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Conformational study of fosinopril sodium in solution using NMR and molecular modeling

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Two conformers of fosinopril sodium in methanol were unambiguously established using 2D NMR methods and variable-temperature NMR experiments. Differences in their conformational structure were shown to be related to the rotational energy barrier about the amide bond and hydrophobic interaction. The relationship between the 3D structure and activity is discussed. It is suggested that the *trans*-conformer may be more biologically active owing to its stacking structure and strong hydrophobic interaction and the *cis*-conformer could be more easily hydrolyzed because of its extended structure. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: NMR; fosinopril sodium; molecular modeling; conformational study

INTRODUCTION

Fosinopril sodium is the only angiotensin-converting enzyme (ACE) inhibitor that depends on a phosphinyl group to inhibit ACE by a zinc ligand relationship.[1,2] It is a pro-drug which is completely de-esterified either during or shortly after absorption to form an active diacid, fosinoprilat.[1] Recently, two conformers of fosinopril sodium, detected in methanol, were found in the course of our research. As an orally active antihypertensive, the solution conformation exerts an influence on its physicochemical properties and consequently affect the absorption in the body.[1,3] Hence a study of the conformational dynamics of fosinopril sodium would not only be helpful for its preservation and formulation, but also be useful for studying interactions between proteins and the drug molecule and assist in drug design.

Many studies on fosinopril sodium have been carried out.[1] A study of polymorphs of this compound in the solid state demonstrated that the two conformers are formed and arise due to *cis–trans* isomerization around the amide bond.[4] Concentration-dependent behavior was observed in aqueous solution.[3] However, the conformers of fosinopril sodium in solution have not been reported. In the present work, the complete ^{1}H and ^{13}C NMR signals were assigned and the 3D structural differences between the two conformers are discussed. The results suggest that the hydrophobic interaction in the *trans*-conformer plays a key role in the stabilization of the conformations.

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EXPERIMENTAL

NMR experiments

The *S*, *R*, *S*, *S*-isomer of fosinopril sodium (Fig. 1) was prepared.[5] About 20 mg of fosinopril sodium were dissolved in 0.5 ml of CD_3OD. The solvent signals were used as references for the chemical shifts (3.14 and 49.5 ppm for the ^{1}H and ^{13}C signals, respectively). All NMR experiments were run on a Varian Inova-600 spectrometer except for the variable-temperature experiments, which were measured on a Varian Mercury-400 spectrometer. The ambient temperature was 295 K. The temperature range of the variable-temperature experiments obtained for ^{31}P and ^{1}H were from 20 to 50 °C and from 20 to 60 °C, respectively.

1D ^{1}H and ^{13}C NMR (BB and DEPT-135) measurements were obtained using standard methods. For all the 2D experiments, spectral widths of 6000 and 30 000 Hz were used for the ^{1}H and ^{13}C dimensions, respectively. The data matrix of 2048 × 512 used in the COSY experiment[6] was zero-filled to 4096 × 4096 data points and multiplied by sine-bell functions in both dimensions. The HMQC experiment was optimized for a proton–carbon coupling constant of 140 Hz. The data matrix of the HMQC was 2048 × 200 and zero-filled to 2048 × 4096 and multiplied by Gaussian functions in both dimensions. For the HMBC experiment,[7] which used a long-range coupling constant of 8.0 Hz, a data matrix of 2048 × 256 points was collected and zero-filled to 2048 × 2048 with sine-bell multiplication. ROESY experiments[8] were carried out with mixing times of 300, 400 and 500 ms, in which a data matrix of 2048 × 512 points was used and zero-filled to 4096 × 4096 with Gaussian multiplication.

Molecular dynamics calculations

According to the isolated spin-pair approximation,[9] the inter-proton distances for the pair H_k–H_l can be derived from

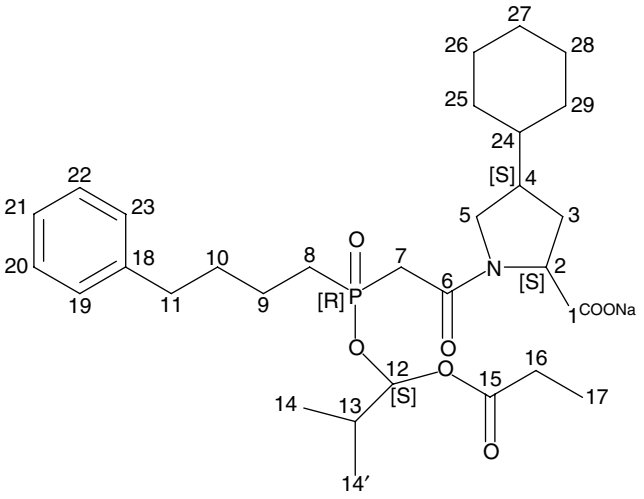

Figure 1. Structural formula of fosinopril sodium (*S*, *R*, *S*, *S*). The chiral centers are labeled in parentheses.

the equation $r_{kl} = r_{ij}(\sigma_{ij}/\sigma_{kl})^{1/6}$, where σ_{ij} and σ_{kl} are the cross-relaxation rates for unknown and calibration distances, respectively.[10,11] In this study, the distance restraints were obtained from the ROESY spectrum with a mixing time of 400 ms. Applying the 1.78 Å distance between the geminal protons at position 5 as a reference, the intensities of the NOE cross peaks were classified as strong, medium and weak, based on the distances <3.0 Å for strong, <4.0 Å for medium and <5.0 Å for weak.[12,13]

All the calculations, including the restrained molecular dynamics and energy minimization, were performed with HyperChem software (version 7.0 for evaluation) on a Pentium III 733M computer using the MM+ force field.[14] The starting structures were built manually using the Model Builder program in HyperChem. The system was first minimized with the Polak–Ribiere (conjugate gradient) method to remove any high-energy contacts.

MD simulations[10,14] were carried out for 100 ps with a step size of 0.5 fs and the trajectory structures were saved every 0.5 ps during the last 50 ps for conformational analysis. Two temperatures, 1000 and 300 K, were employed without temperature baths. The free molecular dynamic simulation at 1000 K was carried out to obtain the optimal structures for restrained molecular dynamic simulation at 300 K. No cutoff distance was used to include all possible interactions and the electrostatic interaction was gained with bond dipoles. The distance restraints were applied with a force constant of 7 kcal mol^{-1} Å^{-2}. Finally, five low-energy conformations minimized with the Polak–Ribiere (conjugate gradient) method were selected as the resulting structures for each group.

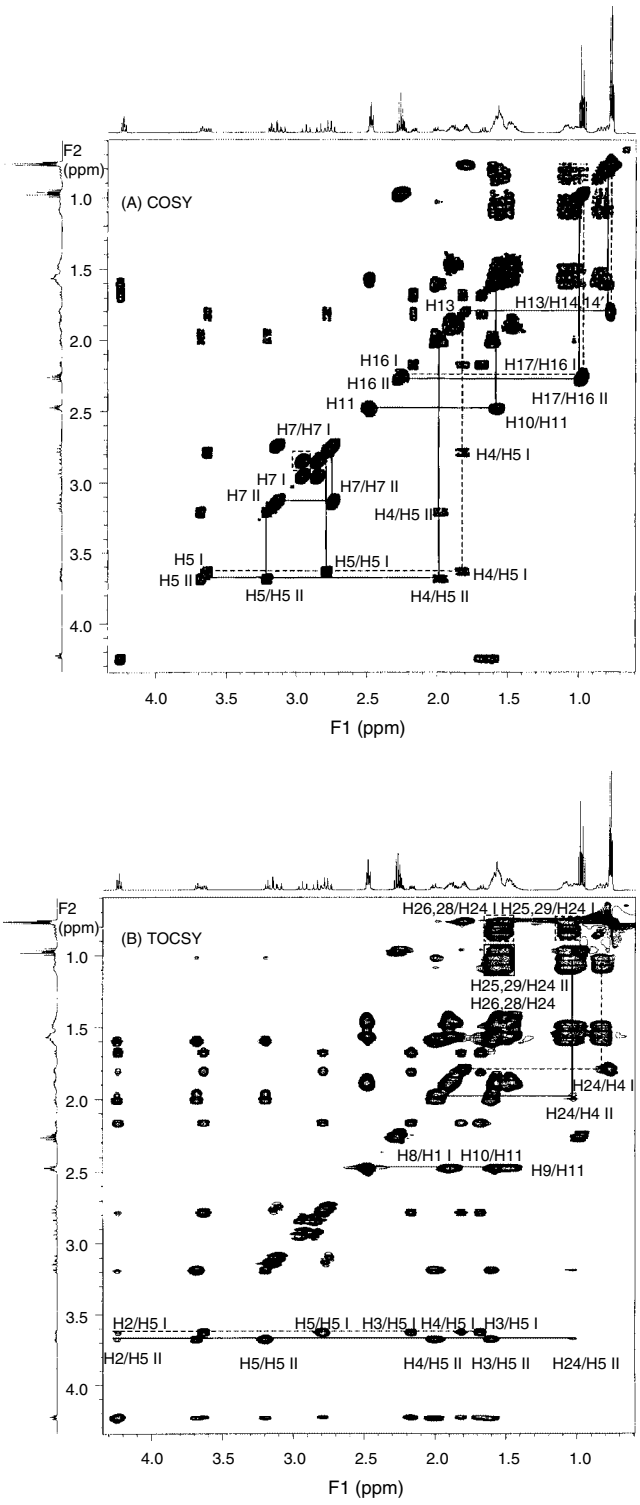

Figure 2. (A) Aliphatic proton region of the COSY spectrum; (B) the same region of the TOCSY spectrum. Sequential walks for conformer I (broken lines) and II (solid lines) are delineated.

RESULTS

NMR assignments of the two conformers

The ^{1}H spin systems were identified and assigned from the COSY and TOCSY spectra (Fig. 2). After all the protons of the conformers had been completely assigned, the chemical shifts of the corresponding carbons were directly assigned from the HMQC spectrum. The quaternary carbon atoms were identified by HMBC. The assignments of ^{1}H and ^{13}C NMR resonances for the two conformers are listed in Table 1.

Variable-temperature ^{31}P and ^{1}H experiments

The ^{31}P NMR signal of fosinopril sodium in CD_3OD was a 'doublet' peak centered at δ 58 at room temperature. As the temperature was elevated, the two phosphorus resonances

Table 1. Assignments of the two conformers of fosinopril sodium in methanol solution

	Conformer I				Conformer II			
Position	δ_H (ppm)	$J_{HH(P)}$ (Hz)	δ_C (ppm)[b]	J_{CP} (Hz)	δ_H (ppm)	$J_{HH(P)}$ (Hz)	δ_C (ppm)[b]	J_{CP} (Hz)
1			179.87				179.01	
2	4.22(d)	8.79	65.37		4.23(d)	8.79	63.92	
3	1.67, 2.17(m)		37.65		1.60, 2.01(m)		36.34	
4	1.79(m)		43.69		1.96(m)		45.32	
5	2.78(dd), 3.63(dd)	11.72, 8.30, 11.72, 8.30	52.71		3.18(dd), 3.67(dd)	9.77, 9.77, 8.79, 9.77	54.57	
6			167.11	3.54			166.33	2.47
7	2.83(dd), 2.94(dd)	16.51[a], 16.51, 16.51[a], 16.51	37.23	86.88	2.76(dd), 3.11(dd)	14.68[a], 14.68, 20.45[a], 14.68	38.13	81.75
8	1.88(m)		30.25	96.49	1.88(m)		30.25	96.49
9	1.48(m)		22.38	3.75	1.48(m)		22.38	3.75
10	1.57(m)		34.05	16.43	1.57(m)		33.90	15.94
11	2.48(t)	7.81	36.77		2.48(t)	7.81	36.73	
12	6.10(dd)	7.81, 4.39	95.93	7.47	6.13(dd)	8.79, 4.39	95.37	7.47
13	1.79(m)		34.90	4.96	1.79(m)		34.96	4.96
14, 14′	0.76(dd)	6.84, 2.11	16.89, 17.17		0.77(d)	6.84	16.91, 17.22	
15			175.01				175.21	
16	2.25(q)	7.81	28.77		2.27(q)	7.81	28.76	
17	0.96(t)	7.81	9.68		0.98(t)	7.81	9.61	
18			143.70				143.65	
19, 23	7.01(d)	7.80	129.98		7.01(d)	7.80	129.98	
20, 22	7.08(dd)	7.80, 7.80	129.86		7.08(dd)	7.80, 7.80	129.86	
21	6.97(dd)	7.80, 7.80	127.32		6.97(dd)	7.80, 7.80	127.32	
24	0.88(m)		43.70		1.02(m)		43.82	
25, 29	1.05(m), 1.08(m)		27.68, 27.95		1.50(m), 1.54(m)		27.98, 27.64	
26, 28	1.53(m)		33.00		1.59(m)		33.56	
27	0.83(m)		33.53		0.79(m)		33.02	

[a] *J* coupling constant for proton and phosphate.
[b] The chemical shifts of peaks split by phosphate were determined as the centers of the doublets.

coalesced into a single peak at δ 58 at 50 °C, and when the temperature was lowered back to room temperature, the phosphorus 'doublet' appeared again. The results are shown in Fig. 3. These results strongly suggest the existence of two conformers in methanol solution, in contrast to that of configurational isomers. The variable-temperature ^{1}H NMR experiments revealed that proton peaks corresponding to individual groups in the two conformers were became slightly closer and broader as the temperature increased. However, no significant exchange broadening was observed at 60 °C (spectra not shown).

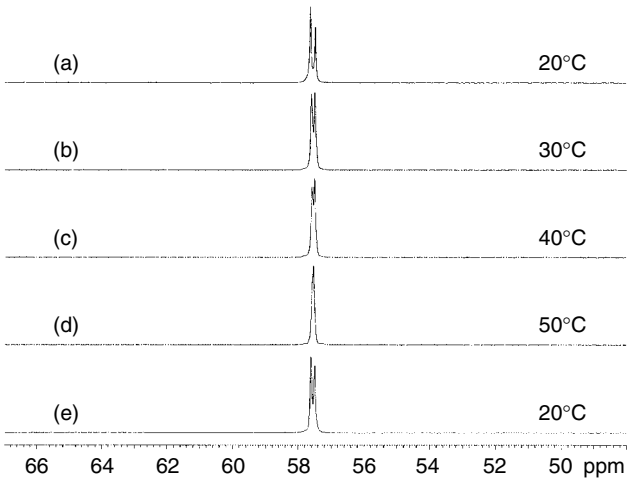

Figure 3. Variable-temperature ^{31}P NMR spectra of fosinopril sodium. In (a)–(d) the temperature was elevated from 20 to 50 °C and in (e) the temperature was decreased to 20 °C again.

J coupling and NOE

Proton–proton coupling constants were directly measured from the ^{1}H NMR spectrum (Table 1). The proximity of the *J*-coupling constants obtained for the two conformers showed that the torsion angles[15–17] of H2—C2—C3—H3, H4—C4—C5—H5, O6—C6—C7—H7 and P—O—C12—H12 are very similar, and therefore the apparent structural differences between them must exist in another fragment of the molecule.

From the results of ROESY experiments (Fig. 4), the inter-proton distance restraints could be obtained and are listed in Table 2. The data revealed that the two conformers adopt disparate 3D structures. For conformer I, H2 was adjacent to H7, whereas for conformer II H5 was close to H7. They are designated as *cis* (conformer I) and *trans* (conformer II), respectively.[2] This demonstrates that conformational

Table 2. Important NOE contacts and corresponding distance constraints for the two conformers

	Conformer I		Conformer II	
Proton pair	NOE intensity	*r* (Å)	NOE intensity	*r* (Å)
H2–H7 (δ 2.94)[a]	Strong	<3.0		
H2–H7 (δ 2.83)[a]	Medium	<4.0		
H3 (δ 1.67)[a]–H17[b]	Medium	<4.0		
H5 (δ 3.67)[a]–H7 (δ 3.11)[a]			Strong	<3.0
H5 (δ 2.78)[a]–H17[b]	Medium	<4.0		
H5 (δ 3.18)[a]–H24			Strong	<3.0
H9–H14, 14′[c]			Weak	<5
H10–H19, 23[b]	Weak	<5	Weak	<5
H8–H10[b]	Weak	<5	Weak	<5
H9–H11[b]	Weak	<5	Weak	<5
H8–H11[b]	Weak	<5	Weak	<5

[a] The proton with the chemical shift specified in parentheses which was determined by the structure of conformer.

[b] The carbon in the group was used for restraint and a correction of 1.1 Å was added.

[c] The carbon in the CH_3 group nearer to C9 and C9 was used for restraint and a correction of 2.0 Å was added.

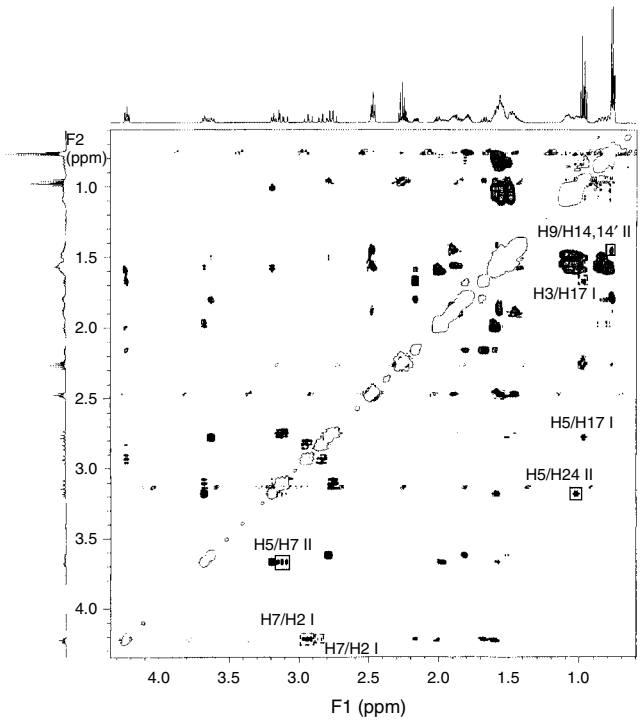

Figure 4. Expansion of the unsymmetrized NOESY spectrum of fosinopril sodium in CD_3OD at 295 K. Mixing time, 400 ms.

differences mainly arise from the isomerization about the amide bond.[2]

Molecular dynamics simulations

To cross energy barriers and obtain the low-energy conformations, free molecular dynamic simulation was performed at 1000 K. The resulting 10 minimum energy conformations could be identified as two groups, and the conformations in each group were fairly similar although conformations in different groups appeared distinct. The highest energy barrier resides in the amide bond, which cannot rotate freely at room temperature, and so two conformers, *cis* and *trans*, are possible. The *trans*-conformer exhibited lower potential energy than the *cis*-conformer. After optimization of these conformations, the lowest potential energy conformation for each conformer was sought and applied to restrained molecular dynamics simulations with NMR-derived distance restraints. The inter-proton distance constraints obtained from ROESY experiments are listed in Table 2. Because some hydrogens could not be identified from the NMR experiment, carbons had been used in methylene and methyl group identification to obtain the location of hydrogens in these groups.[9,12,13] Some corrections had been used in the distance restraints in such cases. We added 2.0 Å for the C9–C14 pair and 1.1 Å for CH_3 and CH_2 groups.[13] The minimum energy conformations within about 3 kcal mol^{-1} (1 kal = 4.184 kJ) relative to the global minimum conformation for conformer II and 1 kcal mol^{-1} for conformer I are summarized in Table 3. The differences between the C2—N—C6—C7 and P—C8—C9—C10 torsion angles were very small for the two conformers, but were consistent with the *J* couplings and NOE results. The average r.m.s.d.s for the mean structure of conformers I and II were 0.706 and 0.953, respectively. This means that conformer I is relatively rigid.

To obtain the relative populations of both conformers, the energies of the global minimum energy conformation for each conformer were calculated and averaged with the Boltzmann distribution. The two global minimum energy conformations are displayed in Fig. 5. The calculated relative content is 0.89, which is very close to the experimental result of 0.81.

DISCUSSION

From the depictions of the two conformers (Fig. 5), it was found that in the *trans*-conformer (II) the cyclohexyl, prolinyl and propionyloxy groups stacked together, as did the phenyl and isopropyl groups, thus exhibiting strong hydrophobic interactions between them. However, the structure of the *cis*-conformer (I) was extended and the

Table 3. Minimum energy conformations of fosinopril sodium calculated from molecular dynamics simulations

	E (kcal mol^{-1})		α_1[a]		α_2[b]		Relative content of the two conformers (I/II)	
Conformation	Group I	Group II	Group I	Group II	Group I	Group II	Experimental	Calculated
1	43.60	39.21	3.3	175.9	179.0	−58.1	0.81	0.89
2	43.74	40.15	5.2	176.4	−177.3	−62.7		
3	44.30	40.42	1.9	174.3	−177.8	−71.0		
4	44.33	40.47	4.3	175.9	179.7	−77.1		
5	44.42	42.00	4.6	177.6	−178.1	−67.0		

[a] The torsion angle α_1 is defined as C2—N—C6—C7.
[b] The torsion angle α_2 is defined as P—C8—C9—C10.

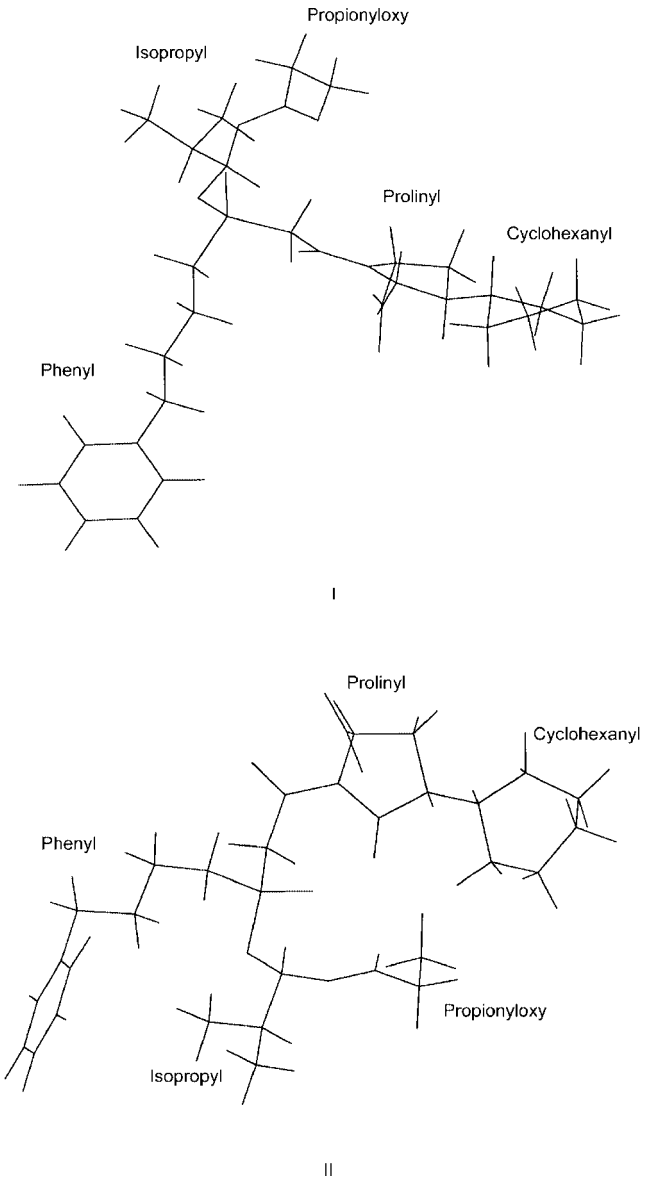

Figure 5. The two global minimum energy conformations of fosinopril sodium. I is designated as the *cis*-conformation and II as the *trans*-conformation about the amide bond.[2]

hydrophobic groups were located remote from one another. In the calculations, no hydrogen bonding was observed in the two conformers. In the *cis*-conformer, the ethyl group was positioned close to the cyclohexyl group, and because of the rotational barrier of the amide bond, precluded it from forming a hydrophobic stacked structure. Thus, the *trans*-conformer has lower potential energy than the *cis*-conformer. The important role of hydrophobic interactions for conformational stabilization has been similarly observed in many proteins and peptides.[18–24]

The phosphinyl group in the *trans*-conformer was encompassed by the phenyl side-chain group and isopropyl group, but the hydrophobic groups in the *cis*-conformer were positioned apart, so that the *cis*-conformer should undergo hydrolysis more easily. Owing to the proximity of the amide carbonyl and phosphorus—oxygen double bond, the *cis*-conformer could form a metal complex more easily, according to the mechanism for the metal ion-mediated degradation of fosinopril sodium postulated by Thakur *et al.*,[25] and thus could more rapidly degrade relative to the *trans*-conformer. This is in agreement with the above result that the *cis*-conformer is more susceptible to attack.

Although the coalescence of the two peaks in the ^{31}P NMR spectrum at 50 °C exhibited a fast exchange rate between conformers, the rate of exchange was not fast enough to make corresponding peaks in the two conformers in the ^{1}H spectrum coalesce even at 60 °C. These results demonstrated a relatively high-energy barrier to rotation around the amide bond that was also observed in enalapril maleate.[26] The ratio of the two rotamers was unlikely to change significantly during the hydrolytic process from fosinopril sodium to fosinoprilat because of the relatively high energy barrier to rotation. Based on the hypothesis that a potent inhibitor may not undergo major conformational changes on binding to an enzyme,[2] fosinoprilat was thought to be bound as its *trans*-rotamer since it is a potent ACE inhibitor.

CONCLUSION

Based on the variable-temperature NMR experiments, in particular the ^{31}P NMR experiments, it was concluded that two distinct conformers exist in a methanol solution of fosinopril sodium. Using various NMR methods (1-D and 2-D techniques), the ^{1}H and ^{13}C NMR signals of the two distinct conformers were unambiguously assigned.

The use of restrained molecular dynamics simulation permits the relative population of the two conformers to be calculated and it was shown to be very close to the experimentally determined result. The simulation demonstrated that the rotational energy barrier of the amide

bond meant that the conformations could not interchange freely at room temperature, and so two conformers, *cis* and *trans*, came into being. The *trans*-conformer is more stable, and may be more biologically active because of its hydrophobic stacking structure.

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