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Stereochemistry–Activity Relationships of ACE Inhibitors. Conformational Studies by ^{1}H and ^{13}C NMR of Perindopril and Selected Stereoisomers

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Perindopril, the *tert*-butylamine salt of 1-{(2*S*)-2-[(1*S*)-(1-carbethoxybutyl)amino]-1-oxopropyl}-(2*S*,3a*S*,7a*S*)-perhydroindole-2-carboxylic acid, is an inhibitor of angiotensin-converting enzyme (ACE) and a new drug for the treatment of hypertension. The interaction between the inhibitor and the enzyme was investigated by studying the active diacid metabolite of perindopril, its stereoisomers and a desmethyl analogue. The pharmacological study allowed the measurement of the *in vitro* activities of the different compounds. The ^{1}H and ^{13}C NMR studies have shown that the *cis–trans* equilibrium about the amide bond is strongly dependent on the configuration of the chiral centres and on the pH of the solution. The pK_a of the different acid–base species were measured. The results show that perhydroindole derivatives are potent inhibitors of ACE as long as they fulfil the following basic requirements: (1) an *S* configuration of the carbon bearing the terminal carboxy group; (2) an *S* methyl substituent in the alanine residue; however, the inhibitor potency is not modified on the replacement of the alanine residue of the perindopril by a glycine residue; and (3) less stringently, an *S* configuration of the C-1 butyl carbon. Under these conditions the Zn binding ligand (chain carboxylate group) is devoid of steric hindrance in the *trans* conformers. No direct relationship appeared between the relative amount of the *trans* form and the activity. The cyclic skeleton of the perhydroindole derivatives provided a strong hydrophobic interaction with the active enzymatic site, whatever the configurations at C-3a and C-7a. Lipophilic interactions involving the different parts of the inhibitor are not independent of each other.

KEY WORDS ACE inhibitors Perindopril stereoisomers SAR RMN

INTRODUCTION

The study of inhibitors of the angiotensin-converting enzyme (ACE) has resulted in the synthesis of new drugs for the treatment of hypertension. Early work on structure–activity relationships centred on the synthesis and testing of a variety of compounds. The structural requisites for an effective ACE inhibitor were shown to be a *C*-terminal carboxyl group, an amide carbonyl group and a third functional group which may act as a potent Zn ligand.[1–5] The well known orally active inhibitors of ACE, captopril[6] and enalapril[7,8] are peptide analogues with an L-proline unit at the *C*-terminal end.

Molecular modelling studies[9–14] of structurally different molecules allowed the definition of a specific geometry for the active site of ACE. It was shown that inhibitors with a *C*-terminal proline residue fulfil well the geometric constraints when they are in the *trans* conformation with respect to the amide bond.

Derivatives of the octahydroindole-2-carboxylic acids, which should enhance the lipophilic interactions of the favourable proline subunit, have been studied.[15,16] In particular this involves a new available drug, the *tert*-butylamine salt of 1-{(2*S*)-2-[(1*S*)-(1-carbethoxybutyl)amino] - 1 - oxopropyl} - (2*S*,3a*S*,7a*S*) - perhydroindole-2-carboxylic acid (perindopril), synthesized by Vincent *et al.*[17]

In a previous paper[18] we described the ^{1}H and ^{13}C NMR study of perindopril. A study of its conformational behaviour in various solvents led to the conclusion that the cyclization at the γ, δ-positions of the proline ring does not hinder the preference for the *trans* conformation about the amide bond.

The active metabolite of perindopril is the dicarboxylic form (Scheme 1, compound **1**). The synthesis of this metabolite and of its stereoisomers (compounds **1–32**) will be described separately (manuscript is in preparation). We have to hand the basis of detailed investigation of the stereochemistry–activity relationships for this class of ACE inhibitors since a systematic study of their inhibitor potency versus ACE have shown values within a range of four orders of magnitude.

In this paper we present the results of a detailed analysis of the physicochemical properties and conformational behaviour of the active metabolite and of selected stereoisomers in relation to their biological activities.[19] The choice was made in order that the influence of the ring junction and that of the configu-

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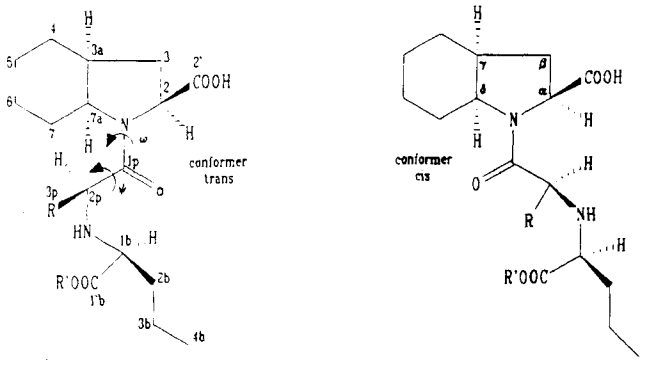


$R = CH_3$ $R' = H$ **1–32** (the absolute configurations are described in Table 1)

$R = CH_3$ $R' = C_2H_5$ **33**

$R = H$ $R' = H$ **34**

Scheme 1

ration of the asymmetric centres of the side chain could be established. Two related compounds were also examined for the purpose of comparison: 1-{(2*S*)-2-[(1*S*)-(1-carbethoxybutyl)amino] - 1 - oxopropyl} - (2*S*,3a*S*,7a*S*) - perhydroindole-2-carboxylic acid (**33**) and the desmethyl analogue of the active metabolite of perindopril, 1 - {2 - [(1*S*) - (1 - carboxybutyl)amino] - 1 - oxoethyl} - (2*S*, 3a*S*,7a*S*)-perhydroindole-2-carboxylic acid (**34**). The various compounds were studied in aqueous solution by means of ^1H and ^{13}C NMR spectroscopy over a wide range of pH to take account of the influence of the ionization states of the multiple acidic and basic sites.

RESULTS

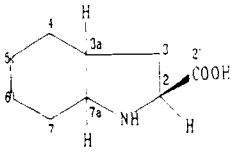
Inhibitor potency

The *in vitro* ACE inhibitor activity was characterized by the concentration of inhibitor in nmol. l^{-1} needed for 50% inhibition of isolated angiotensin converting enzyme. The IC_{50} values were obtained using hippurylhistidylleucine (HHL) as substrate.[20] The results are reported in Table 1. Inspection of Table 1 shows that the compounds with the *R* configuration at C-2 lack significant activity, in fair agreement with previous studies.[5,7,9,21] These isomers were not considered further. The inhibitory potency of the stereoisomers with the *S* configuration at C-2 is strikingly dependent on the configuration at C-2p. As previously observed for captopril or enalapril, the *S* configuration is necessary for significant activity.[7,12,22] The question is whether the configuration at C-2p is determinant (i) through its influence on the conformational behaviour about the amide bond, (ii) by promoting a lipophilic interaction of the methyl group with a hydrophobic site of the enzyme or (iii) through its influence on the orientation of the end part of the chain.

The configuration at C-1b appears to be far less important even if, again, the *S* configuration is slightly more favourable. The configuration at C-1b influences the orientation of the Zn ligand carboxylic group and that of the lipophilic end of the chain. Finally, the configurations of the carbons at the ring junction play only a minor role, if any.

Spectral assignments

In order to obtain references for the assignments of the ^1H and ^{13}C NMR spectra of the selected stereoisomers, the four octahydroindole-2*S*,3a(*S*,*R*),7a(*S*,*R*)-carboxylic acids were first examined (Scheme 2, compounds **35–38**). The ^{13}C signals of the ring carbons were assigned by 2D $\delta\,^{13}C$–$\delta\,^{13}C$ correlation spectroscopy (INADEQUATE).[23] Except for a few protons (H-2, H-7a), the ^1H NMR spectra were assigned by 2D $\delta\,^1H$–$\delta\,^1H$ correlation (COSY)[24] or 2D $\delta\,^1H$–$\delta\,^{13}C$ heteronuclear correlation.[25] The chemical shift data are given under Experimental.



2*S*, 3a*S*, 7a*S* **35**

2*S*, 3a*S*, 7a*R* **36**

2*S*, 3a*R*, 7a*S* **37**

2*S*, 3a*R*, 7a*R* **38**

Scheme 2

The *cis* junction in isomers **35** and **38** allowed conformational flexibility of the cyclic skeleton. The chemical shifts of C-5 and C-6 in the cyclohexane ring equal the

Table 1. *In vitro* activity of the *S SS SS* perindopril metabolite, its stereoisomers and its desmethyl analogue, IC_{50} (nM) hippurylhistidylleucine (HHL) as substrate

Compound[a]	IC_{50} (nM)	Compound[a]	IC_{50} (nM)	Compound[a]	IC_{50} (nM)	Compound[a,b]	IC_{50} (nM)
1 *S SS SS*	1.5	**5** *S RR SS*	3.3	**9** *S RS SS*	1.2	**13** *S SR SS*	1.1
2 *S SS SR*	12	**6** *S RR SR*	40	**10** *S RS SR*	54	**14** *S SR SR*	30
3 *S SS RS*	1100	**7** *S RR RS*	10^5	**11** *S RS RS*	$>10^5$	**15** *S SR RS*	7900
4 *S SS RR*	$>10^5$	**8** *S RR RR*	3.3×10^4	**12** *S RS RR*	10^5	**16** *S SR RR*	2600
17 *R RR SS*	$>10^5$	**21** *R SS SS*	1900	**25** *R SR SS*	6×10^4	**29** *R RS SS*	108
18 *R RR SR*	$>10^5$	**22** *R SS SR*	3.6×10^4	**26** *R SR SR*	5×10^4	**30** *R RS SR*	5500
19 *R RR RS*	10^5	**23** *R SS RS*	$>10^5$	**27** *R SR RS*	$>10^5$	**31** *R RS RS*	7800
20 *R RR RR*	10^5	**24** *R SS RR*	$>10^5$	**28** *R SR RR*	2.3×10^4	**32** *R RS RR*	10^5

[a] The configurations of the five asymmetric centres are given in the order C-2, C-3a, C-7a, C-2p, C-1b.
[b] Compound **34** (desmethyl analogue *S SS –S*), $IC_{50} = 1.9$ nM.

mean of the values calculated for each conformation by the use of characteristic increments.[26] In the ^1H spectra, the coupling constants of H-7a with all its vicinal protons have the same mean value, *ca.* 5.3 Hz. The coupling constants measured for H-2 are mean values of similar magnitude (8 and 7 Hz, respectively, to H-3 and H-3'), and the signal is significantly broadened.

The observation of very different multiplet patterns for H-2 in the isomers with the *trans* ring junction, **36** and **37**, shows that the conformation of the proline ring changes strikingly from one isomer to the other. Equal values of the coupling constants of H-2 with H-3 and H-3' (8.9 Hz) in **36**, are well accounted for if the proline ring adopts a conformation of the N type[27] with C-7a(δ)NC-2(α)C-3(β) nearly coplanar and C-3a(γ) out of this plane, and *exo* with respect to the proline carboxylic group. For **37** these coupling constants differ markedly [J(H-2,H-3) = 10.7 Hz and J(H-2,H-3') = 2.5 Hz], showing that the proline ring adopts a conformation of the S type with C-7a(δ)NC-2(α)C-3(β) nearly coplanar and C-3a(γ) out of this plane, and *endo* with respect to the carboxylic group.

For the active metabolite *S SS SS* (compound **1**) and its stereoisomers the ^1H and ^{13}C NMR spectra reveal, as previously observed for perindopril,[18] two sets of resonances which were distinguished on the basis of their relative intensities. This is the result of the slow rate of exchange between the *cis* and *trans* conformers by rotation about the amide bond. The cyclohexane ring of the perhydroindole derivatives is locked in a single chair conformation, whatever the ring junction.

In the ^1H NMR spectra the assignments were straightforward for the methyl protons and for the tertiary protons. 2D NMR COSY experiments were used for the other protons.

The ^{13}C NMR signals were assigned from DEPT experiments,[28] by comparison with the reference compounds and by heteronuclear two-dimensional correlations. The INADEQUATE method was also used for compound **1**. The carbonyl carbons were assigned by observing heteronuclear correlations through long-range coupling.

The conformations about the amide bond were deduced from the relative positions of the signals of C-3(β) and C-3a(γ) of the proline ring in the two sets of resonances.[29–31] These assignments were confirmed by observing, via the nuclear Overhauser effect, a dipolar interaction between H-7a and H-2p in the *trans* conformers, and between H-2 and H-2p in the *cis* conformers.

The ^1H and ^{13}C chemical shift data measured for solutions in D_2O are collected in Table 2 for compound **1**. The data are included in the supplementary material of this paper for other selected stereoisomers. The ^1H and ^{13}C NMR spectra were also recorded over a wide pH range. The influence of the pH on the chemical shifts of protons or carbons in the neighbourhood of the carboxylic acid or amine groups is well documented.[31–36] This is reflected in the titration curves: deprotonation induces high-field shifts of the ^1H signals and the opposite trend is observed for the ^{13}C signals, except for the C-2p which undergoes a high-field shift, as previously reported for the carbon of alanine or methylalanine.[37]

Table 2. ^1H and ^{13}C chemical shifts for compound 1 in D_2O at pH 2.8 (δ ppm referenced to DSS), *trans/cis* ratio = 83/17

	δ^1H (ppm)		$\delta^{13}C$ (ppm)	
Nucleus	*trans*	*cis*	*trans*	*cis*
2	4.60	4.63	61.9	61.4
2'			177.7	178.4
3	2.40	2.52	32.2	34.2
	2.20	2.29		
3a	2.55	2.44	39.5	37.9
4	1.80	1.80	27.1	27.0
	—	—		
5	1.57	1.54	21.6	21.9
	1.43	1.43		
6	1.78	1.78	25.5	25.6
	1.29	1.29		
7	1.93	2.03	30.2	28.0
	1.59	1.36		
7a	4.03	4.19	62.1	61.7
1p			170.4	170.7
2p	4.49	4.08	56.1	57.7
3p	1.66	1.60	19.0	17.6
1b	3.51	3.73	64.0	63.4
1'b			175.4	175.2
2b	1.90	1.99	34.7	34.6
3b	1.46	1.46	20.3	20.1
4b	1.00	1.02	15.3	15.2

Inspection of the ^1H and ^{13}C NMR spectra shows the presence of some significantly broadened signals for the nuclei in the neighbourhood of the acidic and basic groups, particularly in the pH ranges centred about the pK_a values of the relevant groups. The protonation–deprotonation equilibria play the major role in this phenomenon.

pK_a of the acidic and basic groups

Approximate values can be predicted by use of incremental methods.[38] These are 2.2, 3.8 and 7.6 for the chain carboxylic acid, the proline carboxylic acid and the amine conjugate acid, respectively. Provided the two conformers give distinct sets of resonances over a wide range of pH, the pK_a values of the two species can be determined by use of the pH, ^1H or ^{13}C chemical shifts profiles or by the use of chemical shifts diagrams[37] in the case of similar pK_a values. No attempt was made to measure the low pK_a of the side-chain carboxylic group (pK_1). The values obtained, with error ranges of ± 0.1 for the proline carboxylic group (pK_2) and the amine conjugate acid (pK_3), are given in Table 3.

Table 4 summarizes the various through-space interactions which are expected to arise owing to the chemical structure and their expected influences on the various pK_a values.

Examination of the data in Table 3 reveals that the pK_a values vary from one stereoisomer to the other and from one conformer to the other. The influence of the various parameters can be first deduced by comparing compounds **1–4** (*S SS xy*) with the desmethyl analogue **34**.

The pK_3 value of the amine group in **34** has the same value for both conformers. In the *trans* conformers of

Table 3. pK_a values for the different compounds

Compound	Conformation	pK_3 (NH_2)	pK_2 (proline COOH)
1 *S SS SS*	*trans*	8.2	3.8
	cis	9.3	3.6
2 *S SS SR*	*trans*	8.1	3.8
	cis	8.6	3.2
3 *S SS RS*	*trans*	8.8	3.7
	cis	8.1	<3.7[a]
4 *S SS RR*	*trans*	8.8	3.9
	cis	8.1	<3.9[a]
5 *S RR SS*	*trans*	8.5	3.7
	cis	9.0	<3.7[a]
9 *S RS SS*	*trans*	8.3	3.7
	cis	9.0	3.4
10 *S RS SR*	*trans*	8.1	3.8
	cis	8.4	3.4
11 *S RS RS*	*trans*	8.5	3.5
	cis	7.9	2.9
12 *S RS RR*	*trans*	8.7	3.7
	cis	8.0	3.4
13 *S SR SS*	*trans*	8.8	3.5
	cis	9.1	2.9
33	*trans*	5.7	3.6
	cis	6.5	3.1
34	*trans*	8.5	3.7
	cis	8.5	3.1

[a] In strongly acidic solutions, the *cis* conformer is not present; only an estimate is possible as judged from the relative positions of the titration curves.

1–4 the pK_3 value is raised by replacement of the pro *R* hydrogen at C-2p by the methyl group, and lowered by replacement of the pro *S* hydrogen. The electrostatic interaction between the protonated NH_2^+ group and the proline carboxylate is greater with an *R* methyl group than with an *S* methyl group. The chirality at C-1b has no effect. The reverse trends are observed in the *cis* conformers. The electrostatic interaction is thus favoured by the *S* methyl group, and the chirality at C-1b now has some influence since the pK_3 value is higher for **1** than for **2**. The lowering of the pK_3 when the chirality at C-2p is *R* might result, in part, from a hydrogen-bonding effect.

The differences between the pK_2 values are less important for the proline carboxylic group. The general trend is a higher value for pK_2 in the *trans* conformers. The weaker acidity of the carboxylic proton could be explained by intramolecular hydrogen bonding to the proximate amide carbonyl. The same observation was previously reported for captopril.[6]

The chirality at C-1b has no effect in the *trans* conformers. In the *cis* conformers, when the configuration at C-2p is *S*, the acidity is slightly lower if the configuration of the chiral carbon C-1b is *S*. A weak hydrogen-bonding interaction might occur between the two carboxylic groups.

The same general observations can be made for **9–12** (*S RS xy*). The influence of the conformation about the amide bond on the pK_2 values is reduced for all the compounds with the *R* configuration at C-7a.

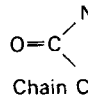
Conformational equilibria

^1H and ^{13}C NMR provide quantitative data on the variation of the *cis–trans* composition with pH. The evolution of the relative populations of the *trans* conformers (%) are shown in Fig. 1.

We first compare **1** with its precursor **33** in which the carboxylic group in the side-chain is esterified, the chiralities being unchanged. The two curves [Fig. 1(a)] display similar characteristics. The overall lower percentage of the *trans* form in the ester probably results from an increase in the steric interactions between the chain and the ring skeleton. For both compounds a true minimum is observed in the medium pH range between the pK_a of the proline carboxylic acid and that of the amine conjugate acid. The *cis* form is more stabilized than the *trans* form by through-space electrostatic interactions between the opposite charges of the protonated amine and the deprotonated proline carboxylate. In **1**, where the two carboxylate groups may be in competition for the interaction with the NH_2^+ group, the stabilization of the *cis* conformer is reduced and the minimum is less pronounced.

The decrease in the *trans* conformer level on deprotonation of the proline carboxylic acid, and its stabilization until the ammonium group is neutralized, depict a general behaviour [Fig. 1(a)–(d)].

Table 4. Through-space interactions between functional groups

Nature	Groups involved[a]		Influence on pK_a values[b]		Conformers concerned	Stabilized conformation
Electrostatic attraction[c]	NH_2^+	Proline COO^-	$pK_3\uparrow$	$pK_2\downarrow$	*cis* and *trans*	*cis* and *trans*
Electrostatic repulsion	Chain COO^-	Proline COO^-	$pK_1\uparrow$	$pK_2\uparrow$	*cis*	*trans*
Hydrogen bonding	Proline COOH		$pK_2\uparrow$		*trans*	*trans*
	Proline COOH	Chain COO^-	$pK_2\uparrow$		*cis*	*cis*
	NH	Proline COO^-	$pK_3\downarrow$		*cis*	*cis*

[a] Chain COOH and proline COOH are used to denote the chain and proline carboxylic groups.
[b] Ref. 38.
[c] The same type of interaction is most likely to occur between the NH_2^+ group and the chain carboxylate, but is expected to have a similar influence whatever the chiralities and the conformation.

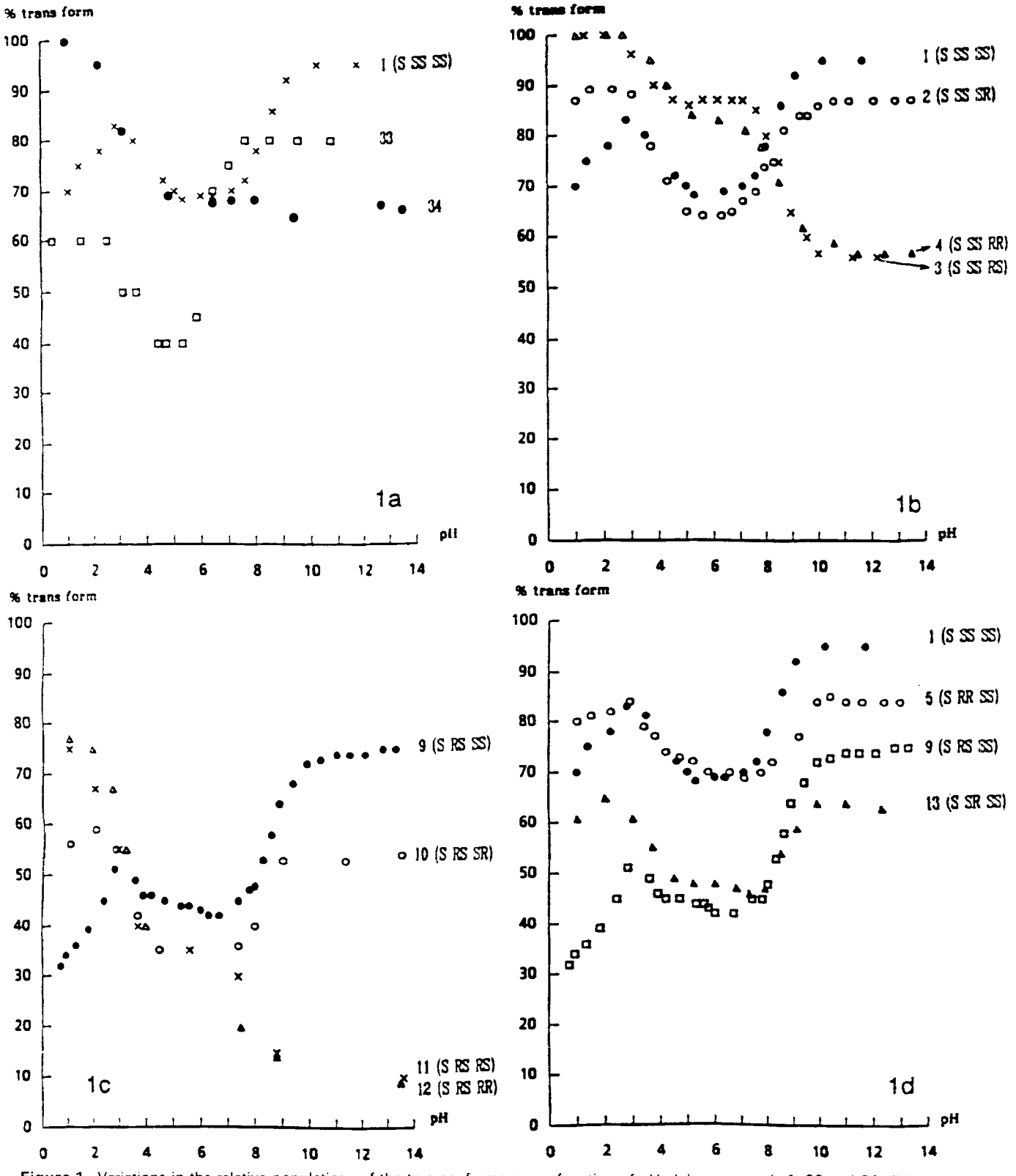


Figure 1. Variations in the relative populations of the two conformers as a function of pH: (a) compounds **1**, **33** and **34**; (b) compounds **1–4**; (c) compounds **9–12**; (d) compounds **1**, **5**, **9** and **13**.

The conformational equilibrium is far more dependent on the configurations of the side-chain carbons in basic and in strongly acidic solutions. Comparison of **1–4** (*S SS xy*) shows that the chirality at C-2p is the dominant factor [Fig. 1(b)], and when this is *R*, the *trans* form is the only species observed in acidic solutions. The population of the *trans* conformer decreases in alkaline solutions. The chirality at C-1b does not seem to play any role.

When the chirality at C-2p is *S* the population of the *trans* form is higher in both acidic and basic solution than in the medium pH range. Further, the conforma-

tional behaviour at the two ends of the pH range becomes dependent on the chirality at C-1b. If the configuration of C-1b is *R*, the population of the *trans* form reaches the same level, *ca.* 85%, in both acidic and basic solutions. Conversely, when the configuration at C-1b is *S*, the level of the *trans* population is higher in alkaline solution, *ca.* 95%, than in the preceding case. In solutions of increasing acidity the percentage of the *trans* conformer reaches a maximum at a slightly lower value than when C-1b is *R*, and then decreases further by *ca.* 15%.

Bearing in mind the conformational stabilizing effects of the various interactions, (see Table 4) the above results are well accounted for if the following assumptions are made: (i) in the medium pH range the stabilization of the *cis* form through electrostatic interaction is favoured by the *S* chirality at C-2p over the *R* chirality; (ii) in alkaline solution hydrogen bonding is the dominant interaction when C-2p is *R* but plays a minor role, if any, when C-2p is *S*; and (iii) in both alkaline and acidic solutions the combination of the *S* configurations at C-2p and C-1b favours interactions between the carboxylic groups.

The above hypotheses are well supported by examination of the desmethyl analogue **34**. As shown in Fig. 1(a), in the medium pH range **34** closely parallels the behaviour of **1**. In strongly acidic solutions it diverges from **1** to approximate the behaviour of **3**. In alkaline solution its behaviour is somewhere between those of these two compounds. The interactions between the two carboxylic groups are missing owing to the lack of the *S* methyl group, and hydrogen bonding between the amine proton and the proline carboxylate is reduced owing to the lack of the *R* methyl group.

The same general trends are found for **9–12** (*S RS xy*) in which the junction of the rings is *trans* [Fig. 1(c)], but the effects of the chiralities in the side-chain are larger. The overall decrease in the population of the *trans* conformer observed for the series **9–12** with respect to the series **1–4** could result from enhanced steric interactions between the chain and the ring skeleton. The same observation can be made for **13** [Fig. 1(d)].

CHEMICAL SHIFTS, COUPLING CONSTANTS AND THROUGH-SPACE INTERACTIONS

Further insight into the various through-space interactions, in particular steric interactions, can be gained from the analysis of small variations in the chemical shifts or coupling constants.

Steric interaction between the ring skeleton and the side-chain

The influence of the configuration at C-2p can be explained by first comparing **1** and **3** (*S SS xS*) to the desmethyl analogue **34**. For all the carbons of the skeleton, except C-2, C-7, C-7a, the chemical shifts in the stereoisomers do not differ by more than 0.6 ppm from those measured for **34**. Carbon C-7 is deshielded whatever the configuration at C-2p. Thus the occurrence of significant steric interactions between the methyl group and the cyclic skeleton can be ruled out in **1–4**. With respect to **34**, the effects of the configuration at C-2p are opposite for C-2 and C-7a: in the *cis* conformer C-2 is deshielded when C-2p is *R* and in the *trans* conformer C-7a is deshielded when C-2p is *S*.

Comparison of **1** and **2** with **5** and **6** shows that the inversion of the two carbons at the ring junction (*SS* → *RR*) results in significant shielding at C-7a and C-3a. Simultaneously, the carbon of the methyl group, C-3p, is also shielded in the *trans* conformer. Similarly, C-7 in the ring skeleton and C-3p in the chain are more shielded in **9** than in **13** (ring junction *RS* → *SR*). These observations reveal increased steric effects between the chain and the cyclohexane ring when the configuration at C-7a is changed from *S* to *R*, i.e. when C-7 moves from the *endo* to the *exo* position with respect to the proline carboxylate.

The conformational changes of the proline ring are best explained by observing the multiplet pattern of H-2. Well defined multiplets are observed in **1–4**, with high values of J(H-2,H-3) and J(H-2,H-3′) (10.4 and 8.3 Hz, respectively, for example in **1**). It is worth noting that in **3** or **4**, where the signals of H-2 in the two conformers about the amide bond are observable, the multiplet patterns are identical. The data are then consistent with the five-membered ring being of the N type conformation in all the series.

When the configurations at the ring junction were changed (*SS* → *RR*) as in **5** or **7** (*S RR SS* or *S RR RS*), H-2 appears as a broad doublet [J(H-2,H-3) ≈ 9 Hz, J(H-2,H-3′) < 1 Hz] in both conformers. These data are consistent with a proline conformation of the S type. Since the conformation of the proline ring does not seem to depend on the conformation of the amide bond in all the compounds with a *cis* ring junction, steric constraints between the chain and the proline ring are of minor importance.

In the **9–12** (*S RS xy*) stereoisomers, the signal of H-2 is an unusual broad doublet owing to the lack of significant coupling of H-2 to H-3′ in an *anti* position [^3J(H-2,H-3) ≈ 8.5 Hz], regardless of the conformation about the amide bond. Thus the proline ring remains of the S type with slight distortions with respect to the model compound **37**, which might result from steric interactions between the chain and the ring skeleton.

In **13** (*S SR SS*), H-2 has the same pattern, a triplet, as in the model compound **36** [J(H-2,H-3) = J(H-2,H-3′) = 8.0 Hz] when the conformation about the amide bond is *cis*. This result seems to indicate the lack of significant steric interaction between the chain and the cyclic skeleton. Conversely, when the conformation is *trans*, the H-2 signal is a doublet of doublets [J(H-2,H-3) = 10 Hz, J(H-2,H-3′) = 7.7 Hz]. In concert with the shielding effects previously outlined, this result suggests steric interactions between the ring skeleton and the chain as the basis of a limited conformational modification of the proline ring. Nevertheless, in both cases the conformation of the proline ring remains in the N region.

The conformation of the proline ring in the perhydroindole derivatives appears to be mainly determined by the configuration at C-3a. The *R* configuration results in an S-type conformation of the proline ring,

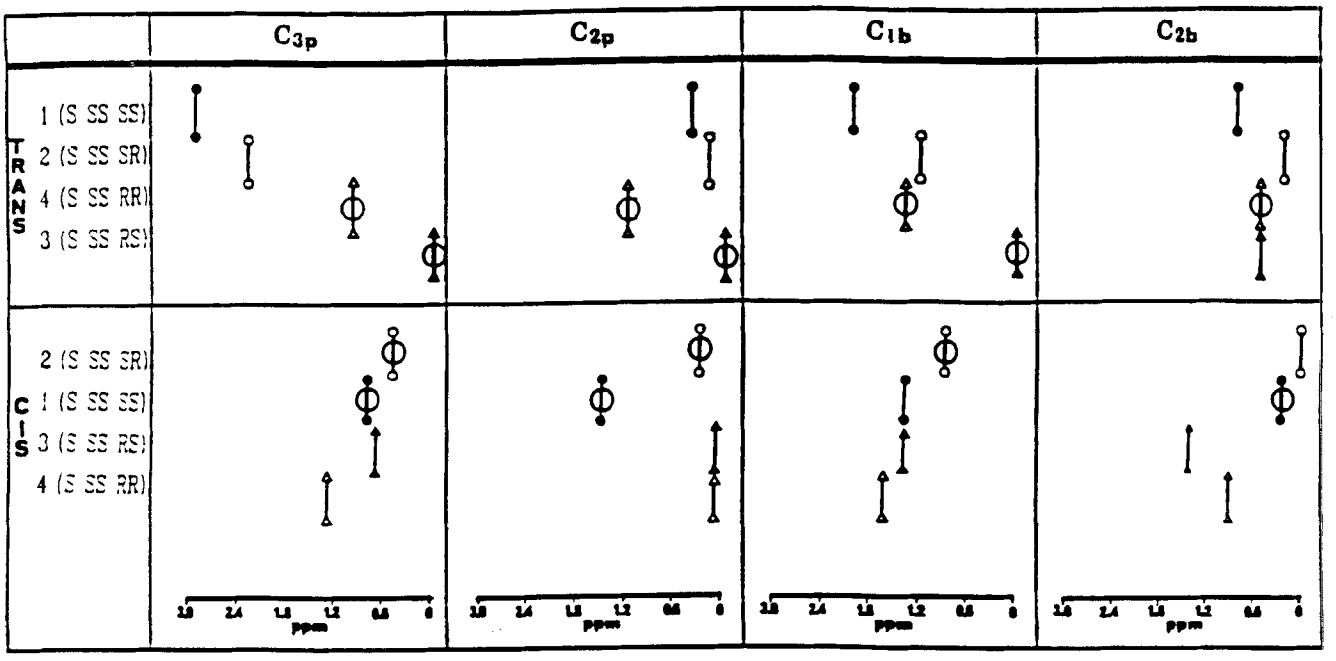


Figure 2. Relative positions ($\Delta\delta$, ppm) of the ^{13}C signals of selected side-chain carbons in the two conformers in the pH range 5–7. Circles denote carbons which undergo simultaneous shielding in the pH range 3–5.

while the *S* configuration results in an N-type conformation.

Intra-chain steric interactions

Many carbons in the side-chain appear to be affected by the protonation state of the proline carboxylic group. Thus, in the pH range where this carboxylic group becomes deprotonated while the amine group remains protonated, the carbon of the methyl group C-3p undergoes a significant shielding in the *trans* conformers if the configuration at C-2p is *R* and in the *cis* conformers if the configuration at C-2p is *S*. The other carbons in the chain, C-2p, C-1b or C-2b, might be similarly affected but to a lesser extent, the shielding effects being simultaneously observed for two carbons as shown in Fig. 2. In contrast, in the desmethyl analogue **34** the chemical shifts of the side-chain carbons are nearly the same for the two conformers. Hence steric interactions involving the methyl group C-3p and another carbon in the chain account well for the above observations.

Other through-space interactions

A systematic increase in the chemical shift of the carboxylic carbon, C-2′, on deprotonation of the amine group is well accounted for by the through-space interactions between the two groups. The magnitudes are different in the two conformers: 0.5 ppm (*trans*) and 0.9 ppm (*cis*). A systematic increase in the chemical shift of the amide carbonyl occurred in the *trans* conformer when the pH of the solution was lowered from about 5 to 2. A similar behaviour was previously reported in the case of dipeptides by Christl and Roberts,[35] and can be explained by hydrogen bonding involving the acidic proton of the *C*-terminal carboxylic acid.

DISCUSSION

The influence of the chirality of C-2p appears to be of paramount importance for the conformational behaviour of the different stereoisomers. The role of the methyl group at C-2p can be explained by using the schematic representations of the molecules drawn in Fig. 3 where, for the sake of simplicity, the end moiety of the side-chain is represented by the symbol Z.

When the configuration at C-2p is *S*, in the *cis* conformation [Fig. 3(a)] which is compatible with previous observations, e.g. dipolar interaction between H-2 and H-2p and strong through-space electrostatic interaction between the charged species, the methyl group is remote from the ring skeleton. The steric interactions which arise between this group and the butyl terminal moiety of the chain are reflected in the shielding of C-3p, C-1b and C-2b.

The *trans* conformation [Fig. 3(b)] in which the methyl group remains remote from the ring skeleton is not compatible with the experimental data. Further rotation of the side-chain about the C-1p–C-2p bond [Fig. 3(c)] brings H-2p into the neighbourhood of H-7a (allowing dipolar interaction) and reduces the distance between the ammonium group and the proline carboxylate (allowing electrostatic interactions).

The methyl group directed towards the cyclic skeleton is devoid of significant steric interactions with the nuclei in the end part of the chain. The enhanced conformational freedom of the terminal part of the chain explains the possibility of interactions between the two carboxylic groups which were detected in strongly acidic and basic solutions. Of course, these possibilities can be modulated by the configuration at C-1b.

The preferred solution conformation shown in Fig.

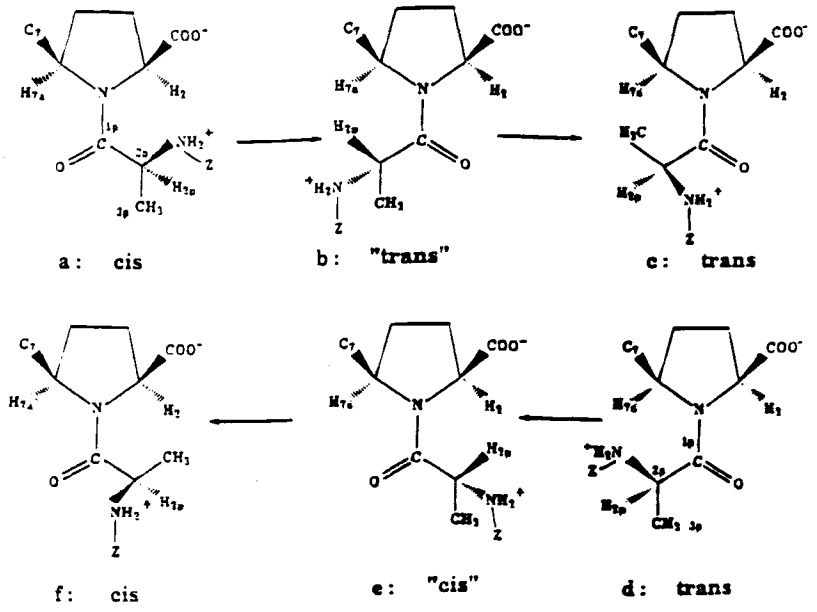


Figure 3. Schematic representations of the *cis* and *trans* conformations: (a–c) for compounds **1** and **2** and (d–f) for compounds **3** and **4**.

3(c) for the *trans* conformer is very similar to the solid-state conformation. For **1** it was shown that the conformation about the amide bond is *trans*, with the amide group nearly planar ($\omega = 178°$), and that the conformation about the C-1p—C-2p bond ($\psi = 153°$) brings the C-2p—C-3p bond nearly parallel to the C-7a—C-7 bond of the cyclohexane ring.[39] Nevertheless, it is stressed at this point that through-space interactions cannot be identical in the solid state and in solution. The zwitterion form which exists in the solid state cannot involve the less acidic proline carboxylic group. In solution, both carboxylic groups are ionized in the medium pH range, and interactions involving the proline carboxylate play a major role.

When the configuration at C-2p is *R*, in the *trans* conformation [Fig. 3(d)] compatible with the observations made previously, e.g. dipolar interactions between H-7a and H-2p and moderate through-space electrostatic interaction between the ammonium group and the proline carboxylate, the methyl group is remote from the cyclic skeleton. It is involved in steric interactions with the end part of the chain, as revealed by the shielding effects for the relevant carbons.

A rotation of 180° about the amide bond [Fig. 3(e)] followed by a rotation of the chain about the C-1p—C-2p bond leads to a representation of the *cis* conformer [Fig. 3(f)] which is fully compatible with the various observations. The methyl group directed towards the cyclic skeleton is devoid of steric interactions with the end part of the chain.

The chirality at C-2p, which determines the orientation of the alanine methyl group for each *cis* or *trans* conformation, has a major influence on the rotational freedom of the end part of the chain.

It is noteworthy that the proposed preferred conformations within the side-chain account well for the relative p*K*a values of the amine group, which increase when the methyl group moves away from the ring skeleton thus allowing stabilization of the amine protonated form by enhanced through-space electrostatic interactions.

The conformations described above are certainly the major forms under biological conditions due to the pK_2 and pK_3 values with respect to the physiological pH.

The effect of the different parameters on the inhibitor potencies of the various stereoisomers can now be analysed as follows. As shown in Fig. 1(d), the amount of *trans* conformer varies, for the potent inhibitors, over a significant range under biological conditions. Whatever the amount of *trans* conformer, the isomers with an *R* methyl group are inactive. Hence the presence of the *R* methyl group is definitely unfavourable owing to the restrictions imposed on the orientation of the terminal part of the chain, rather than to the limitation of the amount of *trans* conformer.

For all potent inhibitors in the *trans* conformation, the *S* methyl group is directed away from the end part of the chain, and there are no restrictions on the position of the carboxylate group of the chain except those depending on the chirality at C-1b. The *S* configuration of C-1b is more favourable than the *R* configuration by an order of magnitude.

Whereas the position of the *R* methyl group, remote from the ring skeleton, would favour a significant interaction with a hydrophobic site of the enzyme, the position of the *S* methyl group directed towards the cyclic skeleton would not allow such an interaction.

Since the *in vitro* activity of the desmethyl analogue **34** falls within the range of the activities of the four more potent inhibitors **1**, **5**, **9** and **13**, it emerges that the *S* methyl group does not play a significant role in the biological activity when the *C*-terminal imino acid is incorporated into a bulky hydrophobic fused-ring system. This proposal is fully supported by comparing the IC_{50} values previously reported for the captoril analogue, in which the proline ring is replaced with a perhydroindole skeleton, and for the corresponding desmethyl compound, 5.2 and 6.4 μM,[16] respectively.

The role of the fused-ring system in the restriction of the overall conformational freedom of the side-chain might decrease as the length of the chain increases. Thus introduction of the fused-ring system in place of proline increases the activity by a factor of 130 in the desmethyl analogue of captoril.[16] Enalaprilat and its perhydroindole derivative analogue have similar activities.[40] However, in the stereoisomer **13** of perindopril, a *trans* junction of the rings was shown to induce increased restrictions on the mobility of the side-chain.

Compounds **1**, **5**, **9** and **13** (*S xy SS*) have very similar inhibitor potencies. Since inversion of the configurations at the chiral centres C-3a and C-7a were shown to induce significant changes in the overall shape of the ring skeleton and, in particular, in that of the proline ring which changes from the S (C-3a, *R*) to the N-type conformation (C-3a, *S*), the shape tolerance of the hydrophobic site of ACE which accommodates the lipophilic group of the *C*-terminal end of the inhibitors is again well demonstrated.[16,40]

Finally, further insight might be gained regarding the role of the lipophilic interactions in the binding of the inhibitors to the ACE active site. In the captopril series a bulky fused-ring imino acid enhances the biological activity by a factor of 2–3 over the proline ring.[16] In enalaprilat-type inhibitors it has no significant effect when the chiralities at the side-chain carbons are optimal (*S*, *S*),[40] but enhances the activity by a factor of 75 when the carbon bearing the carboxylate Zn ligand has the less favourable *R* chirality.[7,16] In the studied series of perindopril type inhibitors it was observed that the biological activity decreases by a factor of 2–3 if the length of the terminal alkyl group is either reduced or enlarged. Hence the lipophilic interactions involving the different parts of the hydrocarbon skeleton of the inhibitor are not independent of each other. In the design of ACE inhibitors which already fulfil the requirements for the functional groups and their spatial distribution, it seems to be necessary to optimize the balance between the different lipophilic interactions.

In the perindopril series, the perhydroindole ring system, whatever the ring junction, and the butyl terminal group might be considered to realize the best compromise in the presence of either an alanine or a glycine residue at the penultimate position.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM 400 and Bruker WP 200 spectrometers at 300 K with sample concentrations of *ca.* 0.1 M in D_2O. Chemical shifts are reported in parts per million (δ) relative to sodium trimethylsilyl-3-propanesulphonate (DSS) as internal reference.

For two-dimensional NMR procedures, the standard pulse sequences were used for the $^{1}H/^{1}H$ COSY,[24] $^{1}H/^{13}C$ COSY,[25] long-range $^{1}H/^{13}C$ COSY[25] and $^{13}C/^{13}C$ INADEQUATE[23] experiments.

The pH values of the solutions were adjusted by adding concentrated DCl or NaOD. Since only the relative values are of interest, the pH reported for the titration curves is the pH meter reading obtained with the usual pH cell with a glass electrode standardized against aqueous buffers. The pD values are given by the relationship pD = pH + 0.4.[41] Owing to the deuterium isotope effects which have been evaluated[42] for dissociation constants of organic acids containing two or more polar groups the pK values measured by use of the titration curves appear to be overestimated by *ca.* 0.1 (pK_2) and 0.2 (pK_3) log unit with respect to their values in natural water.

^{13}C NMR (D_2O) data for the reference compounds are as follows: 2*S*, 3a*S*, 7a*S* octahydroindolecarboxylic acid (**35**), 62.1 (C-2), 34.9 (C-3), 39.4 (C-3a), 27.6 (C-4), 23.4 (C-5), 23.9 (C-6), 26.9 (C-7), 61.7 (C-7a), 177.6 (COOH); 2*S*, 3a*S*, 7a*R*-octahydroindolecarboxylic acid (**36**), 61.6 (C-2), 36.4 (C-3), 45.6 (C-3a), 30.9 (C-4), 26.3 (C-5 or C-6), 26.0 (C-6 or C-5), 29.8 (C-7), 65.8 (C-7a), 176.6 (COOH); 2*S*, 3a*R*, 7a*S*-octahydroindolecarboxylic acid (**37**), 61.9 (C-2), 36.6 (C-3), 44.1 (C-3a), 31.0 (C-4), 26.4 (C-5 or C-6), 26.7 (C-6 or C-5), 30.7 (C-7), 66.6 (C-7a), 177.2 (COOH); and 2*S*, 3a*R*, 7a*R*-octahydroindolecarboxylic acid (**38**), 61.5 (C-2), 35.5 (C-3), 39.9 (C-3a), 27.2 (C-4), 23.1 (C-5), 23.5 (C-6), 26.6 (C-7), 61.7 (C-7a), 177.3 (COOH).

Supplementary material available

Tables of the ^{13}C NMR chemical shifts at various pH values for 12 of the compounds studied are available from the authors on request.

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