Structure and Conformation of (1*S*,2*R*)-*cis*-2-[Hydroxyaminocarbonylmethyl-(*N*-methyl)aminocarbonyl]cyclohexanecarboxylic Acid: X-ray, NMR and Molecular Mechanics Studies

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The structural characterization of idrapril, (1*S*,2*R*)-*cis*-2-[hydroxyaminocarbonylmethyl(*N*-methyl)-aminocarbonyl]cyclohexanecarboxylic acid (**4**), a novel ACE inhibitor and its related benzyloxy derivative (**3**) has been carried out by NMR studies. The crystal structure of **3** has been determined by X-ray diffraction. The NMR spectra indicate the presence of two *cis* and *trans* isomers with respect to the amide bond at room temperature, with rotational barriers of 70 kJ mol^{-1}. The preferred conformation of these compounds is the *trans* rotamer with the carboxylic moiety in the equatorial orientation.

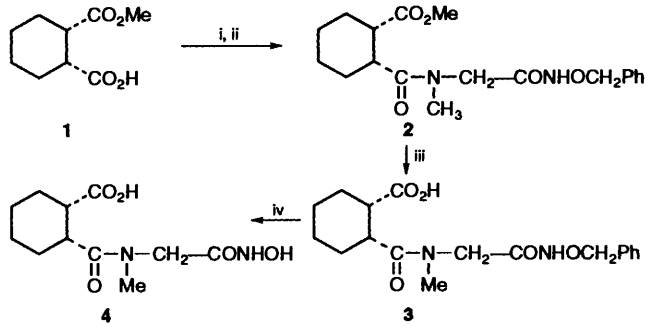
Conformational studies in aqueous solution have been reported for idrapril as a function of pH and the equilibrium constant has been determined. The results suggest the presence of intramolecular hydrogen bonds, as is confirmed by molecular mechanics calculations.

Angiotensin converting enzyme (ACE, EC 3.1.15.1) is the regulatory zinc metallopeptidase in the renin-angiotensin system. ACE converts the decapeptide angiotensin I to the powerful vasoconstrictor angiotensin II and inactivates the vasodilating substance bradykinin. ACE inhibitors are effective drugs in the treatment of essential hypertension and congestive heart failure.[1,2] Captopril, 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, was the first orally active ACE inhibitor with clinical applications.[3,4] Replacement of the zinc-binding mercapto group with carboxy or phosphonic acid ligands enhanced the binding specificity of the more recent inhibitors.[5,6] Idrapril, (1*S*,2*R*)-*cis*-2-[hydroxyaminocarbonylmethyl(*N*-methyl)aminocarbonyl]cyclohexanecarboxylic acid (**4**), is the prototype of a novel class of ACE inhibitors: it is a non-amino acid derivative with the hydroxamic group that binds the zinc atom.[7] Idrapril was shown to be a highly selective and competitive ACE inhibitor *in vitro* and *in vivo*, with a potency comparable to captopril.[8] It is well known that captopril, like other proline and sarcosine-containing peptides,[9,10] exists in solution as a mixture of *cis* and *trans* isomers about the peptide bond, whose proportion is dependent on the state of protonation of the molecule.[11] Thus, idrapril is expected to show the presence of conformational isomerism across the amide bond. NMR spectroscopy provides a convenient method for studying the solution chemistry of these isomers.

In this paper, we report the structural characterization of idrapril and its benzyloxy precursor **3**, as determined by NMR studies in solution and an X-ray analysis of **3**. We have also carried out conformational studies of idrapril in aqueous solution from pH = 1 (IH_2) to pH = 12 (I^{2-}), as the *cis*/*trans* equilibrium could be important in the binding of **4** by ACE. Molecular mechanics calculations were performed for the IH_2, IH^- and I^{2-} forms of **4**.

Results and Discussion

Synthesis.—Idrapril was prepared as previously reported[7] according to Scheme 1, with a stereospecific synthesis starting from (1*R*,2*S*)-*cis*-2-(methoxycarbonyl)cyclohexanecarboxylic acid (**1**).



Scheme 1 *Reagents:* (i), $(COCl)_2$; (ii), $HN(CH_3)$–CH_2–CONH-OCH_2Ph; (iii), NaOH (1 mol dm^{-3}); (iv), H_2, Pd/C

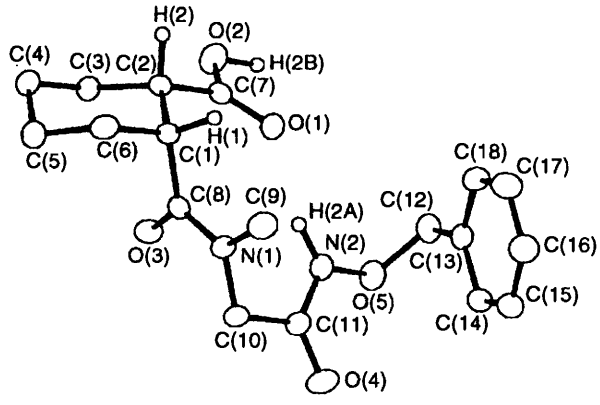


Fig. 1 ORTEP drawing of **3**. For clarity, only the hydrogen atoms H(1), H(2), H(2A) and H(2B) have been drawn.

Description of the Structure.—X-ray crystal structure was determined for the benzyloxy derivative **3**, as we could not obtain single crystals of idrapril. The structure (Fig. 1) consists of discrete molecules of (1*S*,2*R*)-*cis*-2-[benzyloxyaminocarbonylmethyl(*N*-methyl)aminocarbonyl]cyclohexanecarboxylic acid. Table 1 lists selected bond distances and angles. The carboxylic group occupies an equatorial position of the

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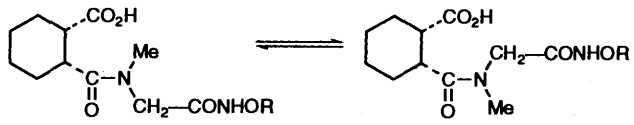
cyclohexane residue, whereas the benzylic chain is an axial substituent. The overall molecular shape is determined by the strong intramolecular hydrogen bond N(2)–H(2A)···O(1) of 2.126(3) Å with angular value of 153.2(3)°. This hydrogen bond causes the creation of a ten-membered ring including all the atoms from O(1) to H(2A). The maximum deviation from the least-square plane described by the ten atoms is 0.37 Å. The C(8)–O(3) and N(1)–C(9) bonds of the amide moiety are almost perpendicular to this mean plane. The hydrogen atom of the carboxylic group is involved in an intramolecular contact with O(1) [O(2)–H(2B)···O(1) 2.25(2) Å], so determining a planar arrangement of the carboxylic group. Because of the involvement of the two hydrogen atoms H(2B) and H(2A) in strong internal hydrogen bonds the molecule does not show any intermolecular strong contact.

NMR Studies.—The ^1H and ^{13}C NMR data of compounds **3** and **4** are summarized in Tables 2 and 3. The assignment of proton and carbon resonances was made on the basis of proton–proton and proton–carbon shift correlation spectra. The combined use of COSY and NOESY spectra helped the conformational study.

We observe a splitting of most ^1H and ^{13}C signals at room temperature attributed to the presence of two conformational *cis* and *trans* isomers (**3a**, **b**, **4a**, **b**) about the amidic C–N bond.



trans
3a R = CH_2Ph
4a R = H

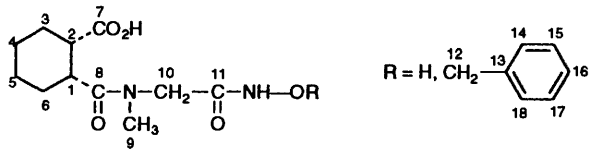
cis
3b R = CH_2Ph
4b R = H

The assignment of the methinic protons H(1) and H(2) was based on the NOESY spectra data and on the signal widths at half-height (see Table 2), which are diagnostic for equatorial (5–10 Hz) and axial (15–30 Hz) protons in disubstituted cyclohexanes.[12] The shielded proton was assigned an axial position ($W_{1/2}$ = 19, 22 Hz) and in the NOESY spectrum no interactions were observed for this proton, except a crosspeak

Table 1 Selected bond lengths (Å) and angles (°) for $C_{18}H_{24}N_2O_5$

O(1)–C(7)	1.212(3)	C(2)–C(7)	1.502(4)
O(2)–C(7)	1.308(3)	C(2)–C(3)	1.534(4)
O(3)–C(8)	1.222(3)	C(3)–C(4)	1.516(4)
O(4)–C(11)	1.220(3)	C(4)–C(5)	1.514(4)
O(5)–N(2)	1.394(3)	C(5)–C(6)	1.523(4)
O(5)–C(12)	1.447(4)	C(10)–C(11)	1.506(4)
N(1)–C(8)	1.359(3)	C(12)–C(13)	1.486(4)
N(1)–C(10)	1.442(3)	C(13)–C(14)	1.383(4)
N(1)–C(9)	1.453(4)	C(13)–C(18)	1.389(4)
N(2)–C(11)	1.333(4)	C(14)–C(15)	1.376(5)
C(1)–C(8)	1.518(4)	C(15)–C(16)	1.373(5)
C(1)–C(2)	1.531(4)	C(16)–C(17)	1.365(5)
C(1)–C(6)	1.537(4)	C(17)–C(18)	1.370(5)
N(2)–O(5)–C(12)	110.2(2)	O(2)–C(7)–C(2)	113.5(2)
C(8)–N(1)–C(10)	118.5(2)	O(3)–C(8)–N(1)	119.9(2)
C(8)–N(1)–C(9)	125.3(2)	O(3)–C(8)–C(1)	121.9(2)
C(10)–N(1)–C(9)	116.1(2)	N(1)–C(8)–C(1)	118.2(2)
C(11)–N(2)–O(5)	116.8(2)	N(1)–C(10)–C(11)	116.3(2)
C(8)–C(1)–C(2)	111.2(2)	O(4)–C(11)–N(2)	124.1(3)
C(8)–C(1)–C(6)	111.4(2)	O(4)–C(11)–C(10)	119.7(3)
C(2)–C(1)–C(6)	111.1(2)	N(2)–C(11)–C(10)	116.2(2)
C(7)–C(2)–C(1)	111.1(2)	O(5)–C(12)–C(13)	114.0(3)
C(7)–C(2)–C(3)	112.9(2)	C(14)–C(13)–C(18)	117.8(3)
C(1)–C(2)–C(3)	114.5(2)	C(14)–C(13)–C(12)	122.0(3)
C(4)–C(3)–C(2)	111.3(3)	C(18)–C(13)–C(12)	120.2(3)
C(3)–C(4)–C(5)	112.2(2)	C(15)–C(14)–C(13)	120.7(3)
C(4)–C(5)–C(6)	110.9(3)	C(16)–C(15)–C(14)	120.4(3)
C(5)–C(6)–C(1)	112.4(2)	C(17)–C(16)–C(15)	119.7(3)
O(1)–C(7)–O(2)	122.4(3)	C(16)–C(17)–C(18)	120.2(3)
O(1)–C(7)–C(2)	124.0(2)	C(17)–C(18)–C(13)	121.3(3)

Table 2 ^1H Chemical shifts (δ) for compounds **3** and **4**[a]



	4		**3**	
	cis	*trans*	*cis*	*trans*
H(1)	3.23 (m, $W_{1/2}$ = 13 Hz)		3.23 (m, $W_{1/2}$ = 10 Hz)	
H(2)	2.41 (m, $W_{1/2}$ = 22 Hz)		2.47 (m, $W_{1/2}$ = 19 Hz)	
H(3)–H(6)	1.05–2.20 (m)		1.10–2.20 (m)	
H(9)	2.73 (s)	3.03 (s)	2.73 (s)	3.01 (s)
H(10a)	3.80 (d)	3.58 (d)	3.80 (d)	3.57 (d)
H(10b)	4.05 (d)	3.96 (d)	4.04 (d)	3.98 (d)
CONH	9.00 (s)	8.80 (s)	10.99 (br s)	
H(12)	—		4.77 (s)	
Ph	—		7.37 (s)	
CO_2H	11.93 (br s)		—	
CONHO*H*	10.65 (s)	10.30 (s)		

[a] Shifts relative to Me_2SO = 2.49 ppm from Me_4Si. Abbreviations: m, multiplet; s, singlet; d, doublet; br, broad.

Table 3 ^{13}C Chemical shifts (δ) for compounds **3** and **4**[a]

	4		**3**	
	cis	*trans*	*cis*	*trans*
C(1)	37.34		37.34	
C(2)	42.18		42.33	42.22
C(3)	25.04	24.73	24.91	24.70
C(4)	23.66	24.06	24.19	23.88
C(5)	22.00	21.69	21.86	21.70
C(6)	26.43	26.09	26.64	26.19
C(7)	175.35		175.45	
C(8)	174.97		174.09	
C(9)	34.01	36.64	34.28	36.64
C(10)	50.10	48.19	50.11	48.10
C(11)	165.15	165.56	165.48	165.90
C(12)	—		76.96	
C(13)	—		135.92	
C(14)–(C18)	—		128.35	128.87

[a] Shifts relative to Me_2SO = 39.50 ppm from Me_4Si.

Table 4 Conformers classification based on the type and number of hydrogen bonds and the *cis–trans* conformation of the amidic bond in IH_2 and IH^-. For each class of conformers the reported energy (kcal) is referred to the most stable isomer.[a]

	Number of hydrogen bonds	Type	E_{trans}	E_{cis}
IH_2	3	O(5)–H(5)···O(4); N(2)–H(2A)···O(3); O(2)–H(2)···O(1)	−8.04	—
	2	N(2)–H(2A)···O(3); O(2)–H(2)···O(1)	−7.39	—
		O(5)–H(5)···O(3); O(2)–H(2)···O(1)	−6.32	—
		O(5)–H(5)···O(4); O(2)–H(2)···O(3)	−1.95	—
		O(5)–H(5)···O(4); N(2)–H(2A)···O(3)	−3.92	—
		O(5)–H(5)···O(4); O(2)–H(2)···O(1)	−6.71	−6.92
	1	O(2)–H(2)···O(3)	−1.05	−2.28
		N(2)–H(2A)···O(3)	−2.62	—
		O(5)–H(5)···O(3)	−1.92	—
		O(2)–H(2)···O(1)	−5.55	−5.39
		O(5)–H(5)···O(4)	−3.83	−3.23
	0	—	−1.81	−1.18
IH^-	3	O(5)–H(5)···O(4); N(2)–H(2A)···O(1); N(2)–H(2A)···O(2)	—	23.2
	2	N(2)–H(2A)···O(3); O(5)–H(5)···O(4)	22.1	—
		N(2)–H(2A)···O(1); N(2)–H(2A)···O(2)	—	22.0
		N(2)–H(2A)···O(1) or O(2); O(5)–H(5)···O(4)	22.9	22.9
	1	N(2)–H(2A)···O(3)	23.5	—
		O(5)–H(5)···O(3)	24.2	—
		O(5)–H(5)···O(4)	—	22.2
		O(5)–H(5)···O(1) or O(2)	24.2	23.1
		N(2)–H(2A)···O(1) or O(2)	21.6	23.0
	0	—	24.0	27.1

[a] The numbering of the atoms is the same as the ORTEP drawing. The Ph–CH_2– group was substituted by H(5). 1 cal = 4.184 J.

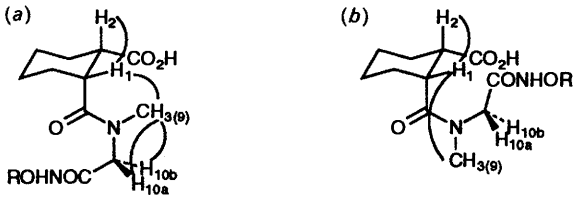


Fig. 2 NOE correlations indicating the preferred conformation of the *trans* (*a*) and *cis* (*b*) isomers

with the other methinic proton, indicating a *trans* orientation with respect to the amide substituent and, in consequence, an equatorial CO_2H group. Therefore the signals at δ 2.41 and 2.47, respectively in the spectra of compounds **4** and **3**, were attributed to the proton H(2). The deshielded proton at 3.23 ppm resulted in equatorial position ($W_{1/2}$ = 10, 13 Hz) and showed a crosspeak with the proton H(9), so it was attributed to H(1).

The deshielded H(9) showed, in addition to interactions with H(10a) and H(10b), a very strong NOE on H(1), indicating the *trans* isomer across the amide bond [Fig. 2(*a*)]. The shielded H(9) showed only a weaker NOE interaction on H(1) indicating the *cis* isomer [Fig. 2(*b*)]. Furthermore, the absence of reciprocal NOE between H(10) and H(1) could suggest that the methylene group is constrained in a position far from the cyclohexane ring in the *cis* conformation.

The *trans*:*cis* ratio as calculated from the peak intensities of the N–CH_3 signals was 7:3 for compounds **3** and **4** in Me_2SO. These results are in agreement with the structure of **3** deduced from X-ray data.

Variable temperature ^1H NMR spectroscopy was used to study rotation about the C–N bond, determining the values of $\Delta G^{\ddagger}$ in Me_2SO for **3** and **4**. Values were calculated using eqn. (1),[13] where T_c is the coalescence temperature and $\Delta\nu$

$$\Delta G^{\ddagger} = -RT_c \ln [\pi h(\Delta\nu)/1.4142\, K_b\, T_c] \qquad (1)$$

refers to the difference in frequency of two non-equivalent signals of the rotamers calculated by extrapolation at T_c.[14] The coalescence temperature for each compound was determined from the N–CH_3 signal behaviour at 2.6–3.1 ppm.

Upon increasing the temperature for compound **4**, some signals due to the partial hydrolysis were observed, but the N–CH_3 peaks were detectable.

Rotational energy barriers calculated for **3** ($\Delta\nu$ = 43.32 Hz, T_c = 341 K) and **4** ($\Delta\nu$ = 50.09 Hz, T_c = 341 K) were 70.95 and 70.54 kJ mol^{-1}, respectively and lie in the typical amide range (50–100 kJ mol^{-1}).

Molecular Mechanics Studies.—Conformers obtained from the systematic conformational search performed on IH_2, IH^- and I^{2-} forms of idrapril have been analysed and classified taking into account the internal hydrogen bonds and the *cis* or *trans* conformation of the amidic bond. Results are reported in Table 4.

IH_2. It is noteworthy that there is not any one conformer which reproduces in a satisfactory way the hydrogen bond and the conformation found in the X-ray structure of the benzyloxy derivative. In fact in all conformers presenting the hydrogen bond N(2)–H(2A)···O(1) this distance is significantly higher than that found in the solid state and the dihedral angle N(1)–C(10)–C(11)–N(2), which is −17.4(4)° in the X-ray structure, assumes more familiar values of about 60°. We can hypothesize this discrepancy between experimental and theoretical findings to be caused by an underestimation of the hydrogen bon

Table 5 Partial charges of hydrogen bonding atoms as calculated by the MNDO method

Atom	IH_2	IH^-	I^{2-}
O(1)	−0.377	−0.598	−0.607
O(2)	−0.300	−0.577	−0.598
O(3)	−0.365	−0.365	−0.412
O(4)	−0.307	−0.330	−0.423
H(2)	0.221	—	—
H(2A)	0.173	0.164	0.000
H(5)	0.192	0.185	—

Table 6 *cis* and *trans* forms' relative population (X) and multiplicity of the CH_2 signals

		H(10)	
pH	X_{trans}/X_{cis}	*cis*	*trans*
0.70	3.10	s	s
3.47	3.56	s	s
5.20	4.15	dd	dd
5.60	4.07	dd	dd
7.60	4.05	dd	dd
8.30	3.80	dd	dd
9.90	2.83	dd	dd
12.19	1.77	dd	dd

stabilizing contribution during the geometry optimization which favours an adjustment of the torsional angle N(1)–C(10)–C(11)–N(2) to more canonical values to the detriment of a very strong hydrogen bond. On the other hand, during a simple energy minimization on the benzyloxy derivative, this hydrogen bond is removed favouring the N(2)–H(2A)···O(3) linkage.

The most stable conformers (see Table 4) having a *trans* conformation of the amide bond present hydrogen bonds often involving the amidic oxygen O(3) besides those 'internal' to the carboxylic and hydroxamic groups. The *cis* conformation of the O(3)–C(8)–N(1)–C(9) torsional angle hinders the presence of the N(2)–H(2A)···O(3) and O(5)–H(5)···O(3) hydrogen bonds allowing a greater conformational freedom to the end moiety of the hydroxamic chain.

IH^-. The most interesting result of the conformational search is that the increased negative charge on the oxygen atoms of the carboxylate group favours the formation of hydrogen bonds with H(2A) and H(5) so making the overall anion rather rigid. Some conformers exist having both carboxylic oxygens linked to the H(2A) hydrogen atom. As expected also for the monocharged species many isomers show the 'internal' hydrogen bond O(5)–H(5)···O(4) and only in the *trans* conformation is O(3) involved in hydrogen bonds.

I^{2-}. The conformational search does not produce isomers having hydrogen bonds because the remaining hydrogen atom H(2A) has a partial atomic charge equal to zero (see Table 5).

Conformational Equilibria of Idrapril in Aqueous Solution.—^1H NMR spectra of **4** in D_2O (pH = 3.6) showed the presence of *cis* and *trans* rotamers with a *trans*:*cis* ratio of 7:3. In order to characterize the conformational equilibria of **4** in aqueous solution and to understand the behaviour of the compound at physiological pH, a ^1H NMR study was carried out at pH 1–12. The relative population of the two *cis* and *trans* forms, obtained from the intensities of the two N–CH_3 singlets and the multiplicity of the CH_2 signals is dependent on the protonation state of the molecule (IH_2, IH^- and I^{2-} forms for idrapril) as shown in Table 6. The fractional concentrations of the two isomers are reported as a function of pH (Fig. 3) and the equilibrium constant for the *trans* $\rightleftharpoons$ *cis* equilibrium, K_{eq} = [*cis*]/[*trans*], is 0.276, 0.246 and 0.565 for IH_2, IH^- and I^{2-}, respectively. The H(10) signals of each rotamer are singlets at low pH values (IH_2 form) and become double doublets on increasing the pH, indicating the change from an A_2 to an AB system of the CH_2 group. This behaviour can be explained with the higher rigidity of idrapril in the IH^- form due to the presence of a negative charge on the carboxylate group that favours the formation of strong intramolecular hydrogen bonds with H(2A) and H(5) (Table 4). The CH_2 signal is a double doublet at pH 12 too, suggesting also in this case molecular rigidity.

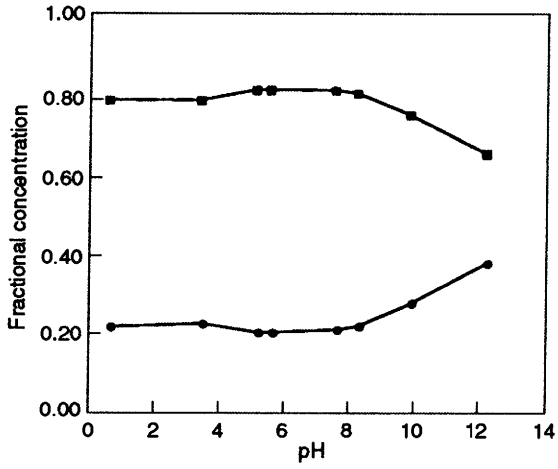


Fig. 3 Fractional concentrations of the *cis* (●) and *trans* (■) isomers of idrapril as a function of pH

At a high pH value we observe a decrease in the *trans*:*cis* ratio that we can attribute to the lack of those hydrogen bonds that stabilize the *trans* rotamer in IH_2 and IH^- forms. These results seem to be in agreement with molecular mechanics studies.

Experimental

All NMR spectra were recorded on a Bruker AC 200 E spectrometer operating at 200.13 and 50.33 MHz for ^1H and ^{13}C, respectively. The temperature was controlled with a Bruker VT 1000 variable temperature unit (± 1 K).

Crystal Data.—$C_{18}H_{24}N_2O_5$, M = 348.4, orthorhombic, a = 6.987(3), b = 11.502(9), c = 22.917(5) Å, U = 1841(2) Å^3 (by least-squares refinement on diffractometer angles for 25 automatically centred reflections, λ = 0.710 69 Å) space group $P2_12_12_1$, Z = 4, D_c = 1.26 g cm^{-3}, $F(000)$ = 744. Crystal dimensions 0.6 × 0.5 × 0.3 mm, μ = 0.86 cm^{-1}.

Data Collection and Processing.—CAD4 diffractometer, θ-2θ mode, with scan width (0.85 + 0.35 tan θ)°, variable scan speed, graphite-monochromated Mo-Kα radiation; 1987 reflections measured ($2.5 < \theta < 25$, $+h$, $+k$, $+l$), 1757 unique reflections; absorption correction with the Walker and Stuart method.[15]

Structure Analysis and Refinement.—Direct methods of SIR88.[16] Full matrix least-squares refinement by using SHELXL-93,[17] with weighting scheme parameters of 0.0600 and 0.3121. The positions of the hydrogen atoms belonging to the carboxylic and amidic groups were found in the ΔF map and refined. The remaining hydrogen atoms were introduced in calculated positions and refined accordingly to the linked atoms. For all the hydrogen atoms an overall isotropic temperature was assigned and refined to a value of U = 0.082 Å^2.

Table 7 Bond angles, rotation step (°) and range (°) involved in the conformational search performed on IH_2, IH^- and I^{2-}

Bond		IH_2		IH^-		I^{2-}	
		Rotation step	Range	Rotation step	Range	Rotation step	Range
C(7)–O(2)	(φ_1)	60	0, 360	—		—	
C(2)–C(7)	(φ_2)	60	0, 360	60	0, 180	60	0, 180
C(1)–C(8)	(φ_3)	60	0, 360	180	0, 360	60	0, 360
C(8)–N(1)	(φ_4)	180	0, 360	60	0, 360	180	0, 360
N(1)–C(9)	(φ_5)	60	0, 120	60	0, 120	60	0. 120
N(1)–C(10)	(φ_6)	60	0, 360	60	0, 360	60	0, 360
C(10)–C(11)	(φ_7)	60	0, 360	180	0, 360	60	0, 360
C(11)–N(2)	(φ_8)	180	0, 360	60	0, 360	180	0, 360
N(2)–O(5)	(φ_9)	60	0, 360		0, 360		

Anisotropic thermal parameters were used for non-hydrogen atoms. A Fourier difference map performed in the last refinement cycle did not show any interesting features, the highest peak being 0.12 e $Å^{-3}$. The final R factor was 0.035 for 1569 reflections having $F_o > 4\sigma(F_o)$, whereas the wR^2 factor was 0.103. The atomic scattering factors and anomalous dispersion corrections for all the atoms from the International Tables of X-ray Crystallography.[18] The final atomic parameters, thermal parameters, atomic coordinates, bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre.‡

2D NOESY.—All measurements were made on $[^2H_6]Me_2SO$ solutions (*ca.* 0.2 mol dm^{-3}) accurately degassed by freeze–pump thaw cycles. The spectra were calibrated relative to the central resonance of $[^2H_6]$DMSO. 2D NOESY were recorded in the phase sensitive mode (TPPI) with the sequence RD–90–t_1–90–t_m–90–AQ. A mixing time of 1 s and a pulse delay of 6 s were used. Arrays of 512 FIDs (8 scans) of 2048 data points were acquired. Zero filling in the F1 dimension was applied to obtain a 2 K × 2 K data point matrix. A Gaussian function was applied to the data in both dimensions.

Theoretical Calculations.—Molecular mechanics calculations were performed on (1*S*,2*R*)-*cis*-2-[hydroxyaminocarbonylmethyl(*N*-methyl)aminocarbonyl]cyclohexanecarboxylic acid (IH_2), its monoanionic (IH^-) and dianionic specie (I^{2-}) by using the software programs of Biosym Technologies: InsightII, Discover, Search-Compare and Analysis.[19] The program package was implemented on an IBM RISC/6000 320H computer. Atomic charges of IH_2, IH^- and I^{2-} obtained by the MNDO program taken from the MOPAC Package (version 6.0)[20] are reported in Table 7. The conformations of a large number of rotamers, for each compound, were accomplished by systematic rotations around the bonds listed in Table 7 with the Search-Compare module. The geometry of each conformer was then optimized by an energy minimization with the aid of the following algorithms: conjugate gradient and Newton-Raphson. The force field used was CFF91 provided by Discover, employing the default value of 1.0 for the relative permittivity.

pH-*Dependence.*—Samples for the measurement of ^1H NMR spectra as a function of pH were prepared by adding idrapril in 99.8% D_2O (*ca.* 0.20 mol dm^{-3}). The resulting pH (uncorrected for the isotope effect) was adjusted to the desired value with some drops of NaOD (40% wt) or CF_3CO_2H respectively. All pH measurements were made at 20 °C with a METROHM Model 691 pH meter equipped with a pH combined electrode. The ^1H NMR spectra were calibrated assigning a shift of 4.72 ppm, with respect to DSS [sodium 3-(trimethylsilyl)propane-1-sulfonate], to the residual HDO signal.

‡ For details of the CCDC deposition scheme, see 'Instructions for Authors (1995)', *J. Chem. Soc., Perkin Trans. 2*, 1995, issue 1.

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References

1 D. McAreavey and J. I. S. Robertson, *Drugs*, 1990, **40**, 326.
2 S. E. O'Connor, *Drug News Perspect.*, 1990, **3** 133.
3 M. A. Ondetti, B. Rubin and D. W. Cushman, *Science*, 1977, **196**, 441.
4 D. W. Cushman, H. S. Cheung, E. F. Sabo and M. A. Ondetti, *Biochemistry*, 1977, **16**, 5484.
5 M. J. Wyratt and A. A. Patchett, *Med. Res. Rev.*, 1985, **5**, 483.
6 H. R. Brunner, J. Nussberger and B. Waeber, *J. Cardiovasc. Pharm.*, 1985, **7**, S2–S11.
7 L. Turbanti, G. Cerbai, C. Di Bugno, R. Giorgi, G. Garzelli, M. Criscuoli, A. R. Renzetti, A. Subissi and G. Bramanti, *J. Med. Chem.*, 1993, **36**, 699.
8 A. Subissi, M. Criscuoli, G. Sardelli, M. Guelfi and A. Giachetti, *J. Cardiovasc. Pharmacol.*, 1992, **20**, 139.
9 C. A. Evans and D. L. Rabenstein, *J. Am. Chem. Soc.*, 1974, **96**, 7312.
10 S. V. S. Mariappan and D. L. Rabenstein, *J. Org. Chem.*, 1992, **57**, 6675.
11 D. L. Rabenstein and A. A. Isab, *Anal. Chem.*, 1982, **54**, 526.
12 W. L. F. Armarego and T. Kobayashi, *J. Chem. Soc. C*, 1969, 1635.
13 I. D. Cunningham, J. Llor and L. Munoz, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1751.
14 Y. H. Lai and P. Chen, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1665.
15 N. Walker and D. D. Stuart, *Acta Crystallogr., Sect. A*, 1983, **39**, 158.
16 M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, G. Polidori, R. Spagna and D. Viterbo, *J. Appl. Crystallogr.*, 1989, **22**, 389.
17 G. M. Sheldrick, in preparation.
18 International Tables for X-Ray Crystallography, Kynoch Press, Birmingham, 1974, vol. 4.
19 Biosym Technologies, Inc. 9685 Scranton Road, San Diego, CA 92121-2777.
20 J. J. P. Stewart, *QCPE* no. 455.

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