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*Research Article*

# Mechanism and Kinetics of Metal Ion-Mediated Degradation of Fosinopril Sodium

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Fosinopril sodium (I), a new angiotensin converting enzyme inhibitor, is a diester prodrug of the active moiety II. We report here a novel transformation of fosinopril into β-ketoamide, III, and a phosphonic acid, IV, mediated through metal ion participation. The interaction of fosinopril with magnesium ions was studied in a solution model system in which methanol was used as the solvent and magnesium acetate as the source of metal ions. Kinetic analysis indicated the degradation to be a bimolecular process, with the rate being first order in both metal ion and fosinopril concentration. The degradation products II, III, and IV effectively retarded the magnesium ion mediated reaction of fosinopril. Based on the results of $^{31}$P-NMR, $^{1}$H-NMR, Mn(II)-EPR spectroscopy experiments and mass spectrometry, a mechanism is postulated for this transformation. A key reactive intermediate has been characterized that supports the proposed mechanism. The results can account for the observed degradation profile of the fosinopril sodium in a prototype tablet formulation.

KEY WORDS: fosinopril sodium; magnesium ions; C–P bond cleavage; kinetics; mechanism; tablet formulation.

## INTRODUCTION

Fosinopril, [1[*S**(*R**)]2α,4β]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl]-acetyl]-L-proline, sodium salt (I) (Scheme I), is a new angiotensin converting enzyme inhibitor marketed under the trade name Monopril® (1). Fosinopril has four chiral centers and theoretically should exist in 16 isomeric forms. However, its synthesis is designed to give 99.9% S R S S isomer. It is a prodrug which is converted *in vivo* into the active moiety II by the hydrolysis of the diester side chain (1). In this communication we report a novel metal ion mediated rearrangement that results in degradation of fosinopril into a β-ketoamide, III, and a phosphonic acid, IV. The degradation product III was isolated from the tablets undergoing accelerated stability testing and was characterized by $^{1}$H NMR and MS. Its structure was confirmed by unambiguous synthesis. Compound IV is reported in the literature (2). We show that the degradation/rearrangement of fosinopril is caused by several metal ions, in particular magnesium. A mechanism invoking metal chelation is proposed for the degradation of fosinopril sodium by this process. The kinetics of the metal ion-mediated degradation were studied by reacting fosinopril sodium with magnesium acetate tetrahydrate in methanol. The kinetic study established that the metal ion-mediated degradation was a second-order reaction between fosinopril and metal ion. The study helped to explain the degradation of fosinopril sodium in a prototype tablet formulation containing magnesium stearate as the lubricant.

## MATERIALS AND METHODS

### Materials

Fosinopril sodium was synthesized at Bristol-Myers Squibb Co. The following metal acetates were obtained from Aldrich Chemical Co.: magnesium acetate tetrahydrate, zinc acetate dihydrate, cobalt(II) acetate tetrahydrate, nickel(II) acetate tetrahydrate, barium acetate, and calcium acetate hydrate. The following metal acetate salts were obtained from Fischer Chemical Co.: potassium acetate, sodium acetate trihydrate, copper(II) acetate, and lithium acetate. Magnesium stearate and iron(II) chloride X · $H_2O$ was obtained from Mallinkrodt, Inc. Iron(III) chloride hexahydrate was obtained from J. T. Baker Chemical Co. All the salts were used as received from the manufacturer. All solvents were of HPLC grade and reagents of analytical purity. The names fosinopril and fosinopril sodium are used synonymously and interchangeably.

### Degradation of Fosinopril Sodium by Metal Acetates in Methanol

Fosinopril sodium was dissolved in methanol at a concentration of 0.0017 *M* and reacted with each of the metal

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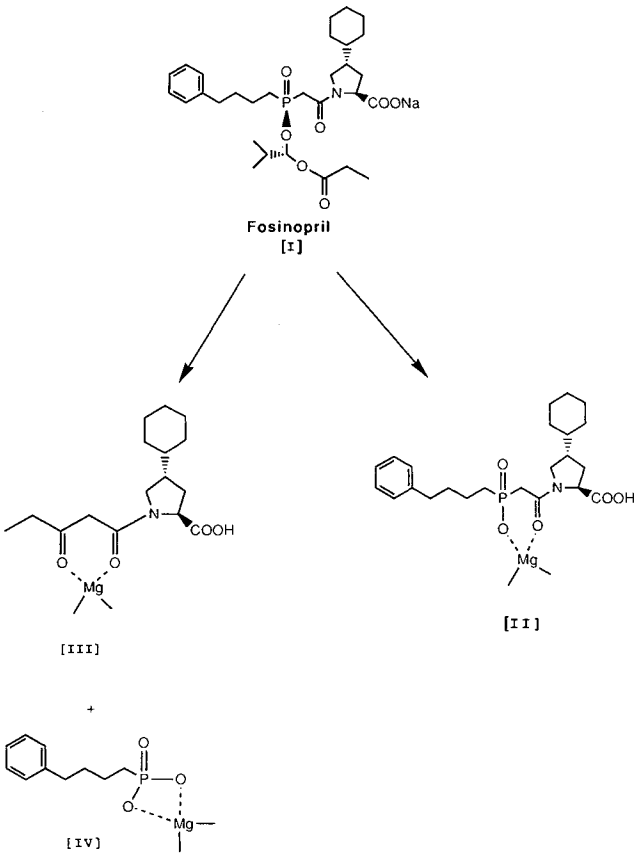



Scheme I. Pathways for degradation of fosinopril sodium. The degradation products II, III, and IV are shown in the form of magnesium ion chelates.

acetates at the same concentration. Acetate salts of calcium, barium, and manganic and magnesium stearate did not dissolve completely in methanol and were used as suspensions. The Fe(II) and Fe(III) salts were reacted as chlorides with potassium acetate added as a base. The reaction was allowed to proceed at 24°C (±1°C) for a specified time and then the contents of the flasks were withdrawn and analyzed by HPLC. Blank controls were solutions of fosinopril sodium in methanol at the same concentration. The data from these experiments were used to rank order the reactivity of each metal ion by calculating the pseudo-first-order rate constants for the degradation of fosinopril sodium.

### Kinetics of Degradation of Fosinopril Sodium in the Presence of Magnesium Acetate in Methanol

For kinetic experiments equimolar stock solutions of fosinopril sodium (MW 585.6) and magnesium acetate tetrahydrate (MW 214.5) were prepared by dissolving separately 100 and 36.5 mg, respectively, in 100 mL of methanol. The two reactant solutions were mixed in a predetermined ratio in Teflon stoppered flasks and appropriately diluted with methanol. A series of flasks were then placed in a constant-temperature bath at 24°C (±1°C) and samples were periodically withdrawn and analyzed by HPLC.

Positive control experiments were performed by reacting fosinopril sodium with potassium acetate (anhydrous) or sodium acetate trihydrate dissolved in methanol in a similar manner. Solutions of fosinopril sodium in methanol without any other additive served as blank controls. The data from these experiments were fitted to a second-order kinetic model (Table II).

### Effect of Additives on Magnesium Ion-Mediated Degradation of Fosinopril

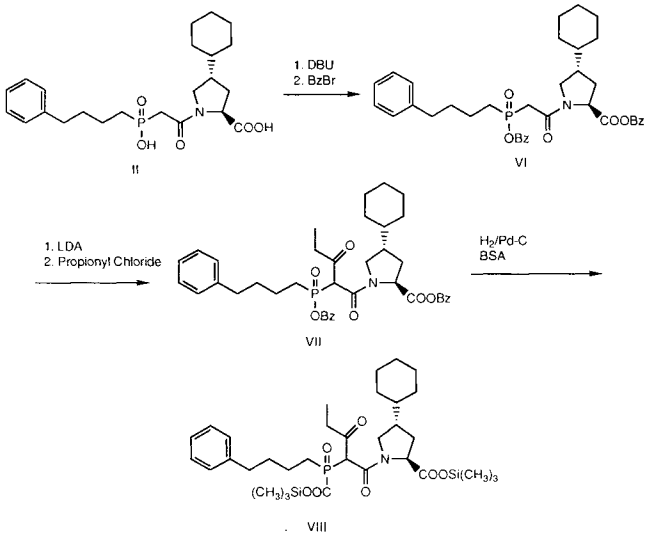
The effect of the II, III, and IV on the magnesium ion-mediated degradation of fosinopril was studied in methanol. Each additive was separately added to a methanolic solution of fosinopril sodium, followed by magnesium acetate in methanol. The molar ratio of the reactants was 1:1:1. The solutions were then placed in a constant-temperature bath at 24°C (±1°C). At periodic intervals, samples were withdrawn and analyzed by HPLC for fosinopril.

### Synthesis of the Bis-Silylated Derivative of Intermediate (V)

The dibenzyl ester VI was prepared from the diacid II using DBU (1,8 diazabicyclo[5.4.0]undec-7-ene) and benzyl bromide (Scheme II). Compound VI was deprotonated with LDA (lithium diisopropylamide) and acylated with propionyl chloride to give the phosphinyldicarbonyl compound VII. Attempted hydrogenolysis of VII afforded an intractable mixture of compounds, however, in the presence of BSA [*N,O*-bis(trimethylsilyl)acetamide], provided the bis-silylated derivative VIII of the key intermediate V. The compound VIII was characterized by mass spectroscopy: ions at $m/z$ = 564 [M + 1 − one $Si(CH_3)3]^+$, 492 [M + 1 − two $Si(CH_3)3]^+$, 562 [M − 1 − one $Si(CH_3)3]^-$, and 490 (M − 1 − two $Si(CH_3)3]^-$. $^{31}$P-NMR of VIII gave a signal at δ 35.9.

### Synthesis of III

β-Ketoamide, III, was obtained by condensation of 4-cyclohexylproline with 3-oxopentanoic acid in the presence of anhydrous 1-hydroxybenzotriazole (HOBT) and *N,N*-dicyclohexylcarbodiimide (DCC) in methylene chloride. The product was isolated as the dicyclohexylammo-




Scheme II. Synthesis of silylated derivative of the intermediate (V).

nium salt, purified by crystallization from methyl isobutyl ketone, and characterized by spectroscopy; m.p. 181°C, *Anal.* Calc. for $C_{28}H_{48}N_2O_4$: C, 70.55; H, 10.16; N, 5.88. Found: C, 70.28; H, 10.11; N, 5.78. MS $(M + H)^+ = 477$. IR (KBr): 2920 (s), 1630 (s), 1655 (m) $cm^{-1}$. $^{13}C$-NMR ($CDCl_3$): carbonyl at δ 205.3, 175.8, and 166.0.

### $^{31}P$-NMR Spectroscopy

$^{31}P$-NMR spectra were obtained at 360 MHz on a JOEL FX-90Q spectrometer using a 5-mm omni probe. Spectral acquisition parameters were 16K points, 6000 Hz (175 ppm), 2-sec repetition rate, and 30° pulse angle. A phosphoric acid external standard served as reference. Eighty pulse spectra were recorded.

### $^1H$-NMR Spectroscopy

Proton spectra were obtained at 400 MHz on a JOEL GX-400 NMR spectrometer with a 5-mm proton probe. Spectral acquisitions parameter were 32K time domain data points, 600-Hz spectral width, 4-msec pulse (90° pulse, 10.5 m · sec), 4.7-sec repetition rate, and 0.2-Hz line broadening factor. A typical spectrum required 40 to 120 pulses to achieve the desired signal-to-noise ratio. All spectra were run at 30°C and referenced to internal TMS standard.

### Mn(II) EPR Spectroscopy

EPR spectra were recorded on a Varian Model E-12 spectrometer operating nominally at 9.5 GHz and at 100-kHz field modulation frequency. Concentration standards of Mn(II) at 5 and 10 m*M* were made from reagent-grade Mn(II) acetate tetrahydrate in methanol. The EPR spectra were compared with spectra recorded at the same concentration after the addition of equimolar fosinopril sodium. Spectra were recorded at ca. 80 K using a gaseous nitrogen cryostat.

### Fast Atom Bombardment (FAB) Mass Spectroscopy

Mass spectra were obtained on a double-focusing magnetic sector mass spectrometer (Model ZAB-1F, VG Analytical) by fast atom bombardment using a 4- to 8-kV xenon source. The sample was dissolved in methanol and taken up in the FAB matrix consisting of a mixture of dithiothreitol, dithioerythreitol, DMSO, and glycerol.

### LC-MS Analysis

LC/MS analysis of the reaction mixture of fosinopril sodium and magnesium acetate at a 1:1 molar ratio in methanol at 24°C was carried out using a SCIEX API-III liquid chromatograph/mass spectrometer. A Waters LC/MS gradient controller system with simultaneous UV detection was employed for the chromatographic run. The eluant was split 20:1, with approximately 60 μL/min directed to the mass spectrometer. The ionization of the eluent was by nebulizer-assisted electrospray. Ions were produced with little internal energy, resulting in intact $(M + H)^+$ and $(M - H)^-$ ions.

### HPLC Analysis

The HPLC system consisted of pump (Model 400, Applied Biosystems, Foster City, CA), an automatic sample processor (Model WISP712, Waters Associates, Milford, MA), and a UV detector (Model 783, Applied Biosystems). Data acquisition and analysis were performed using an HP-1000 computer system (Model A900 with RTE-A and CALS PeakPro Chromatography System, Beckman Instruments, Inc., Allendale, NJ). The chromatographic separations were performed on a phenyl column (Type C-402, 4.6 mm × 30 cm, 10-μm packing, Column Resolution Inc., San Jose CA) using a mixture of methanol and aqueous 0.2% phosphoric acid (72:28). Mobile phase was pumped at a flow rate of 1.5 mL/min. The wavelength of detection was 220 nm. In the experiments using LC-MS analysis, the mobile phase was methanol and aqueous 0.2% trifluoroacetic acid (72:28) pumped at a flow rate of 1.2 mL/min.

### Thin-Layer Chromatography (TLC)

TLC of the reaction mixture of fosinopril sodium with magnesium acetate in methanol was carried out using a stationary phase plate of silica gel-G (Merck, G-60) and a mobile phase of chloroform:methanol:water (60:45:10). The temperature of the chamber was 5°C (±1°C). A reaction mixture containing an equimolar ratio of fosinopril sodium and magnesium acetate was initially monitored by HPLC analysis. After 30 min at room temperature the degradant appeared at an $R_f$ of 0.65 when visualized by iodine vapors. The reaction mixture was then streaked across plates and a front equivalent to 16 cm was developed. A zone corresponding to $R_f$ 0.6–0.7 was scrapped off the plates without visualization and extracted in methanol. The methanol extract was analyzed by fast atom bombardment mass sectroscopy. A similar procedure was followed for the isolation and characterization of degradation product from the tablets that were subjected to accelerated stability testing.

### Molecular Modeling

Computer-generated space filling models of the fosinopril metal ion complex were elicited by using ALEX software (written by Jack Z. Gougoutas of Bristol-Myers Squibb) on an Iris 40/25 Super Turbo (Silicon Graphics) work station.

## RESULTS

### Degradation of Fosinopril Sodium in Methanol by Metal Ions

Figure 1 shows chromatograms of an equimolar mixture of fosinopril sodium and magnesium acetate in methanol. It shows that after a reaction time of 2 hr at 24°C, degradation of fosinopril formed an intermediate degradation product (Fig. 1B). Further heating at 37°C (±1°C) for 12 hr resulted in the formation of II, III, and IV (Fig. 1D). Identity of II, III, and IV was established by comparison to authentic samples. Also shown in Fig. 1 (A and C) are the chromatograms of an equimolar reaction mixture of fosinopril and magnesium stearate after similar treatment in methanol. From the products formed, it was confirmed that both magnesium stearate and magnesium acetate reacted with fosinopril in a similar manner. Other metal ion acetates exhibited similar reactivity towards fosinopril. The relative reactivities of different metal ions are shown in Table I. The reactivities were com-

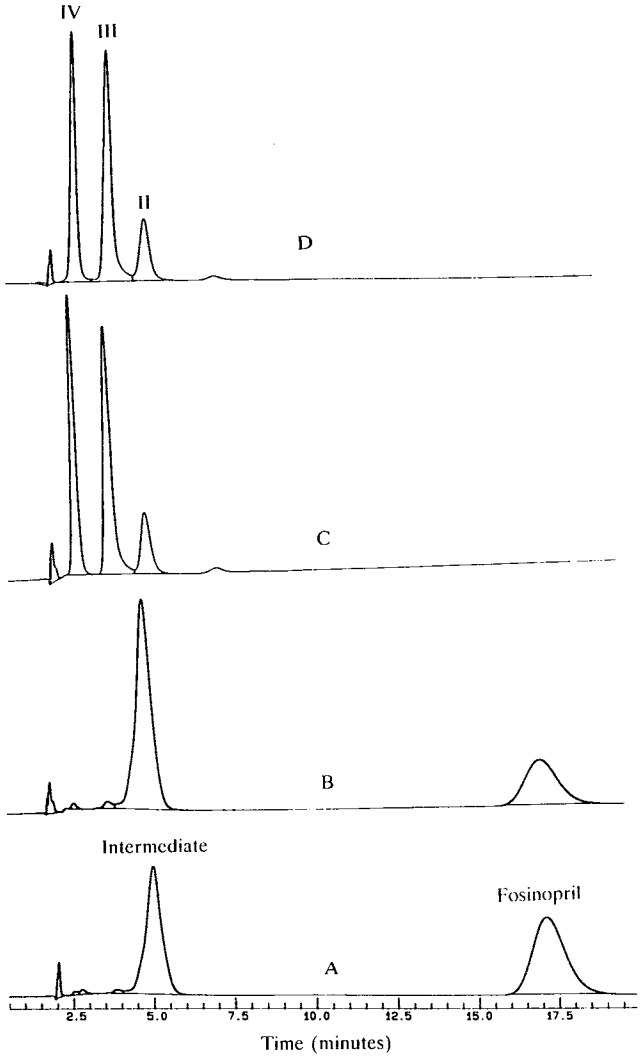



Fig. 1. Chromatograms of reaction mixtures containing 1:1 molar mixtures of fosinopril sodium in methanol, with magnesium stearate (A) at 24°C for 2 hr and (C) at 37°C for 12 hr and with magnesium acetate (B) at 24°C for 2 hr and (D) at 37°C for 12 hr.

pared by measuring the loss of fosinopril caused by each metal ion over a specified time period under identical conditions and then expressing the result as the pseudo-first-order rate constant for each metal ion. It was not clear whether the observed differences were due to inherent activating abilities of the metal ions or to their association constants with fosinopril. On a relative basis, Co(II) and Mn(II) were more reactive than Mg(II), whereas Zn(II), Ni(II), Mn(III), Cu(II), Ca(II), and Ba(II) were less reactive. Reactivity of Fe(III) and Fe(II) ions was determined by reacting their chloride salts since the acetates were not readily available. In these cases, potassium acetate was added as the base. Under the same conditions Fe(II) chloride did not cause degradation of fosinopril, whereas Fe(III) chloride was reactive. Sodium, potassium, and lithium acetates did not cause degradation of fosinopril.

### Influence of Magnesium Ions on the Rate of Degradation of Fosinopril Sodium

Figure 2 shows the effect of varying the magnesium acetate concentration on the degradation of fosinopril sodium from methanolic solution at 24°C. It was observed that a greater loss of fosinopril occurred as the concentration of magnesium acetate was increased and that, after a fixed amount of degradation, the reaction appeared to level off. Likewise, when the reaction was carried out in the presence of a fixed concentration of magnesium acetate and varying fosinopril concentrations, a fixed amount of fosinopril proportional to the metal ion concentration was lost. Thus when the molar ratio of fosinopril:magnesium ion was changed from 1:1 to 1:0.5, the rate of degradation did not change but the reaction stopped after half the drug was consumed. Control experiments with sodium acetate or potassium acetate showed a negligible loss of fosinopril. These results indicated that the degradation of fosinopril by magnesium ions was a bimolecular process. The second-order rate constants at 24°C in methanol at different molar ratios of fosinopril to magnesium ion are shown in Table II. The rate constant did not deviate significantly upon varying the molar ratio of the reactants.

Table I. Relative Reactivities of Metal Ions in the Degradation of Fosinopril in Methanol at 24°C

| Metal ion | % fosinopril remaining at 2.75 hr | Rate ($hr^{-1}$) |
|---|---|---|
| Co(II) | 13 | 0.74 |
| Mn(II) | 16 | 0.69 |
| Mg(II) | 19 | 0.60 |
| Zn(II) | 30 | 0.44 |
| Ni(II) | 31 | 0.43 |
| Mn(III) | 40 | 0.33 |
| Cu(II) | 45 | 0.29 |
| Fe(III) | 69 | 0.13 |
| Ca(II) | 69 | 0.13 |
| Ba(II) | 92 | 0.03 |

### Differentiation of Hydrolytic and Magnesium Ion Pathways of Degradation of Fosinopril Sodium in Methanol

In order to establish the mass balance between the amount of fosinopril lost and the amounts of degradation products formed, a 1:2 molar mixture of fosinopril sodium and magnesium acetate in methanol was reacted at 50°C (±1°C) for 24 hr and II, III, and IV formed in the mixture

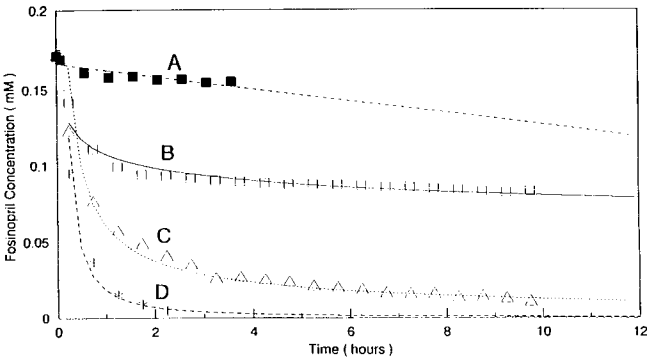



Fig. 2. Degradation of fosinopril sodium in methanol at 24°C, with a varying magnesium acetate-to-fosinopril mole ratio: (A) 0.1; (B) 0.5; (C) 1.0; (D) 1.5.

Table II. Second-Order Rate Constants for the Degradation of Fosinopril at Varying Ratios of Magnesium Acetate in Methanol at 24°C

| Initial molar ratio of Fos:$Mg^{2+}$ | Rate ($mmol^{-1}r^{-1}$) (SD)[a] |
|---|---|
| 1:0.1 | 7.1 (*1.7*) |
| 1.0.5 | 7.3 (*2.3*) |
| 1:1 | 9.7 (*1.1*) |
| 1:1.5 | 9.4 (*0.5*) |
| 1:2 | 7.6 (*1.1*) |

[a] Number of observations = 3.

were quantitatively determined (Table III). Control experiments were performed in which magnesium acetate tetrahydrate was replaced by either sodium acetate or potassium acetate to which known quantities of water were added. The distinct role of magnesium ion and water on the direction of the reaction pathway was established from the product profile. The formation of III and IV was attributed to magnesium ion pathway (88%), while II was formed by the hydrolysis (12%) of fosinopril caused by the water from the tetrahydrate salt form used.

### Effect of Degradation Products on Reaction Kinetics

The second-order rate constants for the degradation of fosinopril by magnesium acetate at 24°C in methanol in the presence of the degradation products II, III, and IV, added separately, are shown in Table IV. The rate constants were calculated by monitoring the loss of fosinopril as a function of time. The magnesium ion-mediated degradation of fosinopril was significantly retarded in the presence of II, III, or IV.

### Mass Spectral Analysis of the Reactive Intermediate

A reaction mixture containing mixture of fosinopril sodium and magnesium acetate at 24°C was subjected to tandem HPLC-MS analysis. The peak corresponding to "intermediate" (Fig. 1) gave ions at $m/z$ = 492 $[M + H]^+$ and 514 $[M + Na]^+$. High-resolution mass spectral analysis (FAB) on a TLC isolate of the intermediate gave a negative ion at $m/z$ = 512.2181 that was assigned to $[M - 2H + Na]^-$. The calculated value suggested an empirical formula of $[C_{26}H_{36}NO_6PNa]$ for the negative ion (theory = 512.2178). This corresponds to a formula of $C_{26}H_{38}NO_6P$ for the neutral molecule, consistent with the proposed structure of the intermediate V (Scheme III).

### $^1$H-NMR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Magnesium Acetate

The methylene protons on C-7 of fosinopril in $CD_3OD$ appear as a multiple at δ 2.85–3.25 (Fig. 3). After the addition of magnesium acetate to the solution, this signal was rapidly depleted. The double quartet at δ 6.25–6.35 due to the single side-chain methine proton on C-12 of fosinopril was also progressively diminished in the presence of magnesium acetate, as a result of the loss of side chain. On reaction with magnesium acetate in $CD_3OD$, fosinopril liberated isobutaraldehyde as its hemiacetal (doublet at δ = 4.16, $J$ = 8 Hz). A small signal for free aldehyde proton was also detected at δ 9.5. With time, more isobutaraldehyde formed as detected by NMR. Formation of isobutyraldehyde was confirmed by spiking. The spectral pattern became complex after further standing, indicative of the formation of several compounds in the solution.

### EPR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Manganese(II) Acetate in Methanol

The EPR spectrum of a reaction mixture containing equimolar Mn(II) acetate and fosinopril sodium in methanol is shown in Fig. 4B. It is shown that the peak height of the signal due to Mn(II) is three times greater than the intensity of the Mn(II) acetate alone in methanol (Fig. 4A) at the same concentration. The line shape is narrower, and "forbidden" hyperfine ($\Delta M_S$ = 1 and $\Delta M_I$ = 1) transitions exist for the initial complex which are not resolved in the broad spectrum of Mn(II) acetate control.

### $^{31}$P-NMR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Magnesium Acetate

The $^{31}$P resonance of fosinopril sodium in $CD_3OD$ occurs as a doublet centered at δ 58.55. Immediately after adding magnesium acetate to the solution, the phosphorous signal appeared as a singlet at δ 58.6 (Fig. 5). After the reaction mixture remained at room temperature, another signal appeared at δ 36.1, which was assigned to the "intermediate" as indicated by the HPLC analysis of the sample.

## DISCUSSION

In extended stability studies of the bulk drug substance, fosinopril sodium does not undergo the postulated rearrangement and degradation reactions. If exposed to high humidity, the ester prodrug undergoes hydrolysis to form the active moiety II. In the formulations containing magnesium stea-

Table III. Contributions of Hydrolysis and Magnesium Ion Pathways in the Degradation of Fosinopril in Methanol at 50°C

| Addition | Fosinopril lost (%)[a] | Degradation products formed (%) | | |
|---|---|---|---|---|
| | | II | III | IV |
| None | 1 | Trace | Trace | Trace |
| Sodium acetate trihydrate | 6 | 6 | Trace | Trace |
| Potassium acetate anhydrous | 1 | 1 | Trace | Trace |
| Sodium acetate + $4H_2O$ | 14 | 11 | 2 | Trace |
| Magnesium acetate tetrahydrate | 100 | 12 | 88 | 88 |

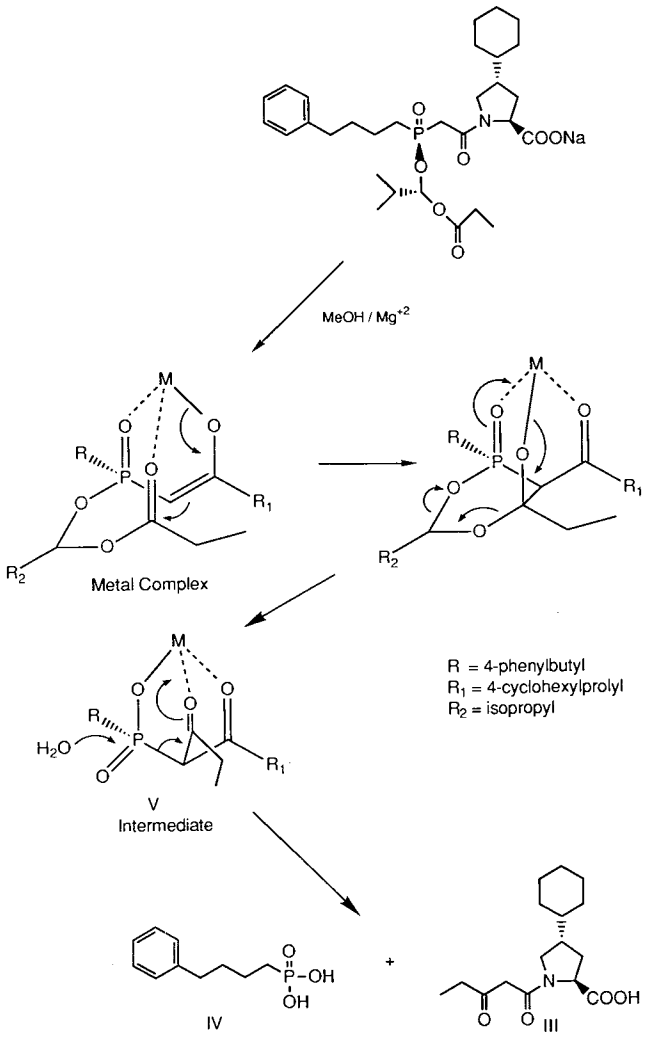
[a] Initial concentrations: fosinopril, 3.4 × $10^{-4}$ mmol/mL; metal acetates, 6.8 × $10^{-4}$ mmol/mL].

Table IV. Effect of II, III, and IV on Magnesium Ion-Mediated Degradation of Fosinopril in Methanol at 24°C[a]

| Addition | Rate ($mmol^{-1}$ $hr^{-1}$) |
|---|---|
| None | 9.7 |
| III | 2.2 |
| IV | 1.5 |
| II | 0.006 |

[a] Molar ratio:fosinopril: magnesium acetate:degradant, 1:1:1.

rate, fosinopril degrades to form not only II, but also small amounts of III and IV. We have studied the degradation of fosinopril in a model system wherein the fosinopril was reacted with a soluble salt form of a metal in methanol. The solid-state behavior of fosinopril in tablet formulation was simulated in solution by substituting freely soluble magnesium acetate for magnesium stearate. We have demonstrated that the reaction was not unique to magnesium ions but occurred with other metal ions as well. There are several advantages to studying the incompatibility between fosinopril and metal ions in a solution system. The reaction could be monitored more accurately and with a precision many fold greater than the solid state. The solution model offers the flexibility of varying the reactant ratios and temperature and allows for the introduction of extraneous additives with minimal changes in the overall setup. Most importantly the reaction could be selectively directed toward the magnesium ion pathway, while minimizing the hydrolytic degradation of the ester prodrug moiety. The kinetic and mechanistic details of the metal ion-mediated reaction of fosinopril are given below.




Scheme III. Proposed mechanism for the degradation of fosinopril sodium by metal ion.

## Directional Control of the Reaction in Methanol as Solvent

Scheme I shows the two pathways postulated for degradation of fosinopril sodium. The ester prodrug is shown to degrade by hydrolysis to form the active agent II and by the metal ion-mediated pathway to III and IV. Dependence of the reaction pathway on the presence of metal ions or water was demonstrated by studying the process in methanol, in which known amounts of water or metal salts were introduced and then heated at 50°C for 24 hr (Table III). Under the experimental conditions 100% of fosinopril degraded in the presence of 2 equiv of magnesium acetate tetrahydrate to form 12% of II, 88% of III, and 88% of IV, whereas in the presence of potassium acetate only 1% of the drug was degraded, forming II. These observations suggested that the formation of III and IV was caused by magnesium ions. When sodium acetate trihydrate was added, it caused a 6% loss of fosinopril, resulting in the formation of II as the major product. When the reaction with anhydrous sodium acetate was carried out in the presence of 4 equiv of water the loss of fosinopril was increased to 14%, with II again being the major product. Thus it was concluded that in the presence of magnesium acetate tetrahydrate, II was formed due to hydrolysis, whereas the formation of III and IV was metal ion mediated. The small amount of III and IV formed in these control experiments in the absence of added magnesium ions was attributed to the presence of trace metal impurities contributed by the system. Formation of equal amounts of III and IV is significant and consistent with the proposal of the reaction proceeding through an "intermediate" because III and IV can be viewed as the molecular fragments of the intermediate (Scheme III).

## Order of the Reaction and Self-Limiting Kinetics

Reactivity of magnesium ions towards fosinopril sodium was increased in methanol as a solvent. In control experiments with an equimolar mixture with magnesium acetate 100% of the initial fosinopril was lost within 2 hr at 50°C in methanol, whereas under the same experimental conditions in water less than 1% loss was observed. Thus, using methanol as the solvent allowed the reaction to progress through several half-lives in a short period of time, facilitating accurate assessment of the kinetics of the process. The kinetic data were fitted to a second-order model:

$$\ln[B_t/A_t] = \ln[B_0/A_0] + (B_0 - A_0)\,kt$$

where $A_0$ and $B_0$ are the initial concentrations of fosinopril sodium and magnesium acetate and $A_t$ and $B_t$ are the residual concentrations at time $t$. Though the rate of the reaction is

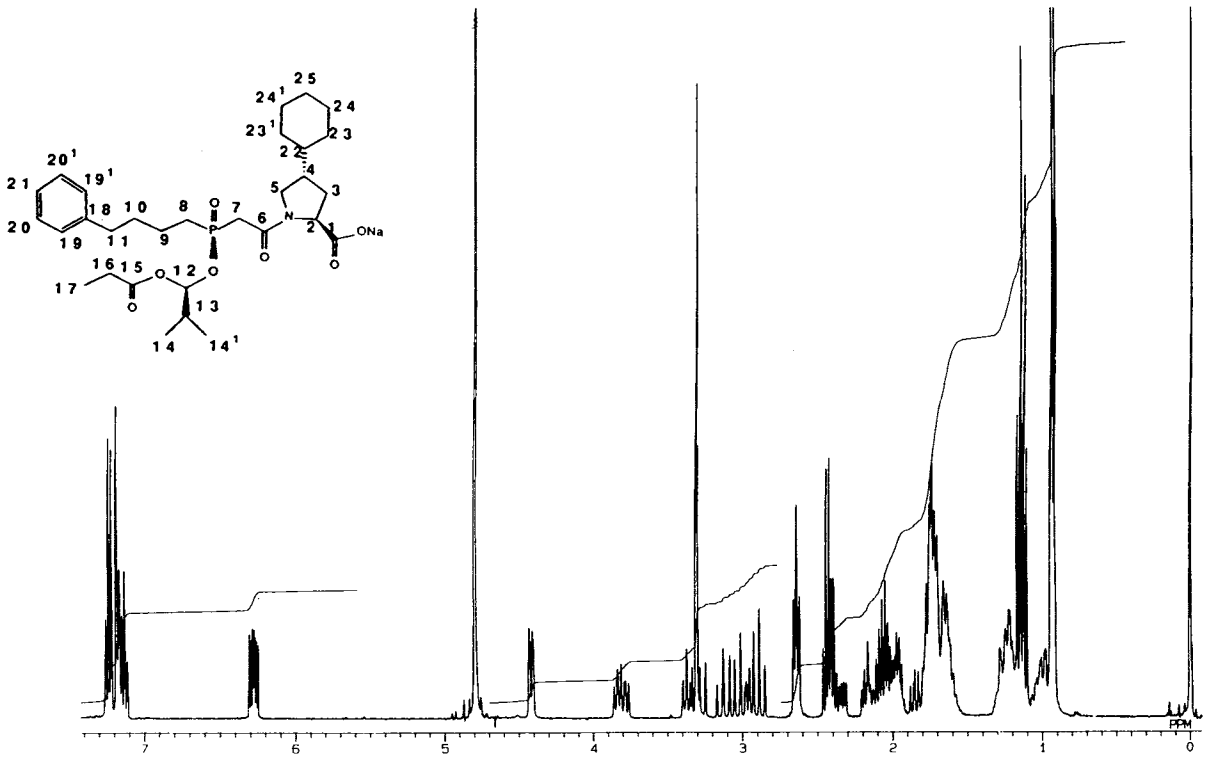



Fig. 3. $^1$H-NMR spectrum of fosinopril sodium in $CD_3OD$.

dependent on the concentration of both fosinopril and magnesium acetate, only the fosinopril concentration was actually measured as a function of time. The actual concentration of magnesium ions remained unchanged as a function of time, though their reactivity toward fosinopril was decreased as shown by the leveling of the reaction with time when less than 1 equiv of magnesium acetate was used. Since the initial concentration of both fosinopril sodium and magnesium acetate was known, the effective concentration of magnesium ions could be calculated (3). The rate constants calculated from these data are shown in Table II. It can be seen that the

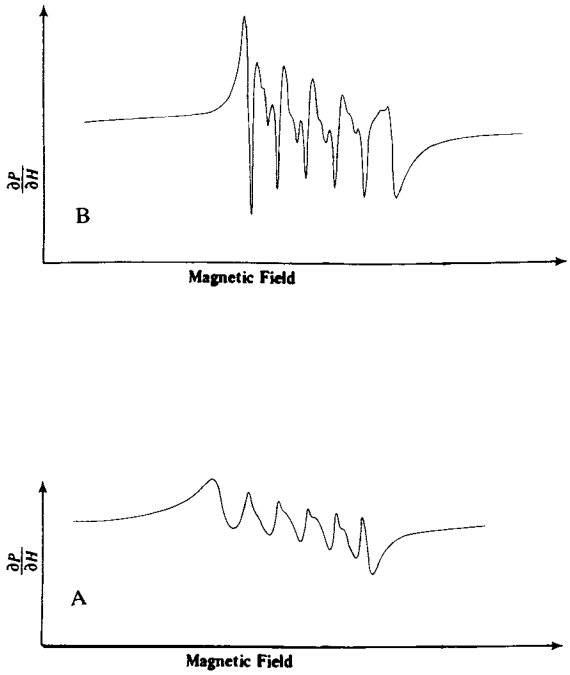



Fig. 4. EPR spectrum: (A) Mn(II) acetate (10 m*M*) in methanol and (B) Mn(II) acetate (10 m*M*) in the presence of equimolar fosinopril sodium in methanol.

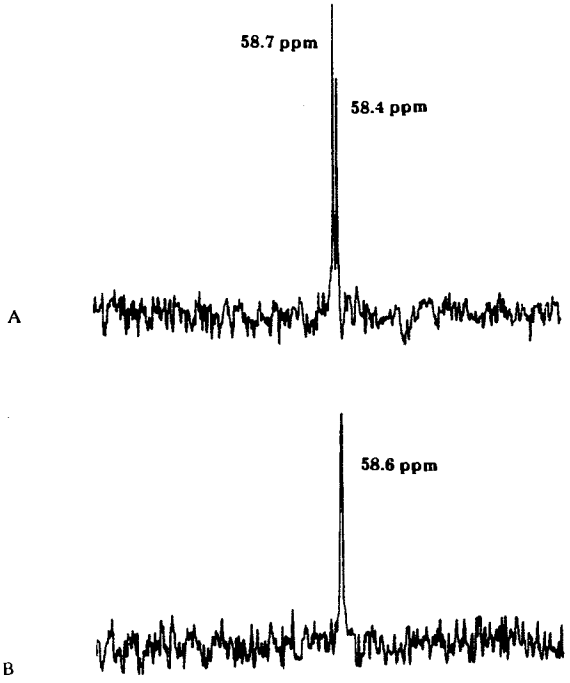



Fig. 5. $^{31}$P-NMR spectrum: (A) fosinopril sodium in $CD_3OD$ and (B) after the addition of equimolar molar magnesium acetate in $CD_3OD$.

value of the rate constant remained relatively invariant even when the ratio of the two reactants was varied over a large range. The second-order kinetics demonstrated the stoichiometric rather than catalytic involvement of magnesium ions in the reaction. This was, at first thought, surprising because the concentration of magnesium ions in the solution does not change with time. However, II, III, and IV are all capable of complexing magnesium ions, hence it is likely that their formation would retard the reaction rate due to removal of the metal ions from further reaction. According to this model, the magnesium ion-mediated degradation of fosinopril is a self-limiting process (Scheme I) and the degradation will "level off" with time in the presence of an excess of fosinopril. This is an unusual instance of incompatibility of a pharmaceutical entity in which there is a first-order dependence of the reaction on the availability of the metal ion. Most reported incompatibilities of drug substances with metal ions in the literature describe the catalytic involvement of metal ions. The reported example of reactions that show leveling trends are usually attributed to reduced availability of reactants such as moisture or oxygen (4–6). However, these examples differ significantly from the present case. In the magnesium ion-mediated degradation of fosinopril, the rate of the reaction is initially promoted by the metal ion but is progressively slowed down due to the effective removal of the reactive metal ions by the products of the reaction. Such involvement of metal ions in the hydrolysis of substrates of biological interest is well documented (7). Self-limiting reaction kinetics are described for Fe(III)-promoted hydrolysis of diester prodrugs of iron chelating agents *N,N'*-bis(2-hydroxybenzyl) and *N,N'*-bis(2-hydroxyphenyl)ethylenediamine diacetic acid by Pitt *et al.* (8). The authors report that overall rate of hydrolysis was second order with respect to both the ligand and the metal ion concentration. We have shown that the degradation of fosinopril is similarly affected in the presence of metal ions.

## Mechanism

The mechanistic steps of the metal ion mediated reaction of fosinopril leading to III and IV are presented in Scheme III. A central feature of this rearrangement is the ability of the phosphodicarbonyl system of fosinopril to form an activated complex with the metal ion. A computer-generated complex between fosinopril and magnesium ion is shown in Figs. 6A and B). The structures were generated by starting with the conformation (translational atomic coordinates) from the solved single crystal structure of fosinopril sodium (9). The geometry was then adjusted by rotating bonds and changing the angles to correspond to the hypothesized structure of the magnesium ion complex. The space filling model displays the appropriate van der Waal radii of the constituent atoms. The stereo line drawing shows the enol form of the proposed complex with half-van der Waal radii representation of the $sp^2$ orbitals at the reactive centers and for the magnesium ion. All the manipulations resulted in allowable conformations with respect to orbital overlap and bond angles.

Involvement of the phosphinyl–dicarbonyl system in the metal complex was suggested from the results of the $^{31}P$ NMR and Mn(II)EPR. The $^{31}P$ NMR spectrum of fosinopril sodium in $CD_3OD$ gives two resonance signals at δ 58.7 and 58.4 (Fig. 4). This is due to two distinct conformations of the molecule around the amide bond. On the addition of magnesium ions, the phosphorous resonance occurs as a singlet centered at δ 58.6, implying that rotation around the amide bond freezes and a single conformation results due to complexation of the metal ion.

As shown in Table I, Mn(II) ions also caused the degradation of fosinopril. Therefore it was thought correct to draw inference on the fosinopril–metal interaction from EPR spectroscopy. The complex formation was studied by reacting Mn(II) acetate with fosinopril in methanol. Mn(II) has a nuclear spin quantum number $I = 5/2$ and hence gives a characteristic six-line EPR signal due to $^{55}Mn$ hyperfine structure. On the addition of fosinopril sodium the peak height of the Mn(II) signal in frozen methanol is increased by a factor of three, while the average line width decreases between 20 and 50% depending on the nuclear state ($M_J = -5/2$ to $+5/2$). The line shape and intensity changes indicate that fosinopril binds to Mn(II) in methanol. We estimate a dissociation constant of $K_D = 0.1$ m*M*, assuming a 1:1 complex. The sharpening of line width upon binding indicates that the coordination environment of Mn(II) acetate in methanol becomes more homogeneous. This feature is expected only if fosinopril binds to create a more uniformly ordered coordination site.

The key steps in the degradation of fosinopril by metal ions are the formation of the complex, followed by deprotonation to give the magnesium enolate. The enhanced acidity of the protons on the C-7 was demonstrated by NMR, wherein a rapid exchange was observed with the solvent on the addition of magnesium acetate. The complexation of the metal serves to bring the C-7 and C-16 into the correct spatial orientation for orbital overlap to occur in the bond forming process (Fig. 6B). The attack of the enolate C-7 on the carbonyl C-16 gives a six-member ring transition state which collapses to give the proposed reactive intermediate V (Scheme III). The formation of V in the solution was inferred from the chromatographic and spectral analysis. But its isolation as a solid was not successful. The literature on aldol reactions is replete with examples of metal ion involvement in facilitating attack of enolate on the carbonyl group (10,11). Solladie *et al.* report an stereoselective addition of an α-sulfinic ester to an aldehyde (12) that bears a similarity to the proposed reaction of fosinopril with metal ion. The strategy that Nicolaou *et al.* (13) have employed, utilizing the keto phosphonate–aldehyde system in the ring closure reaction in the synthesis of macrolides tylosin and ampholide, is cited in support of the reaction mechanism proposed for the degradation of fosinopril by metal ions. This transformation of fosinopril under the mediacy of metal ion bears a topographic resemblance to the enolate Claisen rearrangement (14) and related Carroll rearrangement (15).

The driving force for the rearrangement/degradation cascade is chelation of metal ion by fosinopril. The metal ion presumably facilitates the cleavage reaction through binding to the intermediate, providing a conformation which maximizes the orbital overlap thus lowering the overall reaction barrier. The hydrolysis of the intermediate V (Scheme III) by the attack of water at the phosphinyl group of the intermediate affords the products III and IV in equimolar quantities.

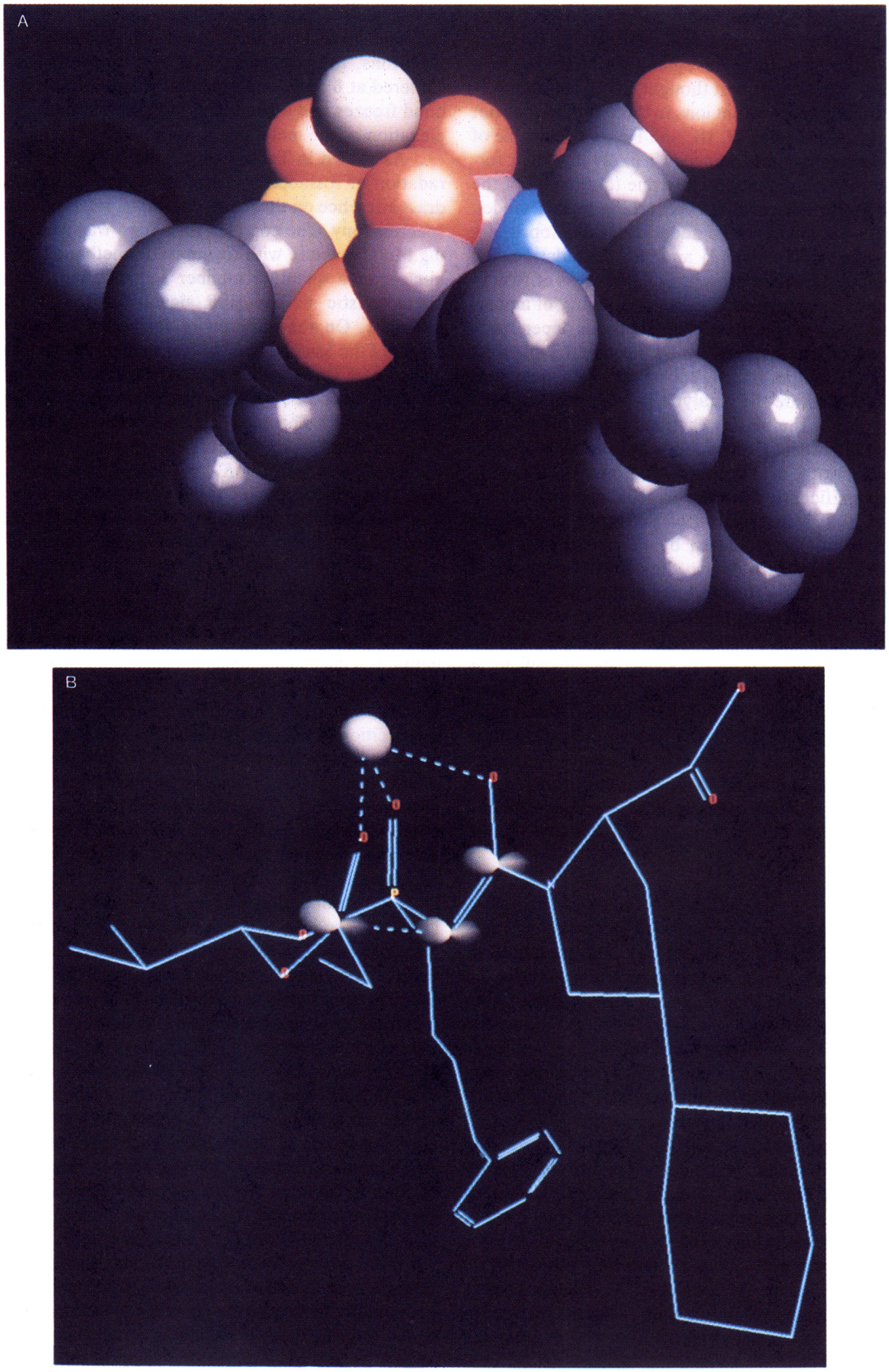



**Fig. 6.** Structural representation of interaction of fosinopril with magnesium ion. (A) Space filling model: magnesium ion (off white), phosphorus (yellow), oxygen (red), nitrogen (blue) and carbon (gray). (B) Stereo projections.

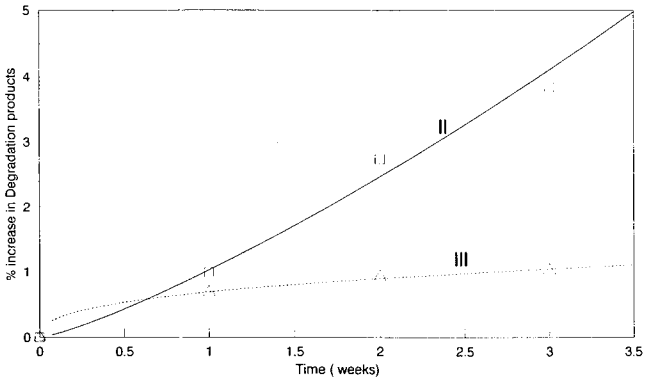



Fig. 7. Degradation of fosinopril sodium in a prototype tablet formulation containing magnesium stearate after storage at 50°C and 100% relative humidity, the showing formation of degradation products II and II as a function of time.

A notable feature of this reaction is the breaking of a carbon–phosphorous sigma bond under remarkably mild conditions. This process is analogous to metal-catalyzed dephosphonylation of 2-amino-3-phosphonopropionic acid by pyridoxal (16). The facile cleavage of the C–P bond is likely a result of the stabilization of the enolate of III by the metal ion. Thus the metal ion plays a central role in this transformation by bringing the respective reaction centers into the correct orientation and proximity. The net effect is the acylation of an active methylene group. The mediacy of the metal ion facilitating this transformation is reminiscent of enzyme mimetics (17,18). We intend to investigate the generality of this reaction with other molecules bearing similar structural features.

### Significance to Tablet Formation

The results of this study helped rationalize the degradation of fosinopril in the tablet formulation lubricated with magnesium stearate. It clearly identified two distinct pathways of degradation, i.e., magnesium ion mediated and hydrolysis. In the tablet formulation the amount of the lubricant is low compared to drug and hence the magnesium ion-mediated degradation would occur only to a small extent as predicted by the second-order kinetic model. However, the formation of acidic degradation products would enhance the acid catalyzed degradation of the ester prodrug. In Fig. 7 stability data from an experimental fosinopril tablet formulation containing magnesium stearate as lubricant and stored at 50°C and 100% relative humidity are shown. The formation of magnesium ion-mediated product III levels off, whereas the formation of hydrolysis product II continues with time of storage. The data thus validate the predictions of the model.

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