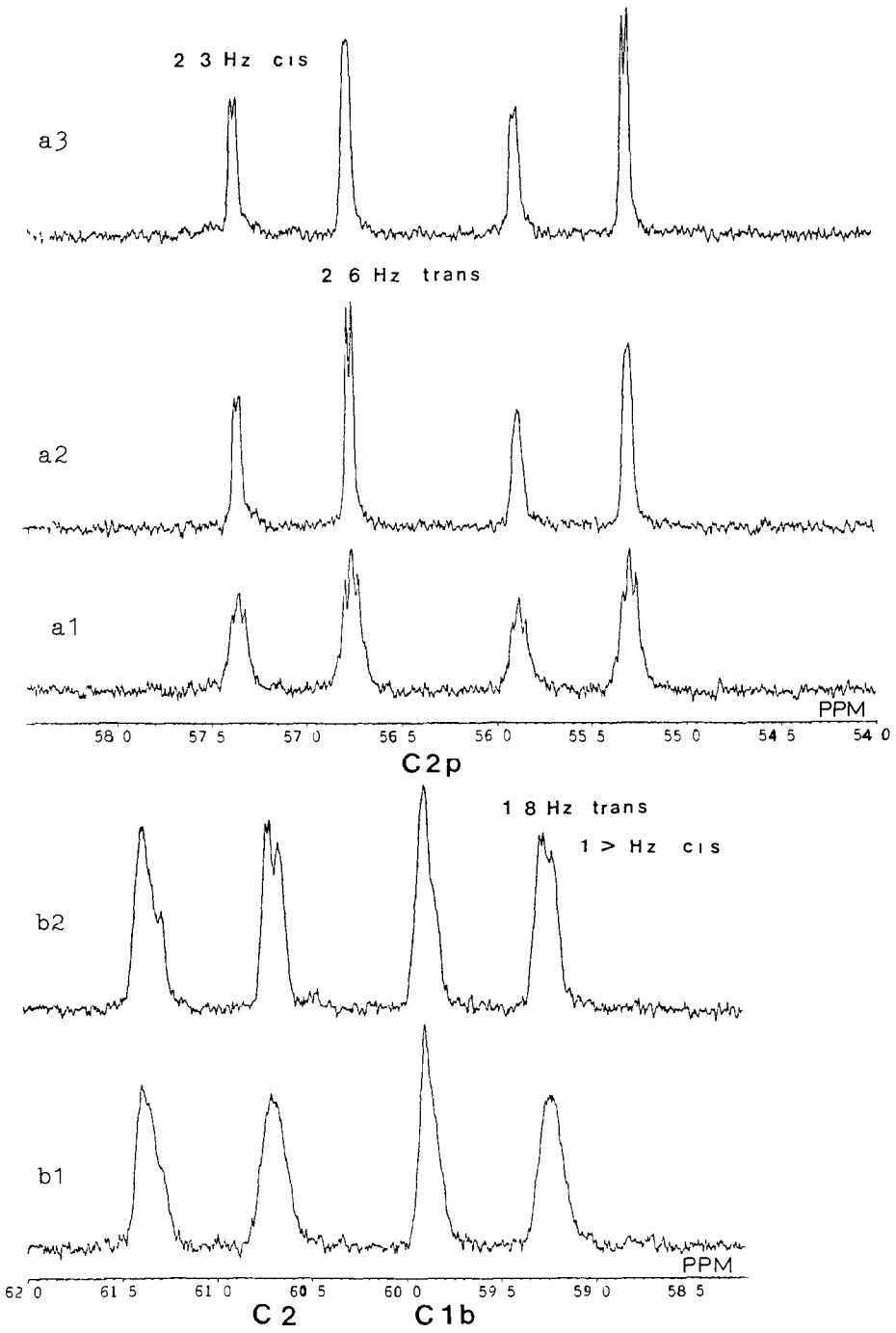


Fig 2 (a1) ^{13}C NMR spectra of C2p by gated decoupling with NOE, (a2) with selective irradiation of H3p, *trans* form, (a3) with selective irradiation of H3p, *cis* form (b1) ^{13}C NMR spectra of C1b by gated decoupling with NOE, (b2) with selective triple irradiation of H2b and H3b, *cis* and *trans* forms

The value of ω_1 was determined by the calculation of internuclear distances by the difference NOE NMR spectra as shown in Fig. 3.

Enhancement of the H2 signals by irradiation of the H3p proton was estimated to be 5 0 and 0 7% in the *cis* and *trans* forms, respectively, based on the enhancement of the H2p signal (8 3%) by the irradiation Coupling constants of H2p and H3p and the difference NOE spectra gave information on the internuclear distance between H3p and H2 The distance between H3p and H2p was 2 96 Å in the *cis* form, which was considered as a criterion of distance between the H3p and H2 protons of the *trans* and *cis* forms NOE is proportional to $1/r^6$

$$\frac{0\,7}{8\,3}=\frac{2\,96^6}{r^6}, \qquad r=4.47\ (4\,5\,\mathring{A})\ trans$$

$$\frac{5.0}{8\,3}=\frac{2.96^6}{r^6}, \qquad r=3\,22\ (3\,2\,\mathring{A})\ cis$$

From these relations, the distance between the $3pCH_3$ and the H2 proton of the *trans* form was found to be 4.5 Å and that of the *cis* form was 3 2 Å. From these results, it seems reasonable to assume that the dihedral angle (ω_1) of the amide bond in the *cis* form is 0° and that in the *trans* form is 180°

Using the program GONCHAN we predict the *trans* and *cis* forms to be as shown in Figs. 4a and 4b. The coupling constants and difference NOE spectra gave information on the conformation in solution.

Structure–activity studies have shown that particular functional groups are essential for the biological activity of MK-421. It seems reasonable to assume that, in the binding of MK-421 to ACE, the functional groups are situated on the same side In the *trans* form, ethoxycarbonyl, carbonyl and carboxyl, which form active regions, are situated on the same side, whereas in the *cis* form the carboxyl group is situated on the opposite side to ethoxycarbonyl and carbonyl groups Therefore, the *trans* conformation should be preferred for biological activity

Conformation of the proline ring

The detailed conformation of the proline ring in solution was estimated

In the proline ring, *S*-type and *N*-type isomers exist in solution We determined the occurrence ratio of these isomers. From Fig. 5, the values of the dihedral angle for the diaxial and diequatorial forms were determined to be 87° and 150°, respectively The dihedral angle H2–C2–C3–H3′ was estimated to be equatorial for the *S*-type isomer and axial for the *N*-type isomer (Fig 6)

By using the Karplus equation (1), the value of $^3J_{H2,H3}$ was found to be 2 Hz for the *S*-type isomer and 10 Hz for the *N*-type isomer For the amide bond the values for the *trans* and *cis* forms in solution were 8.8 Hz and 6 3 Hz, respectively (Table 2)

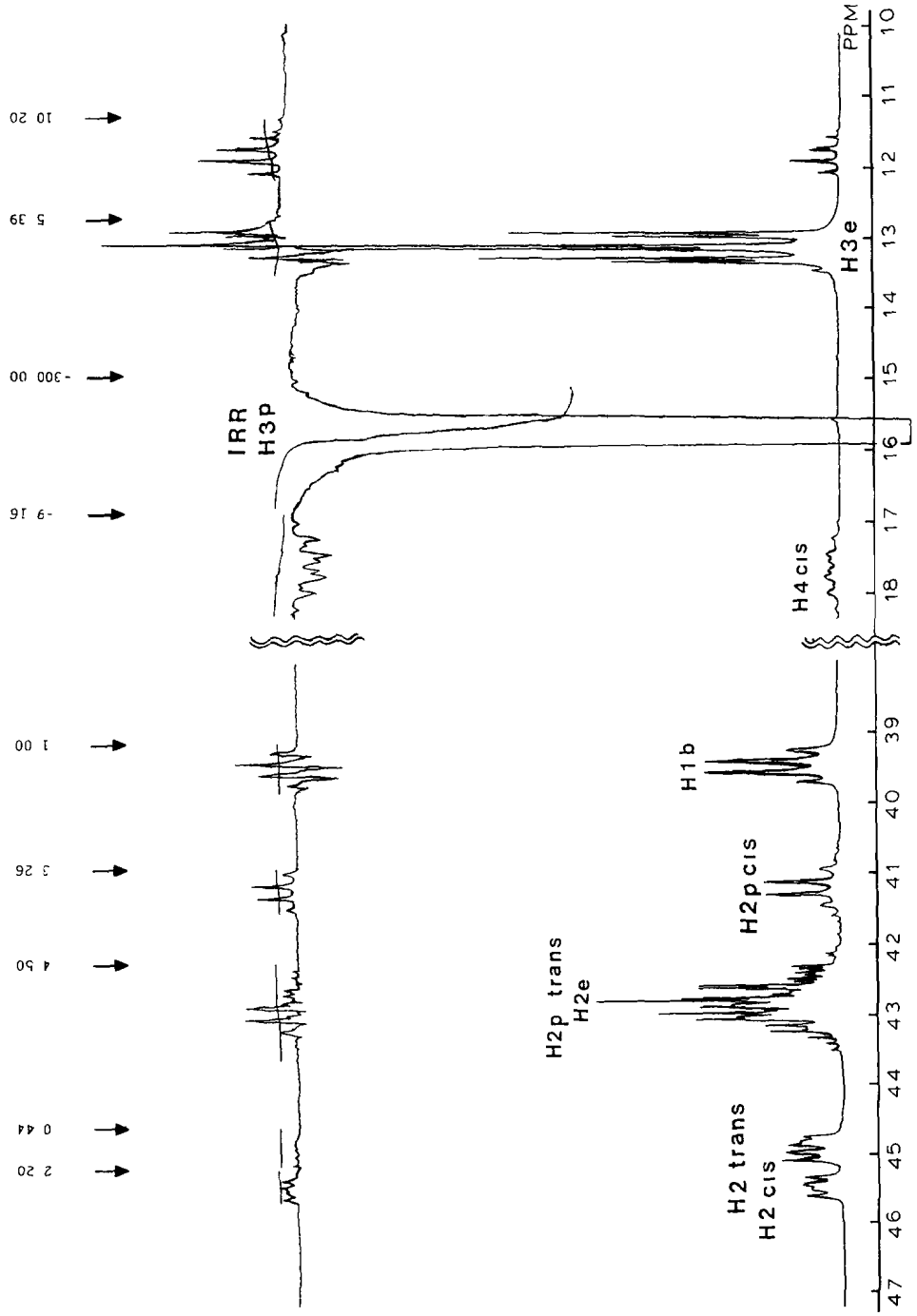
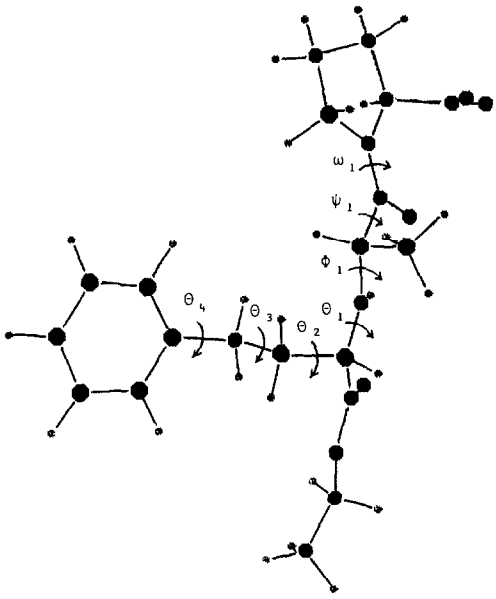
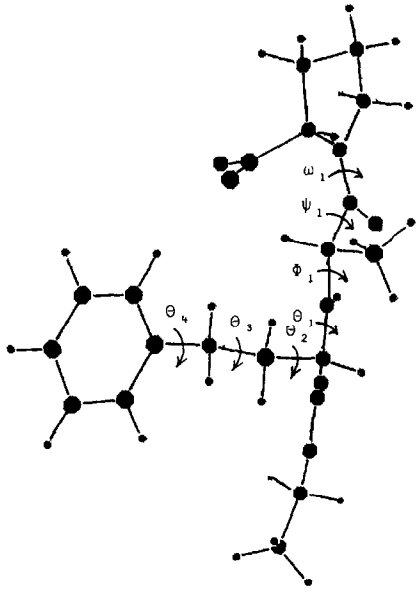


Fig 3 Difference NOE spectra with selective irradiation of H3p (top), ^{1}H NMR spectra with selective irradiation of H3p (bottom)



(a) *trans*



(b) *cis*

Fig 4 Stereoscopic views of the molecular in CD_3OD (a) *trans* form, (b) *cis* form

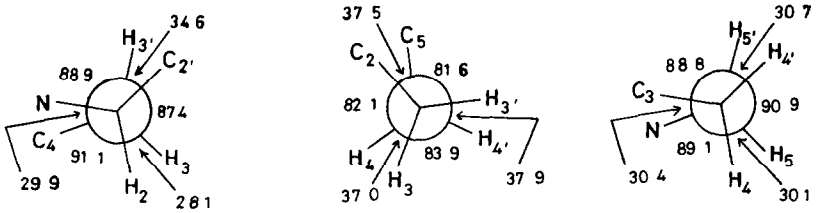


Fig 5 Newman projections along (a) C2-C3, (b) C3-C4, and (c) C4-C5

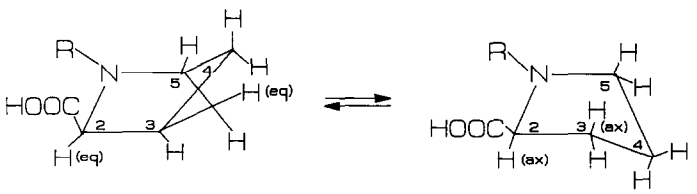


Fig 6 Structure of *S*- and *N*-types of proline ring in MK-421

If one supposes that α_1 and α_2 are the molar ratios of *S*-type isomer in *trans* and *cis* conformations, respectively, the following equations are obtained

$$2(\mathrm{Hz})\alpha_1 + 10(\mathrm{Hz})(1-\alpha_1) = 8\,8(\mathrm{Hz}) \qquad \alpha_1 = 0\,15(trans)$$

$$2(\mathrm{Hz})\alpha_2 + 10(\mathrm{Hz})(1-\alpha_2) = 6\,3(\mathrm{Hz}) \qquad \alpha_2 = 0.46(cis)$$

In the *trans* form, the occurrence ratio of *S*- and *N*-type isomers is 15 85 The prevalence of *N*-type isomer over *S*-type isomer may be due to the tendency of the carbonyl group to hydrogen bond with the carboxyl group In the *cis* form, the corresponding ratio is 46 54, that is, *S*- and *N*-type isomers are present in almost the same proportion (in contrast to the case of the *trans* form) because the carbonyl and carboxyl groups in the *cis* form are oriented in opposite directions, and they do not influence each other

The comparison of the conformations of *trans* and *cis* forms obviously confirms results obtained using the NMR techniques in solution

CONCLUSIONS

Use of bond angles and bond lengths obtained for MK-421 from NMR studies in solution have enabled us to postulate the favoured spatial orientation of functional groups in solution which are important for binding to the enzyme ACE. We conclude that the *trans* conformation of MK-421 is favoured for binding to angiotensin converting enzyme

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