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Registry No. 1, 4474-91-3; 2, 126898-68-8; 3, 126898-69-9; 4a, 126898-70-2; 4b, 126898-71-3; 5a, 126898-72-4; 5b, 126898-73-5; 6a, 126999-84-6; 6b, 126999-85-7; 7, 126925-39-1; 7 (cyclic), 126925-36-8; 8, 126925-40-4; 8 (cyclic), 126925-37-9; 9, 126898-76-8; 9 (cyclic), 126925-38-0; 10, 126898-77-9; 10 (cyclic), 126898-74-6; 11, 126898-78-0; 11 (cyclic), 126898-75-7; 12, 126898-79-1; 12 (cyclic), 126898-66-6; 13, 126898-80-4; 13 (cyclic), 126898-67-7.

Supplementary Material Available: Table listing the amino acid analysis and FABMS for compounds 2-13 (1 page). Ordering information is given on any current masthead page.

Crystallographic Studies of Angiotensin Converting Enzyme Inhibitors and Analysis of Preferred Zinc Coordination Geometry

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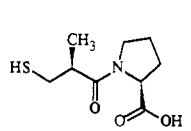
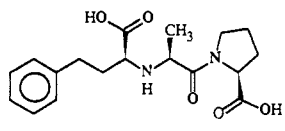
The molecular structures of two potent inhibitors of angiotensin converting enzyme (ACE, EC 3.4.15.1, dipeptidyl carboxypeptidase), ketoace, (5*S*)-5-benzamido-4-oxo-6-phenylhexanoyl-L-proline, and (1*S*,2*R*)-1-[[2-(benzoylthio)-cyclopentyl]carbonyl]-L-proline were determined by X-ray diffraction methods. The distances between the binding functions in both crystal structures are in agreement with the experimental results for the hypertension drug captopril and the enzyme substrate hippuryl-L-histidyl-L-leucine. The modified peptide skeletons of both inhibitors adopt extended conformations with the proline amide bond trans. Crystallographic data have been used to determine the coordination geometry for zinc–sulfhydryl and zinc–carbonyl interactions. Coordination distances and bond angles are found to be different from values assumed in models of the angiotensin converting enzyme active site. No preferred torsion angles for a zinc–sulfhydryl inhibitor interaction can be identified. Superposition of the crystallographic structures of four ACE ligands shows that the observed extended conformations place the pharmacophores, zinc atom ligand, carbonyl oxygen atom, and carboxyl group, in juxtaposition and provide an alternative model for the interaction of ligands with the ACE active site.

Angiotensin converting enzyme (ACE, EC 3.4.15.1, dipeptidyl carboxypeptidase) is the regulatory zinc protease in the renin–angiotensin system. ACE converts the decapeptide Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu to the vasoconstrictive octapeptide angiotensin II by cleaving the C-terminal dipeptide.[1] In addition, the converting enzyme hydrolyzes the nonapeptide bradykinin, which has hypotensive activity.[2] Thus, both enzymatic reactions result in elevation of blood pressure. Clinical studies have shown that inhibitors of the converting enzyme are effective in the treatment of essential hypertension and congestive heart failure. Captopril (1),[3,4] a mercaptoalkanoyl amino acid, was the first orally active ACE inhibitor that possessed clinical usefulness. Substitution of the zinc binding sulfhydryl moiety in captopril by carboxyl,[5] carbonyl,[6] or phosphonic acid ligands[7] enhanced both activity and binding specificity for the inhibitors, such as for the hypertension drug enalaprilat (2).[5]

We report the first crystal structure for a representative of the carbonyl ligand class, ketoace (3),[6] and the structure

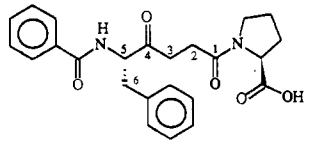


1 captopril

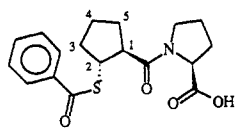


2 enalaprilat

for a semirigid analogue of captopril, 4.[8] Both molecular structures assist in the prediction of the binding conformation for ACE inhibitors and in the modeling of the active-site architecture of the converting enzyme. Ketoace, (5*S*)-5-benzamido-4-oxo-6-phenylhexanoyl-L-proline (3), is a derivative of the tripeptide ACE inhibitor Bz-Phe-Gly-Pro, in which the labile peptide amide bond of the model peptide is replaced by a more stable ketomethylene group. The nonpeptide analogue is 130 times more potent



3 ketoace



4

in vitro than the peptide[6] and 3-fold more potent than captopril,[9] although it has slow binding kinetics in vitro.[10] In vivo activity has been observed by continuous iv infusion;[9,11] however, ketoace is less active when given either

(1) Skeggs, L. T.; Marsh, W. H.; Kahn, J. R., Shumway, N. P. *J. Exp. Med.* **1954**, *99*, 275–282.
(2) Yang, H. Y. T.; Erdoes, E. G.; Levin, Y. *Biochem. Biophys. Acta* **1970**, *214*, 374–376.
(3) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441–444.
(4) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484–5491.
(5) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, N. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrack, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R. F.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280–283.
(6) Almquist, R. G.; Chao, W. R.; Ellis, M. E.; Johnson, H. R. *J. Med. Chem.* **1980**, *23*, 1392–1398.
(7) Petrillo, E. W., Jr.; Powell, J. R.; Cushman, D. W.; Ondetti, M. A. *Clin. Exp. Hypertens., Part A* **1987**, *A9*, 235–241.
(8) Ciabatti, R.; Padova, G.; Bellasio, E.; Tarzia, G.; Depaoli, A.; Battaglia, F.; Cellentani, M.; Barone, D.; Baldoli, E. *J. Med. Chem.* **1986**, *29*, 411–417.
(9) Almquist, R. G.; Crase, J.; Jennings-White, C.; Meyer, R. F.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1982**, *25*, 1292–1299.
(10) Grobelny, D.; Galardy, E. *Biochemistry* **1986**, *25*, 1072–1078.
(11) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964–969.

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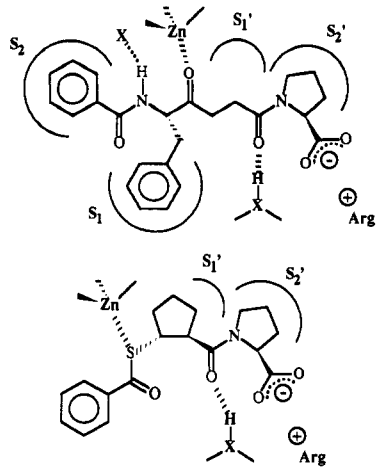


Figure 1. (a) Ketoace in an idealized ACE active site which demonstrates the putative binding interactions and hydrophobic sites. (b) Compound 4 in an idealized ACE active site which demonstrates the putative binding interactions and hydrophobic sites.

by iv bolus injection or by an oral route. The poor bioavailability of this compound, as compared to captopril, is probably due to faster degradation and/or clearance.

The postulated interactions of ketoace with the enzyme are summarized in Figure 1a. The carbonyl oxygen atom of the ketomethyl fragment presumably coordinates to the active site zinc ion; conversion to the oxime or to an hydroxyl, greatly lowers the potency.[9,11] The benzamido function is essential for binding; deletion of this group decreases inhibition by 105-fold.[12] Activity is lowered by 28-fold by replacing the phenyl group by methyl[11] and by 44000-fold by substitution of the amide moiety with an ethylene group;[12] thus both the amide function and the hydrophobic part of the benzamido group are important.

The importance of the benzyl group in ketoace is paralleled in the carboxyl inhibitor enalaprilat (2). Removal of the benzyl group in 3 leads to a 750-fold loss in potency;[12] in enalaprilat, deletion of the hydrophobic side chain leads to a 2000-fold loss in potency. In contrast to these inhibitors which coordinate zinc through oxygen atoms, sulfhydryl inhibitors like captopril (1) do not contain corresponding hydrophobic groups. The high potency of the sulfhydryl inhibitors is presumably achieved by much stronger coordination of the zinc by the sulfur ligand.

The semirigid captopril derivative 4, (1*S*,2*R*)-1-[[2-(benzoylthio)cyclopentyl]carbonyl]-L-proline, shows not only a slightly higher in vitro activity than the hypertension drug captopril (1) but also has significant hypertensive activity in vivo,[8] in contrast to ketoace. Several structure–activity studies for sulfhydryl inhibitors, such as captopril and 4, revealed the significance of three pharmacophores: the sulfur atom, the amide oxygen atom, and the C-terminal carboxyl group,[4,13-15] as shown in Figure 1b for compound 4. Conversion of the benzoylthio group in 4 to the sulfhydryl derivative leads to 6, (1*S*,2*R*)-1-[(2-mercaptocyclopentyl)carbonyl]-L-proline, with a 2-fold lower inhibitory activity. The additional cyclopentane moiety of 4 restricts the conformational flexibility of the portion of the inhibitor that binds to zinc. Both the ring size and the 1*S*,2*R* configuration of the cyclopentane portion are ideal for binding,[8] which makes this constrained inhibitor a most valuable model compound.

Several models for the active-site geometry of ACE have appeared. Early studies by Hassall et al.[16,17] developed a model that was based on the X-ray structure of captopril, NMR conformational studies, molecular mechanics, and the design of active, constrained analogues. To obtain a model for the active conformation of inhibitors, a trans peptide bond, a Zn···S–C angle of 124°, and a trans conformation for the critical Zn···S–CH_2–C torsion angle were assumed. This model predicts an axial orientation of the terminal carboxyl group and, when the overlap of active compounds is taken into account, predicts two preferred positions for the sulfhydryl ligand.

Andrews et al.[18] used conformation searching on nine ACE inhibitors to define the torsion angles that determine the conformation of the peptide bond, the orientation of the S_1' subsite methyl group, Ψ_1, and the position of the zinc ligand, Φ_1. (Angles defined[18,19] in parallel to the numbering of inhibitor 6 such that Ψ_1 is C2–C3–C4–N6 and Φ_1 is S1–C2–C3–C4.) The calculations were done with fixed values of bond lengths and bond angles and ignoring electrostatic interactions. This model determines that the trans peptide bond is preferred and that the active inhibitor conformation has $\Psi_1 = 165°$. Thorsett et al.,[19] in a more extensive modeling study of lactam inhibitors, widened the range for tight-binding to Ψ_1 values in the range 130–170°. On the basis of low energy conformers and constrained analogues, the optimal value for the torsion angle that determines the position of the zinc ligand, Φ_1, was proposed by Andrews to be 300° (or −60°). A position for the zinc site was predicted for all inhibitors by superimposing the proposed active conformers and assuming a Zn···S distance of 2.0 Å and the coordination bond and torsion angles used by Hassall et al.[16,17] The predicted zinc position led to a coordination geometry for ketoace which placed the carbonyl zinc ligand at a Zn to O distance of 1.7 Å, a Zn···O=C bond angle of 97° and a Zn···O=C—C torsion angle of 120°. The most recent model for the pharmacophore of ACE inhibitors[20-22] was developed by a systematic search of the conformational space with use of 28 ACE inhibitors. A position for the zinc ion was proposed[20] by using the Zn coordination geometry of Andrews et al.[18] The model[22] postulates an active site geometry that is defined by five distances between the zinc atom, the amide carbonyl oxygen and

(12) Gordon, E. M.; Godfrey, J. D.; Weller, H. N.; Natarajan, S.; Pluscec, J.; Rom, M. B.; Niemela, K.; Sabo, E. F.; Cushman, D. W. *Bioorg. Chem.* **1986**, *14*, 148–156.
(13) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441.
(14) Rubin, B.; Laffan, R. J.; Kotler, D. G.; O'Keefe, E. H.; Demaio, D. A.; Goldberg, M. E.; Morton, E. *J. Pharmacol. Exp. Ther.* **1978**, *204*, 271–280.
(15) Natarajan, S.; Condon, M. E.; Cohen, M. S.; Reid, J.; Cushman, D. W.; Rubin, B.; Ondetti, M. A. *Peptides, Structure and Biological Function, Proceedings of the American Peptide Symposium, 6th* by Gross, E., Meienhofer, J., Eds.; Elsevier: Amsterdam, 1979; pp 463–466.
(16) Hassall, C. H.; Kröhn, A.; Moody, C. J.; Thomas, W. A. *FEBS Lett.* **1982**, *147*, 175–179.
(17) Hassall, C. H.; Kröhn, A.; Moody, C. J.; Thomas, W. A. *J. Chem. Soc., Perkin Trans. 1* **1984**, 155–164.
(18) Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R. *J. Med. Chem.* **1985**, *28*, 393–399.
(19) Thorsett, E. D.; Harris, E. E.; Aster, S. D.; Peterson, E. R.; Snyder, J. P.; Springer, J. P.; Hieshfield, J.; Tristram, E. W.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *J. Med. Chem.* **1986**, *29*, 251–260.
(20) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. *Quant. Struct.-Act. Relat. Pharmacol., Chem., Biol.* **1986**, *5*, 138–152.
(21) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski, J. *Quant. Struct.-Act. Relat. Pharmacol., Chem., Biol.* **1986**, *5*, 99–105.
(22) Mayer, D.; Naylor, C. B.; Motoc, I. M.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 3–16.

carbon atom, and one oxygen atom of the carboxyl group; these distances are highly dependent on the choice of zinc coordination geometry, on the positions of the pharmacophore points relative to the molecular backbone and on the position of the oxygen atom in the relatively freely rotating terminal carboxyl group. Since the zinc coordination geometry is critical to modeling the ACE active site, a search of the crystallographic database for both macromolecules and for zinc complexes is reported herein to establish the experimentally observed geometry for zinc interactions. Vedani et al.[23] established Zn coordination distances by this method; we extend their work to an examination of bond angles and torsion angles.

These data on zinc coordination geometry will be used with the observed crystal structures of ketoace and the semirigid captopril analogue, 4, to examine the models for the ACE active site geometry.

Database Analysis of Zinc Coordination Geometry

Modeling the interaction of ACE inhibitors with the enzyme active site requires knowledge of the geometry of the coordination to the zinc ion. The typical geometric parameters observed in crystallographic determinations of Zn–S complexes was obtained by searching the Cambridge Structural Database (CSD)[24] for the coordination geometry of Zn···S–CH_2–C fragments. The criteria for accepting a CSD entry were tetrahedral coordination for the zinc ion, a Zn···S coordination distance less than 2.6 Å, and a crystallographic R factor less than 0.06. Twenty Zn···S–CH_2–C fragments were retrieved which satisfied these requirements; of these 15 arose from bidentate coordination of the zinc atom and were excluded from the torsion angle analysis. The Zn···S distances and Zn···S–CH_2 bond angles have a narrow variance. The Zn···S distances lie in the range from 2.247 to 2.456 Å with a mean value of 2.319 ± 0.053 Å. The Zn···S–CH_2 bond angle varied over the range 91.4–104.8° with an average value of 97.0 ± 4.5°. These findings agree with the Zn···S distances found by Vedani et al.[23] However, the Zn coordination observed in crystal structures is significantly different from that assumed in modeling studies of ACE inhibitors; indeed, the values assumed in the models are *outside the ranges* of observed distances and angles. The mean Zn···S distance found in this study is ca. 0.32 Å longer than the assumed model values, and the mean Zn···S–CH_2 angle is ca. 27° smaller than the model values.[16,18]

Data for both small molecule structures from the CSD and for complexes of Zn in a protein environment were used for describing the preferred torsion angle for the Zn···S–CH_2–C fragment. In the refined, high-resolution X-ray structures for two complexes between sulfhydryl inhibitors and thermolysin, the angles were −35.1° for the (2-benzyl-3-mercaptopropanoyl)-L-alanylglycinamide complex[25] and −40.9° for the thiorphan complex.[26] In addition, the Cd/Zn-metallothionein structure[27] provides examples of the geometry for tetrahedral coordination of zinc ions with cysteine ligands; the torsion angles are 42.5, 48.7, 77.7, 143.0, 149.9, 153.6, and −154.4°. These macromolecular data are included in the plot of torsion angles found in the Cambridge Structural Database and presented in Figure 2. The distribution for the torsion angles of the Zn···S–CH_2–C fragment shown in Figure 2 does not support the modeling assumption[16] of a strictly trans arrangement for the coordination of the active site zinc ion with inhibitors of the sulfhydryl ligand class. The observed torsion angles indicate that, while the trans value of 180° is observed, there is a wide distribution of observed conformations for Zn···S–CH_2–C. Notably, the protein data indicate that torsion angles of 40° are also favorable and are observed in two examples of zinc-inhibitor complexes. Thus, although the coordination distance Zn···S and the coordination angle Zn···S–CH_2 can be obtained with some confidence, modeling of the zinc position in the ACE active site is significantly hampered by the lack of any conformational preference for the torsion angle Zn···S–CH_2–C which defines the zinc coordination. Therefore, previous modeling studies which have restricted the coordination torsion angle must be regarded as incomplete.

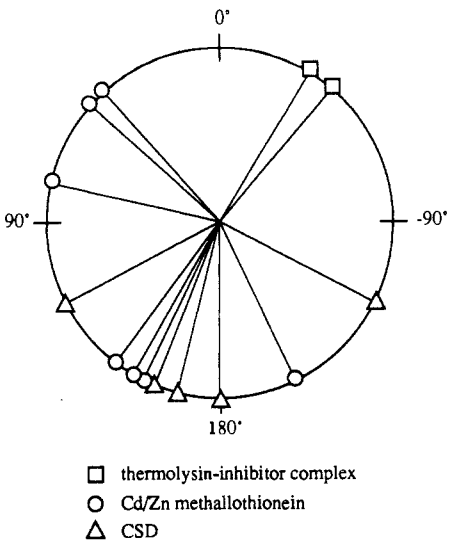


Figure 2. Torsion angles for Zn···S–CH_2–C fragments in the crystal structures of thermolysin-inhibitor complexes, Cd/Zn metallothionein and those found by a search of the Cambridge Structural Database (CSD).[24]

A similar database search was done for the carbonyl zinc ligand. Twenty-one examples of the fragment Zn···O=$C(C)_2$ with the Zn···O distance less than 2.6 Å and crystallographic $R \leq 0.09$ were found; of these, only four had a tetrahedrally coordinated zinc ion. The Zn···O distances range from 1.896 to 2.128 Å with a mean value of 2.038 ± 0.070 Å; if only the tetrahedral examples are averaged, the mean distance is 1.943 ± 0.035 Å. These values are similar to those reported by Vedani et al.,[23] who also found that the distance varies slightly with coordination. The Zn···O=C bond angle for all examples range from 122.3 to 136.2° with a mean value of 128.1 ± 4.7°; the mean value for a tetrahedral zinc complex is 126.0 ± 4.1°. Thus, models for the interaction of zinc with carbonyl ligands, like that found in ketoace (**3**), should use values of 1.94 Å for the zinc distance and 126° for the Zn···O=C bond angle. These values differ from those obtained from the Andrews et al.[18] model of the ACE active site by 0.24 Å in Zn···O distance and 29° in Zn···O=C bond angle.

The coordination torsion angles for the Zn···O=C—C fragment cluster in two populations. One, the cis cluster, has a range of values from −17.6 to 18.7° and a mean value of 0.4 ± 10.9°. The other, the trans cluster, has a range of values from −164.8 to 162.5° and a mean value of 179.7

(23) Vedani, A.; Dobler, M.; Dunitz, J. D. *J. Comput. Chem.* **1986**, *7*, 701–710.
(24) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwrite, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. F.; Kennard, O.; Motherwell, W. D. S.; Rogers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331–2339.
(25) Monzinga, A. F.; Matthews, B. W. *Biochemistry* **1982**, *21*, 3390–3394.
(26) Roderick, S. L.; Fournie-Zaluski, M. C.; Roques, B. P.; Matthews, B. W. *Biochemistry* **1989**, *28*, 1493–1497.
(27) Furey, W. F.; Robbins, A. H.; Clancy, L. L.; Winge, D. R.; Wang, B. C.; Stout, B. C. *Science* **1986**, *231*, 704–710.

Table I. Crystallographic Data for Ketoace (3) and for Compound 4

	3	4
mol formula	$C_{24}H_{26}N_2O_5$	$C_{18}H_{21}NO_4S$
mol wt	422.485	347.436
crystal size (mm)	0.1 × 0.1 × 0.5	0.1 × 0.1 × 0.6
space group, Z	$P2_12_12_1$, 4	$P2_1$, 2
a (Å)	10.184 (1)	14.666 (3)
b (Å)	11.146 (1)	6.605 (1)
c (Å)	19.683 (2)	8.854 (1)
V (Å^3)	2234 (5)	845.6 (2)
β (deg)		90.014 (10)
calcd density (g cm^{-3})	1.256	1.349
meas density (g cm^{-3})	1.267	1.350
Θ_{max} (deg)	70	75
unique reflections	2411	1915
R	0.048	0.040
R_w	0.064	0.059
goodness of fit (S)	1.113	1.135

± 10.9°. These data include 21 fragments which are either mono or bidentate. If the bidentate ligands are excluded from consideration, only three fragments remain and trends are undefinable; even so, the torsion angle ranges for the two clusters remain essentially the same for the monodentate ligands.

The REFCODES used in the CSD searches are available (see paragraph at the end of the paper regarding supplementary material).

Crystallographic Experimental Section

A sample of ketoace (3) was provided by Dr. R. G. Almquist of SRI International of Stanford, CA and the semirigid analogue of captopril (4) was provided by Dr. R. Ciabatti of Gruppo Lepetit S.p.A., Milano, Italy. Single crystals of 3 were grown by slow evaporation of a methanol/water solution; compound 4 was crystallized from a saturated ethyl acetate solution by using vapor diffusion with petroleum ether as the precipitant. The density for each crystal was measured by suspending single crystals in a toluene/CCl_4 mixture.

The crystallographic data for both compounds are summarized in Table I. The orientation angles and unit cell parameters for each crystal were obtained from the positional parameters of 25, individually centered reflections with θ angles between 24–38°. The diffraction data were collected at room temperature on an Enraf-Nonius CAD-4F automated diffractometer, using $\omega/2\theta$ scans and Cu Kα radiation (λ = 1.5418 Å, Ni filter, 20 mA, 45 kV). Both Lorentz and polarization corrections were applied to the data sets, but corrections to absorption and secondary extinction were not necessary.

The structures were solved by direct methods by using MULTAN-80[28] for 3 and SHELXS-86[29] for 4. The hydrogen atoms were included in the model at positions found from a difference Fourier synthesis and with isotropic thermal parameters defined as 1.2 × B_{eq} of the attached atom. After a few cycles of refinement, the hydrogen atom bound by C15 of ketoace and six hydrogen atoms of 4 adopted positions with poor geometry and were, therefore, placed in calculated positions with idealized geometry. The parameters for the idealized hydrogen atoms were not refined. All other positional parameters, the anisotropic thermal parameters for the non-hydrogen atoms, and the isotropic thermal parameters for the remaining hydrogen atoms were refined by weighted nonlinear least-squares analysis. The function minimized was $\Sigma w(|F_o - F_c|)^2$ where $w^{-1} = \sigma^2(F_o) + 0.001(F_o)^2$ and $\sigma(F_o)$ was defined by counting statistics. The unobserved reflections were defined as those with $I < 2.5\sigma(I)$; unobserved reflections were included in the least-squares refinement if $F_c > 2.5\sigma(F_o)$. The final reliability indices are given in Table I. Except for the direct

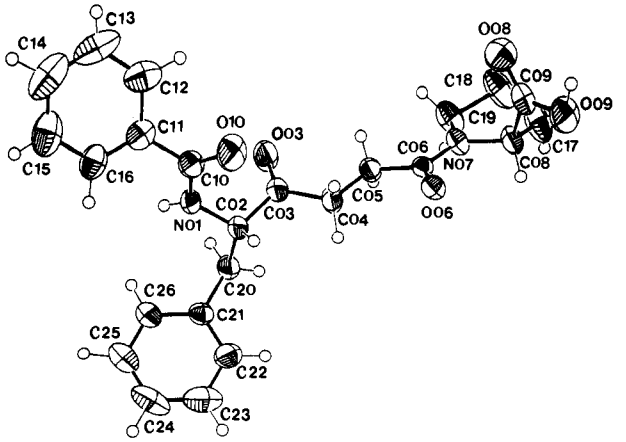


Figure 3. Molecular structure and atomic labeling scheme for ketoace. This drawing was prepared by the computer program ORTEP.[43]

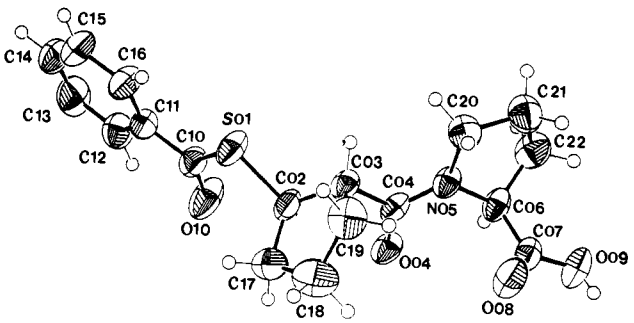


Figure 4. Molecular structure and atomic labeling scheme for compound 4. This drawing was prepared by the computer program ORTEP.[43]

method, the programs used were those of the XRAY76 system, by J. M. Stewart.[30] The scattering factors used were those of Cromer and Mann,[31] except for the hydrogen atom scattering factors, which were from R. F. Stewart et al.[32]

The positional parameters for the hydrogen atoms, the thermal parameters for all atoms, and lists of structure factors for both structures are available (see paragraph at the end of the paper regarding supplementary material).

Calculation Methodology

The conformations of several molecules were obtained by MNDO[39] and MMP2[41] calculations. The MNDO program was used from the MOPAC package (Versions 2.08 and 4.0). The MNDO calculations were performed on a CDC CYBER 205 supercomputer with a virtual storage operating system (VSOS). The calculation of a large number of rotamers was accomplished by a driver program written by R.J.H.;[44] the program generated the internal coordinates for a full rotation about a specified torsion angle. Appropriate modifications to read in the coordinates were required for the MNDO main routine.

The molecular mechanics calculations were performed by using MMP2 (Release 11.0). The program was implemented on a Honeywell Multics (HIS DPS 8/70M) mainframe computer. Conformational space for the main chain of an inhibitor was explored by systematic variation of the torsion angles S1–C2–C3–C4 and C2–C3–C4–N6 with the torsion angle driver option of MMP2. At each step,

(28) Main, P. *MULTAN80* Department of Physics, University of York, York, England.
(29) Sheldrick, G. M. In *Crystallographic Computing 3*; Sheldrick, G. M., Krueger, C., Goddard, R., Eds.; Oxford University Press: London, 1985, pp 175–189.
(30) Stewart, J. M. *The XRAY system of crystallographic programs*, Computer Science Center, University of Maryland, College Park, MD, 1976.
(31) Cromer, D. J.; Mann, J. B. *Acta Crystallogr.* **1968**, *A24*, 321–324.
(32) Stewart, R. F.; Davison, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175–3187.

Table II. Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters (×10) for the Non-Hydrogen Atoms of Ketoace [B_{eq} Is Defined as $^1/_3(B_{11} + B_{22} + B_{33})$]

atom	x/a	y/b	z/c	B_{eq}
C01	2750 (3)	5720 (3)	4637 (1)	32 (1)
C02	3613 (3)	6755 (3)	4589 (2)	32 (1)
C03	136 (3)	7780 (4)	5038 (2)	36 (2)
O03	1987 (3)	8006 (3)	5092 (2)	60 (2)
C04	4188 (4)	8498 (3)	5388 (2)	38 (2)
C05	3732 (3)	9663 (3)	5708 (2)	34 (1)
C06	4674 (3)	10128 (3)	6235 (1)	27 (1)
O06	5706 (2)	9602 (2)	6377 (1)	32 (1)
N07	4355 (3)	11154 (3)	6541 (1)	33 (1)
C08	5266 (4)	11740 (3)	7014 (2)	36 (2)
C09	5268 (4)	11147 (3)	7704 (2)	36 (2)
O08	4319 (3)	10696 (3)	7969 (1)	51 (1)
O09	6428 (3)	11254 (3)	8000 (1)	54 (1)
C10	2590 (4)	5192 (4)	5233 (2)	39 (2)
O10	3206 (3)	5522 (4)	5742 (1)	65 (2)
C11	1661 (4)	4148 (4)	5279 (2)	39 (2)
C12	878 (5)	4045 (5)	5858 (2)	56 (2)
C13	108 (5)	3046 (7)	5938 (3)	77 (3)
C14	123 (6)	2129 (6)	5460 (4)	76 (3)
C15	865 (5)	2254 (5)	4882 (3)	64 (3)
C16	1629 (4)	3271 (4)	4786 (2)	47 (2)
C17	4718 (6)	13009 (4)	7072 (2)	56 (2)
C18	3270 (6)	12856 (4)	6961 (2)	60 (2)
C19	3181 (4)	11895 (4)	6407 (2)	45 (2)
C20	3687 (4)	7204 (3)	3847 (2)	36 (1)
C21	4478 (3)	6402 (3)	3378 (2)	33 (1)
C22	5795 (4)	6641 (4)	3249 (2)	44 (1)
C23	6507 (5)	5914 (5)	2819 (2)	62 (3)
C24	5937 (5)	4943 (5)	2521 (2)	64 (3)
C25	4648 (5)	4683 (4)	2648 (2)	56 (2)
C26	3902 (4)	5411 (3)	3072 (2)	42 (2)

Table III. Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters (×10) for the Non-Hydrogen Atoms of 4 [B_{eq} Is Defined as $^1/_3(B_{11} + B_{22} + B_{33})$]

atom	x/a	y/b	z/c	B_{eq}
S01	6767 (1)	1786[a]	6049 (1)	50 (0)
C02	7728 (2)	1714 (7)	4739 (4)	45 (1)
C03	7869 (2)	3794 (5)	3998 (3)	38 (1)
C04	8186 (2)	3511 (5)	2370 (3)	36 (1)
O04	8517 (2)	1880 (5)	1962 (3)	46 (1)
N05	8108 (2)	5077 (5)	1422 (3)	38 (1)
C06	8467 (2)	4984 (6)	−124 (3)	40 (1)
C07	9468 (2)	5613 (6)	−142 (4)	40 (1)
O08	9927 (2)	5849 (7)	954 (3)	60 (1)
O09	9760 (2)	5805 (6)	−1538 (3)	54 (1)
C10	6392 (2)	−703 (6)	5967 (4)	43 (1)
O10	6745 (2)	−1936 (5)	5150 (4)	71 (2)
C11	5592 (2)	−1221 (6)	6931 (4)	42 (1)
C12	5223 (3)	−3146 (9)	6796 (4)	55 (2)
C13	4454 (3)	−3668 (9)	7678 (6)	66 (2)
C14	4089 (3)	−2310 (10)	8679 (5)	63 (2)
C15	4459 (3)	−428 (10)	8827 (5)	64 (2)
C16	5211 (3)	143 (8)	7936 (4)	52 (2)
C17	8637 (3)	1224 (10)	5571 (7)	79 (3)
C18	9265 (3)	2995 (10)	5357 (6)	69 (3)
C19	8670 (3)	4750 (7)	4901 (4)	56 (2)
C20	7736 (3)	7096 (6)	1747 (4)	49 (2)
C21	7634 (3)	8092 (8)	196 (4)	57 (2)
C22	7846 (3)	6446 (9)	−959 (4)	56 (2)

[a] The y coordinate was fixed to define the origin on the b axis.

the input coordinates were taken from the geometry of the preceding rotamer.

Results

The molecular conformations and atomic labeling schemes for ketoace (3) and for 4 are shown in Figures 3 and 4, respectively. The positional parameters and equivalent isotropic thermal parameters are given in Tables II and III.

The proline amide bond in ketoace adopts a trans arrangement which facilitates two intermolecular hydrogen bonds involving the peptide carbonyl oxygen atom, O06, and the carboxyl oxygen atom, O09. The constrained captopril analogue 4 also is in a trans conformation and forms hydrogen bonds involving the peptide carbonyl oxygen atom, O04, and the carboxyl oxygen atom O09. Even though a trans conformation is observed in both of these structures, the conformational preference of the peptide bond adjacent to proline is ambiguous. A cis conformation of the proline peptide bond is observed in the crystal structures of the related proline analogues: *N*-propionylproline,[33] *N*-(*tert*-butyloxycarbonyl)-D-prolyl-D-prolylproline,[34] and two indoline analogues of captopril,[35,36] in addition, Benedetti[45] et al. have shown a strong preference for the cis conformer in Boc-Pro peptides. Solution studies indicate that the transition between the two conformations is facile: the carbon-13 NMR spectrum of ketoace in both $CDCl_3$ and D_2O demonstrates the occurrence of both cis and trans isomers for the acylproline bond.[6,10] Also, solution experimental data indicate that, while the trans conformation is preferred by alanylproline peptides, the conformation is pH dependent.[19] However, ACE inhibitors, MDL27,088[37] and cilazapril,[38] are constrained to a trans conformation and have high inhibitory potency, thereby indicating that the actual enzyme binding conformation is trans, as is found in these two crystal structures.

No intramolecular hydrogen bonds are found in the two crystal structures of ketoace and 4. Overall, both molecules adopt an extended conformation stabilized by intermolecular hydrogen bonds between inhibitor molecules. The hydrogen-bond geometry is tabulated in Table IV. In ketoace, there are two unique hydrogen bonds: from the carboxyl hydrogen atom H091 to the benzamido carbonyl atom, O10, of a neighboring molecule and from the benzamido amide hydrogen atom, H011, to the peptide chain, carbonyl oxygen atom, O06, of a second neighboring molecule. These two hydrogen bonds are repeated by crystallographic symmetry to form a network of four bonds which connects each inhibitor molecule to four neighboring molecules. The only hydrogen atom acceptor which does not form a hydrogen bond is O03, the putative zinc coordination ligand; the lack of this interaction is probably due to the shortage of hydrogen donors in the structure and to the fact that the amide carbonyl oxygen atoms are better hydrogen acceptors.

In the crystal structure of the semirigid captopril analogue, 4, each molecule forms a strong intermolecular hydrogen bond (see Table IV) between the peptide chain, carbonyl oxygen atom, O04, and the carboxyl hydrogen atom, H091, of a molecule related by the screw axis. This hydrogen bond is repeated by the 2_1 axis so that the conformation of the molecule is stabilized by hydrogen bonds with two neighboring molecules. As in the case of ketoace,

(33) Kamwaya, M. E.; Oster, O.; Bradaczek, H. *Acta. Crystallogr.* **1981**, *B37*, 364–367.
(34) Bavoso, A.; Benedetti, E.; DiBlasio, B.; Pavone, V.; Pedone, C.; Taniolo, C.; Bonora, G. M. *Macromolecules* **1982**, *15*, 54–59.
(35) Vrielink, A. M.Sc. Thesis, University of Calgary, Alberta, Canada, 1985.
(36) Vrielink, A.; Codding, P. W. *Peptides, Structure and Biological Function, Proceedings of the American Peptide Symposium, 9th* Deber, C. M., Hruby, V. J., Kapple, K. D., Eds.; Pierce Chem. Co.: Rockford, IL, 1985; 783–786.
(37) Flynn, G. A.; Giroux, E. L.; Dage, R. C. *J. Am. Chem. Soc.* **1987**, *109*, 7914–7915.
(38) Attwood, M. R.; Hassall, C. H.; Kröhn, A.; Lawton, G.; Redshaw, S. *J. Chem. Soc., Perkin Trans. 1* **1986**, *6*, 1011–1019.

Table IV. Geometry of the Intermolecular Hydrogen Bonds in Ketoace (3) and 4 (The Equivalent Position Refers to the Hydrogen Atom Acceptor)

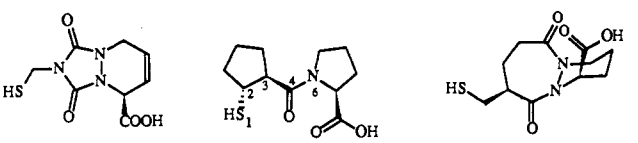
X-H···Y	X-H, Å	X···Y, Å	H···Y, Å	X-H···Y, deg	equivalent position
		Compound 3			
N01-H011···O06	0.96 (3)	2.906 (4)	1.96 (3)	170 (3)	$x - 0.5, 1.5 - y, 1 - z$
O09-H091···O10	0.89 (4)	2.633 (4)	1.81 (4)	153 (4)	$1 - x, 0.5 + y, 1.5 - z$
		Compound 4			
O09-H091···O04	0.79 (5)	2.652 (4)	1.88 (4)	166 (4)	$2 - x, 0.5 + y, - z$

the putative zinc ligand and, as well, the benzoyl carbonyl oxygen atom are not involved in hydrogen bonding due to a shortage of hydrogen donors.

The effects of hydrogen bonding and crystal packing on the molecular conformation of ketoace (3) and the constrained captopril analogue (4) were investigated by MNDO[39] minimization of the observed molecular conformation. In each case, the two structures (MNDO and X-ray) were similar, with extended backbone conformations. In ketoace (3), the differences between the MNDO-calculated structure and that observed in the crystal are mostly due to the formation of the intermolecular hydrogen bonds in the crystal: thus, the two major changes in torsion angles involve the benzamido group and the carbonyl group, C06=O06. The torsion angle C10-N01-C02-C03 changed by 19° from -60.8 (4)° in the crystal to -79.8° in the MNDO result and the torsion angle C03-C04-C05-C06 changed from 160.8 (3)° to -177.1°.

In the semirigid captopril analogue (4), the MNDO minimization resulted in the same basic conformation of the molecular backbone as found in the observed structure; however, the conformation of the cyclopentane ring and the relative orientation of the phenyl ring in the benzoyl fragment were changed. In the crystal, the cyclopentane ring adopts an envelope conformation with C19 at the flap (0.50 (2) Å from the plane) and the bond C03-C19 approximately perpendicular to the plane of the molecular backbone C02-C03-C04-N05-C06. The minimized MNDO structure has an alternative envelope conformation for the cyclopentane ring in which C02 is out of the plane by 0.178 Å and the ring is flattened in comparison to the X-ray structure; yet, the relative orientation of the C3-C19 bond to the molecular backbone is similar to that found in the crystal. The change in ring conformation does not change the separation between C19 (which is designed to occupy the S_1' hydrophobic pocket), the amide carbonyl group, and the carbon atom of the carboxyl group by more than 0.21 Å. The intermolecular hydrogen bonds found in the crystal seem to affect the orientation of the carboxyl group such that the angle N05-C06-C07-O08 is 10.4 (5)° in the crystal and -34.5° in the MNDO calculation. MNDO minimization forced the phenyl ring of the benzoyl group of the conjugation with the carbonyl portion of that fragment: this appears to be an artifact of the approximations made in the calculation, the crystal structure shows a conjugated benzoyl group.

To further investigate the inhibitor conformation, the conformational flexibility of the zinc binding portion of the constrained inhibitor 6,[8] was probed[44] by systematic variation of the torsion angles along the S1-C2-C3-C4-N6 bond sequence by using MMP2 minimizations[41] and full geometry optimization. All of the rotamers that had a conformational energy within 10 kcal/mol of the minimum had torsion angles between 165 and -35° for S1-C2-C3-C4 (Φ_1); those within 2 kcal/mol of the minimum had torsion angles between -165 and -60° for S1-C2-C3-C4 (Φ_1) and between 65 and -170° for C2-C3-C4-N6 (Ψ_1). In fact, the potential energy surface for compound 6 shows that binding conformations for sulfhydryl inhibitors with Φ_1 torsion angles between 0 and 165° and between -35 and 0° can be excluded with confidence on the basis of large steric energies. The calculations also show that the torsion angle, S1-C2-C3-C4 = -144.8 (2)°, which is observed in the crystal structure of the semirigid inhibitor 4 falls in the low-energy range found for the structurally related ACE inhibitor 6.



5 6 7

The barrier to rotation of the carboxyl group or, alternatively, the carboxylate group in captopril and the potent ACE inhibitor 7,[42] were investigated[44] by MNDO[39] calculations. The relative potential energy profiles were obtained from the calculated free energies of formation by rotation of the carboxyl group or carboxylate group in 10° intervals with full optimization at each step. For captopril, the barrier height is 1.5 kcal/mol for the carboxylate group and 3.8 kcal/mol for the carboxyl group with minima at 150 and -30° in each. For compound 7, the barrier height is 2.2 kcal/mol for the carboxylate group and 2.5 kcal/mol for the carboxyl group with minima at 110 and -60°. Thus, the energy gained from formation of one hydrogen bond would be sufficient to overcome the energy barrier for rotation of this group and differences in the position of the carboxyl atoms found in models and crystal structures can easily be overcome by the facile rotation of this group.

Discussion

The observed crystallographic geometry of zinc coordination with sulfur and with carbonyl oxygen is quite different from the zinc-sulfur geometry assumed in prior modeling studies. Differences of 0.32 Å in Zn···S distance and ~27° in Zn···S-CH_2 angle lead to a change in the position of the Zn atom by 1.0 Å for any given inhibitor conformation. In addition, the difference of 29° between the observed zinc-carbonyl bond angle and the angle that resulted from the modeled conformation of ketoace[18]

(39) Quantum Chemistry Program Exchange, Program No. 455, Chemistry Department, Indiana University, Bloomington, IN 47405.
(40) Fujinaga, M.; James, M. N. G. *Acta. Crystallogr.* **1980**, *B36*, 3196-3199.
(41) Allinger, N. L. *MMP2: Molecular Mechanics*, 1982, Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN 47405, 1982.
(42) Hassall, C. H.; Lawton, G.; Redshaw, S. Eur. Pat. Appl. EP 172,552 (CL C07D487/04), 26 Feb 1986, GB Appl. 84/21,493, 24 Aug 1984.
(43) Johnson, C. K. ORTEP-II. Report ORNL-5138, Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(44) Hausin, R. J. Ph.D. Thesis, University of Calgary, Calgary, Alberta, Canada, 1989.
(45) Benedetti, E.; Pedone, C.; Toniolo, C.; Némethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1980**, *16*, 156-172.

suggests that the modeled ketoace–zinc coordination is unlikely.

Most importantly, as shown in Figure 2, there is little indication of a preferred value for the critical torsion angle Zn···S–CH_2–C. A wide range of values is observed, and the observation of gauche (40°) as well as anti (180°) conformations in the protein data indicate that this torsion angle should not be restricted to one value in a model. Instead, it is important to incorporate the apparent lack of strong directional preference for the fragment into any modeling study.

In contrast, the crystallographic data indicate that the carbonyl group orientation is strongly preferential. Two values of the torsion angle Zn···O=C—C are found: 0.4 and 179.7°, constituting a cis and a trans arrangement. These data show that the zinc atom is coordinated by the lone pairs of electrons of the carbonyl oxygen atom and is, therefore, positioned in the plane of the carbonyl group either cis or trans to the terminal carbon atom in the fragment Zn···O=C—C.

The dihedral angles along the C2–C3–C4–C5–C6–N7–C8 bond sequence of ketoace establish an extended conformation for the modified peptide backbone with an average deviation of only 9.5 (3)° from a perfect, all-trans conformation. A similar, extended conformation is observed for the mercaptoalkanoyl fragment of the semirigid captopril analogue (4). Two other sulfhydryl ACE inhibitors, captopril[40] and the indoline analogue of captopril, WY44221,[35] have extended conformations in the crystal.

The observed extended conformations are in sharp contrast to the folded binding conformation with a Φ_1 (S1–C2–C3–C4) angle of 300° (–60°) proposed by Andrews and co-workers[18] for ketoace and captopril. In their modeling studies, the extended, minimum energy conformation (Φ_1 = 180°) was excluded from the model, because an extended or trans conformer of compound 5 is unfavorable. Compound 5, however, is 100 times less potent than captopril[16,17] which conflicts with the fact that it preferentially adopts the folded low-energy conformation of the model and that it is a constrained analogue which should suffer less entropy loss on binding. The poorer inhibitory potency of 5 could, however, be explained if an extended conformation (which would have higher conformational energy for this compound) is required for binding, or could be due to a poorer fit of 5 to an active site that recognizes extended peptides. Even though the observed value for the Φ angle in 4 is different from that predicted in the Andrews model,[18] our calculation (see Results) of the potential energy profile of the related compound 6, shows that the crystal structure is a low energy conformer and thus a possible binding conformation.

In contrast to the difference in Φ_1 angle, the crystal structure of 4 has a Ψ_1 (C2–C3–C4–N6) value in the range defined by Andrews et al.[18] and Thorsett et al.[19] In 4, Ψ_1 is 162.0 (3)°, approximately the value found by Andrews et al.;[18] in ketoace the same angle is 179.6 (3)°, which is 9.6° greater than the range found by Thorsett. The different Ψ_1 value in ketoace may reflect the lack of a S_1' subsite ligand in this inhibitor.

Comparison of the crystal structures to the model defined by pharmacophore validation[20-22] has to be done by comparing the distances between the pharmacophores (zinc atom, amide carbonyl group, and one carboxyl oxygen atom) because five distances are the only metric data given in the model. Such a comparison requires two assumptions: first, that the distribution of pharmacophores relative to the molecular backbone is the same in the crystal structure as in the "active" conformation of the model and second, that the zinc coordination bond distances, bond angles, and torsion angles are known. The first assumption arises from the fact that the five distances between the zinc atom, carbonyl carbon atom, carbonyl oxygen atom, and carboxylate oxygen atom do not identify a single arrangement of 4 points; however, visual inspection of the crystal structures and the "active" conformation[20-22] suggests that the pharmacophores are similarly arranged. The second assumption regarding zinc coordination geometry is critical since, as discussed above, the crystallographic data indicate that observed Zn···S distances and Zn···S–CH_2 bond angles are different from the values assumed in the modeling and that the Zn···S–CH_2–C torsion angle varies over a wide range of values.

Table V. Crystallographically Observed Distances between the Zinc Ligand (L), the C-Terminal Amide Carbonyl Oxygen Atom (O), and the Carboxyl Carbon Atom (C) for ACE Binding Molecules

crystal structure	d(L···C), Å	d(L···O), Å	d(C···O), Å
ketoace (3)	7.06	4.89	3.16
4	7.23	4.44	3.40
captopril[40] (1)	7.15	4.66	3.02
HHL[35] (7)	7.32	4.89	3.64

Comparison of the crystal structure of 4 and the model[20-22] shows that the two distances that define the relative positions of the carbonyl group and the carboxyl oxygen atom are within 0.5 Å of one another; this difference in carboxyl oxygen atom position between the structure and the model is due to rotation of the carboxyl group; as shown in the results section, this group has a low energy barrier to rotation. This is evident in comparison of the MNDO-optimized structure of 4 and the model[20-22] where the two distances dependent on the position of the carboxyl oxygen atom are within 0.1 Å of one another.

If the Zn geometry assumed in the model[20-22] is used to place the zinc atom in the crystal structure conformation of 4, the separation between the zinc atom and the carboxyl oxygen is 1.64 Å longer than in the model. A similar discrepancy is found between the MNDO optimized structure of 4 and the model. The difference between the model and the crystal structure is undoubtedly due to the extended conformation observed in the solid state.

Thus, the observed molecular structures of ketoace and 4 do not fit the three models for the ACE active site. The difference arises mainly from the consistent, extended conformation found in the crystal as opposed to the folded conformation proposed in the models and from the incorrect zinc coordination geometry assumed in the models.

Even though the conformations of 4 and ketoace differ from the proposed active-site models, the separations between the zinc ligand, the carbonyl group, and the carboxyl group in these structures are similar to those found in other crystallographic studies of ACE inhibitors. For example, in ketoace the distances between the zinc ligand, O3; the hydrogen bonding atom, O6; and the C-terminal group centered at C9 are in remarkable agreement with the corresponding pharmacophore separations found in the crystal structures of captopril[40] and the substrate analogue hippuryl-L-histidyl-L-leucine, HHL.[35] The average difference between the pharmacophore distances in ketoace and captopril is less than 0.16 Å and between ketoace and the substrate analogue is 0.25 Å. Similarly, in the constrained captopril analogue (4), the distances between zinc ligand, S_1; the hydrogen bonding atom, O4; and the C-terminal group centered at C7 are in close agreement with the corresponding pharmacophore separations found in the

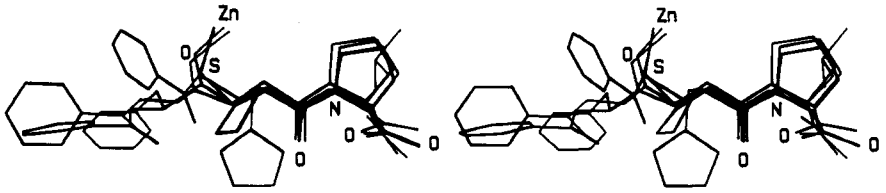


Figure 5. Stereographic view showing a superposition for the crystal structures of ketoace, compound 4, captopril, and HHL.

crystal structures of captopril, ketoace, and the ACE substrate (Table V). The average difference between the corresponding pharmacophore distances for 4 compared to captopril, ketoace, and the substrate analogue are 0.32, 0.19, and 0.26 Å, respectively. Thus, with the addition of these two new inhibitor structures, a consistent, extended conformation is found which differs from that proposed in the several ACE active-site models and suggests that alternative binding conformations should be considered.

To examine the similarity among the crystallographic conformations of the inhibitors, least-squares superpositions of the molecules were calculated, and the zinc atom position was predicted for the carbonyl ligands: ketoace and the ACE substrate analogue HHL. The zinc–carbonyl coordination geometry was taken from the mean values found in the CSD for the geometry of tetrahedrally coordinated Zn···O=C(C)$_2$ fragments; i.e. a Zn···O distance of 1.94 Å, a coordination angle Zn···O=C of 126°, and a trans planar arrangement of the Zn···O=C—C fragment. The carboxyl carbon atoms, the C-terminal amide carbonyl oxygen atoms and the zinc positions for the zinc–ketoace and zinc–HHL complexes were superimposed by least squares. For the two structures, the separations between the carboxyl carbon atoms, the carbonyl oxygen atoms, and the zinc positions were 0.288, 0.240, and 0.186 Å, respectively. A similar least-squares superposition of the crystallographic conformations of the two inhibitors of the sulfur ligand class, captopril, and compound 4 was done by fitting the zinc ligands rather than the zinc position since no conformational preference for the Zn···S–CH_2–C fragment was found by the database search. The separations between the carboxyl carbon atoms, the carbonyl oxygen atoms, and the sulfur atoms were 0.163, 0.218, and 0.065 Å, respectively.

Interactive computer graphics was used to search for an active-site geometry which would be consistent with both the crystallographic results for all four ACE binding molecules and the mean values for the zinc coordination geometries for carbonyl and sulfur ligands. To accomplish this, the Zn···S–CH_2–C fragment for captopril and inhibitor 4 was constructed using 2.32 Å for the coordination distance Zn···S and 97° for the coordination angle Zn···S–CH_2. The two pairs of molecules which had been separately superimposed, ketoace–HHL and captopril–4, were then graphically superimposed and only the torsion angle Zn···S–CH_2–C for the two sulfhydryl ligands was varied to optimize the overlap between the carboxyl carbon atoms, the amide carbonyl oxygen atoms, and the zinc positions. With the exception of this single torsion angle, the molecules were rigidly constrained to their crystallographic conformations. Figure 5 contains a stereodrawing of the result of this superposition. The average separation between the carboxyl carbon atoms, the amide carbonyl oxygen atoms, and the zinc position were 0.45, 0.25, and 0.35 Å, respectively (the maximum separations were 0.66, 0.42, and 0.44 Å, respectively). The torsion angle (Zn··· S–CH_2–C) was changed to optimize the overlap of the zinc positions in the sulfur ligands with the zinc atom position fixed by the preferred geometry for the carbonyl ligands; the angles obtained in the superposition were 4.6° for captopril and −25.7° for compound 4, these are reasonable when compared to the observed values shown in Figure 2. Clearly, the small differences in the pharmacophore positions listed above can be decreased by minor adjustments of the crystallographic conformations.

The successful superposition of these four crystallographic conformations and the close approach of the pharmacophores indicate that this extended conformation is not only a low energy form for ACE inhibitors but also a viable possible binding conformation. Implications of this conformation for active site modeling are under investigation.

In conclusion, crystallographic data indicate that zinc coordination geometry can be well-defined in terms of bond distance and bond angle but that the conformation of the Zn···S–CH_2–C fragment is variable. The crystallographic values for zinc geometry differ from those used in ACE active site models. The crystal structures of ketoace and 4 show an extended conformation consistent with the structures of captopril and a substrate analogue. The crystallographic conformations can be superimposed with a close approach of the three pharmacophore points: carbonyl oxygen, carboxylate oxygen, and zinc atom. The results provide an alternative conformation for ACE inhibitors that should be considered in active site modeling studies.

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Supplementary Material Available: REFCODES from the CSD search, and anisotropic thermal parameters for the nonhydrogen atoms, positional and isotropic parameters for the hydrogen atoms, and lists of bond distances, bond angles, and torsion angles for 3 and 4 (17 pages); structure factors for 4 (9 pages). Ordering information is given on any current masthead page.