Notes

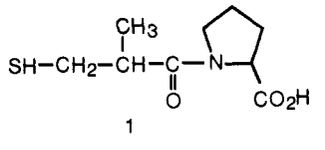
Conformational Analysis and Active Site Modelling of Angiotensin-Converting Enzyme Inhibitors

P. R. Andrews,*† J. M. Carson,‡ A. Caselli,† M. J. Spark,† and R. Woods§

School of Pharmaceutical Chemistry, Victorian College of Pharmacy Ltd, Parkville 3052, The Basin Medical Centre, The Basin 3154, and the Institute of Drug Technology Ltd, Parkville 3052, Australia. *Received April 27, 1984*

The discovery of captopril as a potent, orally active inhibitor of angiotensin-converting enzyme (ACE) led to the recent development of many series of novel structures with similar biological activity. To date, however, all of these inhibitors are flexible or semiflexible molecules, and there is therefore no clear definition of the conformational requirements for ACE inhibition. In an effort to solve this problem, we have carried out conformational energy calculations on a series of eight structurally diverse ACE inhibitors. Comparison of the low-energy conformations available to these molecules leads to the conclusion that there is a common low-energy conformation throughout the series. The calculations thus define the structural and conformational requirements for ACE inhibition. Expansion of this model to the receptor level has been achieved by considering possible alternative receptor sites for each of the molecules in its proposed biologically active conformation and leads to an active-site model for ACE which may be useful for the design of further inhibitors.

Useful antihypertensive activity has recently been demonstrated in the potent angiotensin-converting enzyme inhibitors captopril (**1**)[1] and enalapril (**2**).[2] In a series of structure–activity studies,[3-5] Ondetti et al. have established the importance of the terminal carboxyl, the amide carbonyl, and a zinc-binding function (thiol in **1** carboxyl in the active form of **2**) for the interaction of ACE inhibitors with the active site. Hassall et al.[6] and Thorsett et al.[7] have also used conformational analysis of specific inhibitors as an aid in the design of new ACE inhibitors. As yet, however, no conformational analyses of a wider range of ACE inhibitors have appeared in the literature, and no three-dimensional model of the active site is therefore available.



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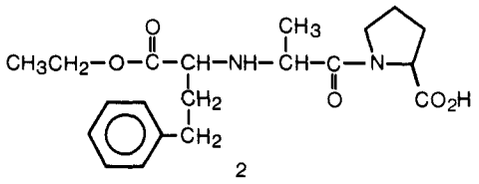

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In this paper we have used classical potential energy calculations to obtain complete conformational analyses of each of the eight ACE inhibitors listed in Table I. These data, together with the activities of a number of semirigid analogues, are shown to be sufficient to define the structural and conformational requirements for binding to the active site of angiotensin-converting enzyme.

Experimental Section

The set of torsion angles which specify the conformation of each of the inhibitors studied is indicated in Table I and further defined with respect to the biologically active form of enalapril, MK422 (**7**), in Figure 1. The torsion angles are defined by clockwise rotations around the appropriate bonds according to the convention of Klyne and Prelog.[8] Calculations were based on molecular geometries obtained from the crystal structures of these and related molecules,[9] supplemented where necessary with bond lengths and angles from standard compilations.[10]

Calculations were performed on a Cyber 73 computer at the Royal Melbourne Institute of Technology. The program COMOL[11] was used to perform classical potential energy calculations by pairwise summation of the van der Waals interactions between nonbonded atoms, together with electrostatic and torsional potentials. The parameterization, which was developed by Giglio[12] on the basis of hydrocarbon and amide structures, has been used in our laboratory to study a number of systems of biological interest.[13-15] The calculations were carried out at fixed values

† Victorian College of Pharmacy Ltd.
‡ The Basin Medical Centre.
§ Institute of Drug Technology Ltd.

(1) M. A. Ondetti, B. Rubin, and D. W. Cushman, *Science*, **196**, 441 (1977).
(2) A. A. Patchett, E. Harris, E. W. Tristram, M. J. Wyvratt, M. T. Wu, D. Taub, E. R. Peterson, T. J. Ikeler, J. ten Broeke, L. G. Payne, D. L. Ondeyka, E. D. Thorsett, W. J. Greenlee, N. S. Lohr, R. D. Hoffsommer, H. Joshua, W. V. Ruyle, J. W. Rothrock, S. D. Aster, A. L. Maycock, F. M. Robinson, and R. Hirschmann, *Nature (London)* **288**, 280 (1980).
(3) D. W. Cushman and M. A. Ondetti in "Progress in Medicinal Chemistry", G. P. Ellis and G. B. West, Eds., Elsevier, Amsterdam, 1980, Vol. 17, p 41.
(4) E. W. Petrillo and M. A. Ondetti, *Med. Res. Rev.* **2**, 1 (1982).
(5) M. E. Condon, E. W. Petrillo, Jr., D. E. Ryono, J. A Reid, R. Neubeck, M. Puar, J. E. Heikes, E. F. Sabo, K. A. Losee, D. W. Cushman, and M. A. Ondetti, *J. Med. Chem.*, **25**, 250 (1982).
(6) C. H. Hassall, A. Krohn, C. J. Moody, and W. A. Thomas, *FEBS Lett.*, **147**, 175 (1982).
(7) E. D. Thorsett, E. E. Harris, S. Aster, E. R. Peterson, D. Taub, and A. A. Patchett, *Biochem. Biophys. Res. Commun.* 111, 166 (1983).
(8) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).
(9) F. H. Allen, S. Bellard, M. D. Brice, B. A. Cartwright, A. Doubleday, H. Higgs, T. Hummelink-Peters, O. Kennard, W. D. S. Motherwell, J. R. Rodgers, and D. G. Watson, *Acta Crystallogr., Sect. B*, **35B**, 2331 (1979).
(10) L. E. Sutton, Ed., "Tables of Interatomic Distances", Special Publication No. 11 and 18, The Chemical Society, Burlington House, London, 1958 and 1965.
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(12) E. Giglio, *Nature (London)*, **222**, 339 (1969).
(13) G. P. Jones and P. R. Andrews, *J. Med. Chem.*, **23**, 444 (1980).
(14) L. Pierri, I. H. Pitman, I. D. Rae, D. A. Winkler, and P. R. Andrews, *J. Med. Chem.*, **25**, 937 (1982).

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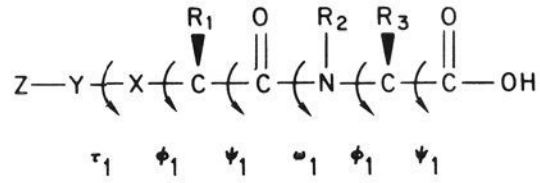
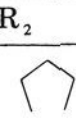
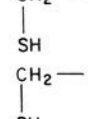
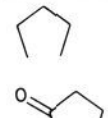
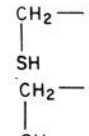
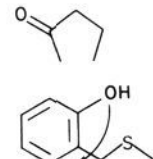
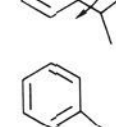
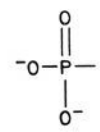
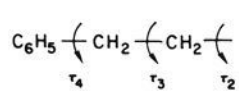
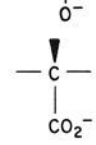
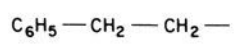
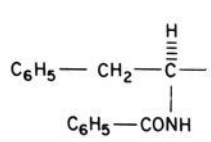
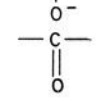
Table I. Structures, Activities, and Conformational Variables of ACE Inhibitors Studied



compd	Z	Y	X	R_1	R_2	R_3	I_{50},[a] M
captopril (1)[b]				CH_3			2×10^{-8}
5-oxocaptopril (3)[b]				CH_3			9×10^{-9}
SA446 (4)[c]				H			6.5×10^{-8}
WY44221 (5)[d]				CH_3			3.7×10^{-9}
6[e]				CH_3			1.4×10^{-9} [f]
MK422 (7)[g]				CH_3			1.2×10^{-9}
8[e]				CH_3			0.5×10^{-9} [f]
9[h]				H			3.2×10^{-9}

[a] Reference 4. [b] Reference 5. [c] J. Iwao, M. Oya, E. Kato, Y. Kawashima, H. Masuda, T. Iso, and T. Chiba, Brit. U.K. Patent Appl. 2 027 025. [d] D. H. Kim, C. J. Guinesso, G. C. Buzby, D. R. Herbst, R. J. McCaully, T. C. Wicks, and R. L. Wendt, *J. Med. Chem.*, **26**, 394 (1983). [e] R. E. Galardy, V. Kontoyiannidou-Ostrem, and Z. P. Kortylewicz, *Biochemistry* **22**, 1990 (1983). [f] K_i value. [g] Reference 2. [h] R. F. Meyer, E. D. Nicolaides, F. J. Tinney, E. A. Lunney, A. Holmes, M. L. Hoefle, R. D. Smith, A. D. Essenburg, H. R. Kaplan, and R. G. Almquist, *J. Med. Chem.*, **24**, 964 (1981).

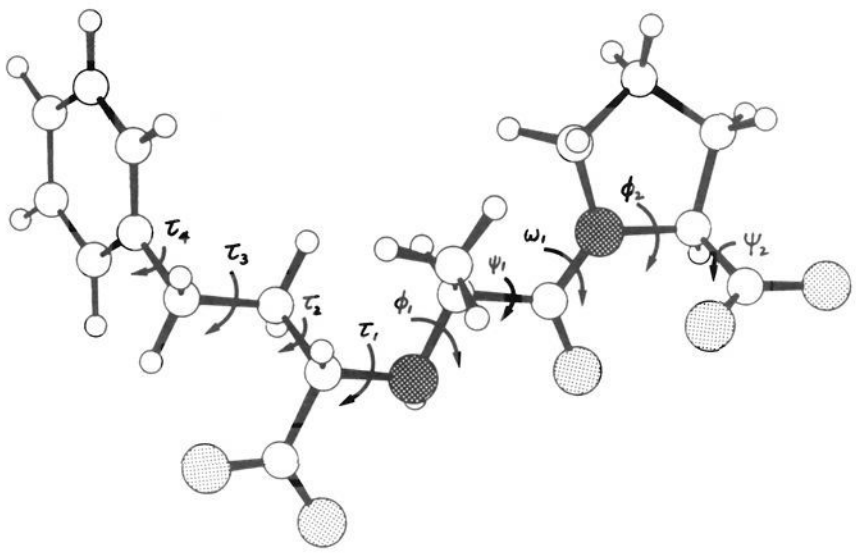


Figure 1. Conformational variables for the molecules under study defined with respect to MK422 (**7**). Torsion angles are defined by clockwise rotations of backbone atoms around the appropriate bonds; in this illustration $\psi_2 = \omega_1 = \psi_1 = \phi_1 = \tau_2 = \tau_3 = 180°$, $\phi_2 = -63°$, $\tau_1 = 60°$ and $\tau_4 = 90°$. Light and dark shadings represent oxygen and nitrogen atoms, respectively.

of all bond lengths and bond angles, ignoring electrostatic charges. It was shown that these approximations have no effect on the qualitative nature of the results but have the great advantage of allowing very rapid determination of all alternative biologically active conformations. We stress, however, that relaxation of nontorsional degrees of freedom reduces both the energy differences and the rotational barriers between alternative conformations. The quantitative energy differences therefore should not be used, for example, to calculate relative conformer populations.

For each molecule, the conformational variables were considered either two or three at a time, and the potential energy surfaces calculated with use of rotation intervals of 10° for each variable. In molecules with more than three consecutive conformational variables the interactions between variables were determined by considering overlapping groups of torsion angles. In the case of MK422 (Figure 1), for example, three-dimensional energy surfaces were computed for (ψ_1, ϕ_1, τ_1), (ϕ_1, τ_1, τ_2), (τ_1, τ_2, τ_3), and (τ_2, τ_3, τ_4), as well as ψ_2 and ω_1. The results were plotted as potential energy maps by using a modification of the contouring program KONTOR.[16]

Molecular comparisons and superimpositions were performed by using the Victorian College of Pharmacy Ltd molecular modelling system MORPHEUS,[17] which minimizes the sum of the squares of the distances between corresponding atoms in the two or more molecules being compared.

Results and Discussion

Calculations were done for all possible geometric and optical isomers of each of the molecules in Table I, but detailed results are reported here only for the most active isomers. For simplicity, the results and discussion will be presented in terms of the conformational preferences of the four groups most clearly involved in binding to the active site, viz., the carboxyl terminal, the amide carbonyl, the zinc-binding function, and the aromatic binding group.

(15) P. R. Andrews, L. C. Mark, D. A. Winkler, and G. P. Jones, *J. Med. Chem.*, **26**, 1223 (1983).
(16) J. A. B. Palmer, *Aust. Comp. J.*, **2**, 27 (1970).
(17) P. R. Andrews and E. J. Lloyd, *Med. Res. Rev.*, **2**, 355 (1982).

Table II. Relative Energies of Cis and Trans Isomers

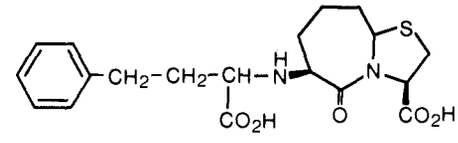
compd	rel energy,[a] kcal/mol		compd	rel energy,[a] kcal/mol	
	cis	trans		cis	trans
1	2.1	0	6	4.8	0
3	16.9	0	7	2.3	0
4	0	2.1	8	4.7	0
5	0	3.6	9	1.6	0

[a] Calculated for lowest energy conformation of each isomer but without full geometry optimization. Experimental differences should be smaller but of the same relative magnitudes.

For this purpose, the eight molecules in Table I will be considered as a group, with attention being focused in turn on each of the conformational variables.

Carboxyl Terminal (ψ_2, ϕ_2). In all of the compounds being studied, there is virtually free rotation of the carboxyl group (ψ_2, <5 kcal/mol), and rotation around ϕ_2 is restricted by the proline ring to the region around −60°. The importance of the orientation of the carboxyl group is illustrated by the relative activities[4] of the *R* (ϕ_2 = 67°, I_{50} = 0.2 μM) and *S* (ϕ_2 = −67°, I_{50} = 1800 μM) isomers of desmethylcaptopril and that of its dehydro derivative (ϕ_2 = 0°, I_{50} = 0.65 μM). In general, it appears that a ϕ_2 value in the range −45° ± 45° is required for activity.

Amide Carbonyl Group (ω). The potential for cis–trans isomerism of the peptide bond means that the carbonyl group may adopt two alternative orientations relative to the carboxyl group. Indeed, this situation has already been observed experimentally for several inhibitors,[5,18] although no clear correlation between inhibitory potencies and relative cis–trans populations was observed.[5] The relative energies of the cis and trans forms, given in Table II, favor the trans structure for all but **4** and **5**, in which repulsive interactions between the aromatic groups and the α_1 carbon substituents make the trans configuration marginally less stable. The energy data thus imply that the trans structure is the biologically active form, and this is confirmed by the fixed trans configuration in two potent semirigid ACE inhibitors recently synthesized at Merck[19] (**10**, $I_{50} = 6 \times 10^{-10}$ M) and Ciba-Geigy[20] (**11**, $I_{50} = 3 \times 10^{-9}$ M).



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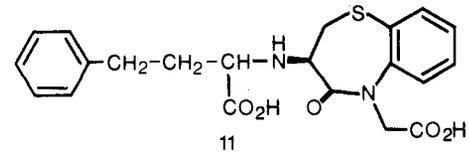

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Zinc-Binding Function (ψ_1, ϕ_1, τ_1). For captopril (**1**) and its thiol analogues **3**–**5**, the orientation of the zinc-binding sulfur atom is defined solely by ψ_1 and ϕ_1. Contour maps of ψ_1 vs. ϕ_1 for **1**, **3**, and **4** are given in Figure 2. The corresponding map for **5** is intermediate between those for **1** and **3**.

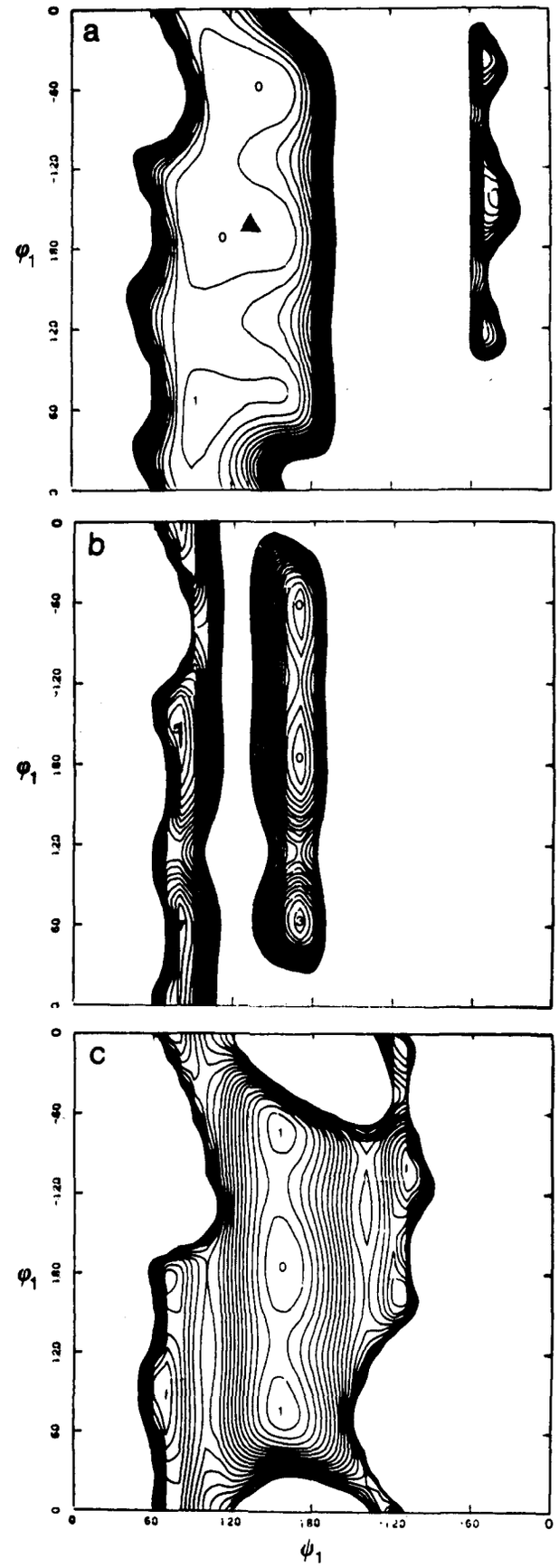


Figure 2. Conformational energy maps of ψ_1 vs. ϕ_1 for captopril (a) and its analogues **3** (b) and **4** (c). The first 20 2.5 kcal/mol contour intervals are shown and the relative energies of the global and secondary minima are as indicated. The conformation adopted by captopril in the single crystal (ref 28) is as marked (▲); a similar conformation is found for captopril when bound to penicillopepsin (ref 28).

(18) R. G. Almquist, W-R. Chao, M. E. Ellis, and H. L. Johnson, *J. Med. Chem.*, **23**, 1392 (1980).
(19) M. J. Wyvratt, M. H. Tischler, T. J. Ikeler, J. P. Springer, E. W. Tristram, and A. A. Patchett, *Proc. 8th Am. Peptide Symp.*, 551 (1983).
(20) G. Mazzenga, D. Ben-David, and J. Stanton, private communication.

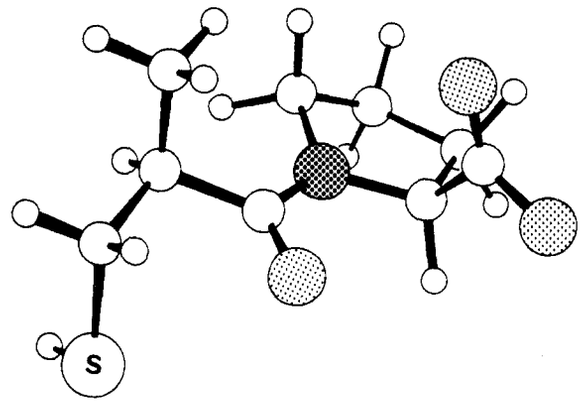
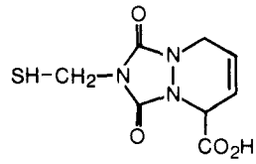


Figure 3. The proposed biologically active conformation of captopril: $\psi_1 = 165°$, $\phi_1 = 300°$.

The map for captopril (Figure 2a) shows good agreement with crystallographic data for the conformation of 1 in the single crystal or when bound to the aspartyl protease penicillopepsin. It is clear from Figure 2, however, that ψ_1 must fall near either 80° or 165° to accommodate each of these inhibitors, and in the case of 4, the energy difference is sufficient to indicate that $\psi_1 \simeq 165°$ is the active form. The same conclusion can be drawn from the semirigid analogues 10 and 11, both of which can adopt, among others, a conformation of $\psi_1 = 165°$, but neither of their active stereoisomers can approach $\psi_1 = 80°$. A similar conclusion has been reached by Thorsett et al.,[21] who suggest a biologically active range of 130–170° for ψ_1 in a series of conformationally restricted lactams. It should be noted, however, that the conformational data reported by these workers appear to apply only to the *S* isomers, whereas the biological data refer to the racemic mixtures. Inclusion of the mirror image *R* isomers in the data set shows that the less active compounds can also attain ψ_1 values near 130–140°, thus implying that the conformations needed for activity are nearer $\psi_1 = 160$–170°, as suggested above.

The ψ_1–ϕ_1 maps also indicate that ϕ_1 in captopril and its analogues can fall near 60°, 180°, or 300°. Of these, the conformation with ϕ_1 near 60° is somewhat higher in energy, at least in 3, and that with $\phi_1 = 180°$ is virtually excluded by the observed activity of another bicyclic captopril analogue 12,[6] in which $\phi_1 = 180°$ is significantly less stable. It can thus be inferred that the biologically active conformation of captopril is most likely to be that in which $\psi_1 \simeq 165°$ and $\phi_1 \simeq 300°$ (Figure 3).



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Further definition of the zinc-binding site may be obtained by fixing ψ_1 in the remaining compounds, 6–9, at 165°, which is invariably a low-energy energy conformation, while ϕ_1 and τ_1 are varied. The resulting ϕ_1–τ_1 maps for 7–9 are shown in Figure 4; the results for 6 are almost identical with those for 8. As indicated in these maps, there are only relatively few orientations of the zinc-binding groups accessible to these molecules, and superimposition of the alternatives on the proposed active

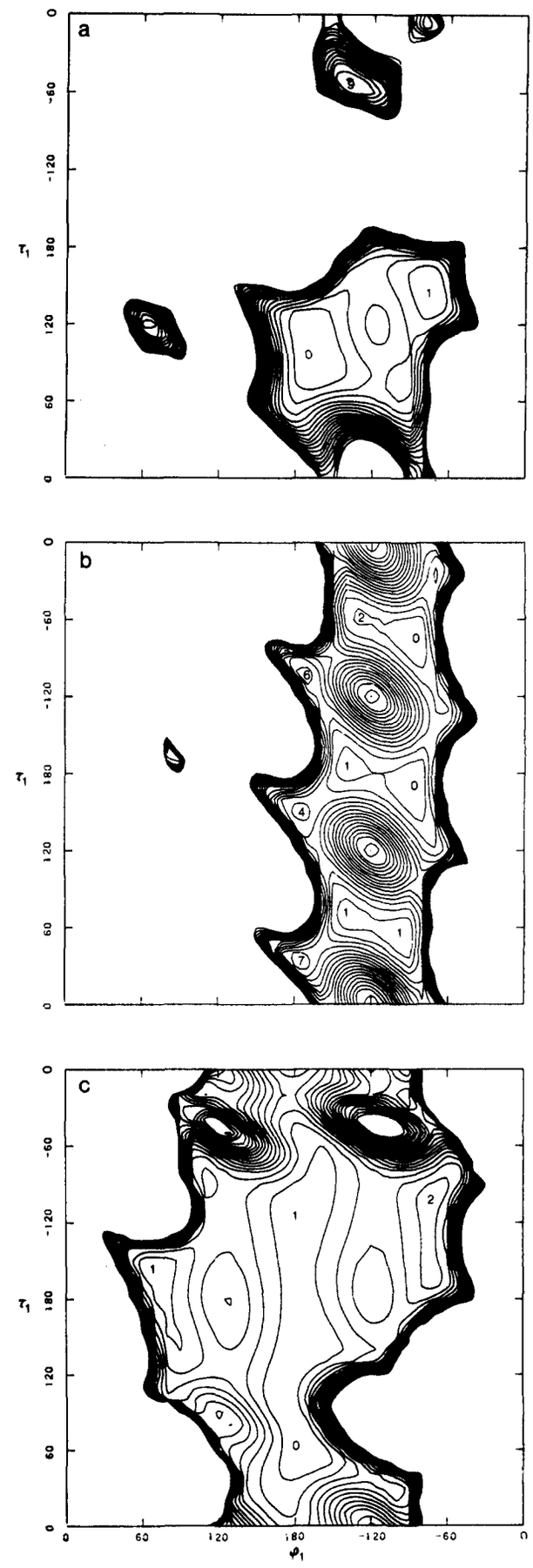


Figure 4. Contour maps obtained for rotations around ϕ_1 and τ_1 in (a) MK422, (b) 8, (c) 9. The contour interval is 2.5 kcal/mol and the first 20 contour lines are shown. The relative energies of the global and secondary minima are as indicated.

(21) E. D. Thorsett, E. E. Harris, S. D. Aster, E. R. Peterson, E. W. Tristram, J. P. Snyder, J. P. Springer, and A. A. Patchett, *Proc. 8th Am. Peptide Symp.*, 555 (1983).

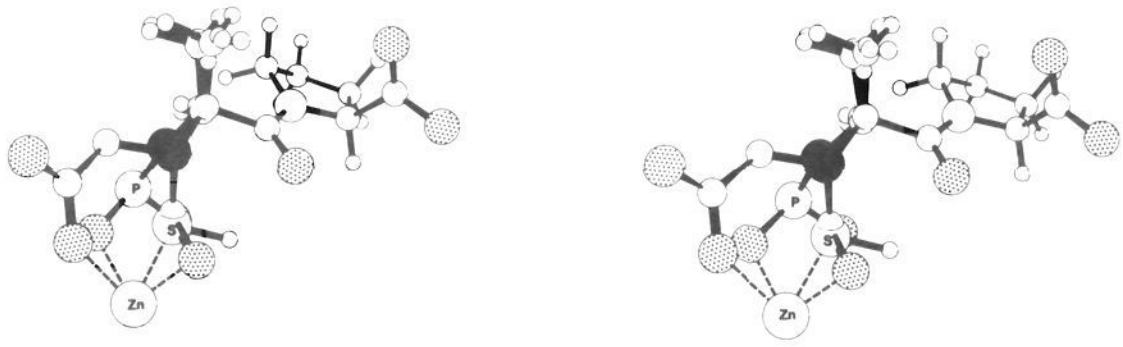


Figure 5. Stereoscopic view of the proposed interaction of inhibitors **1**, **6**, **7**, and **9** with the zinc atom in the active site of ACE. The groups beyond the zinc-binding region in **7** and **9** have been removed for clarity.

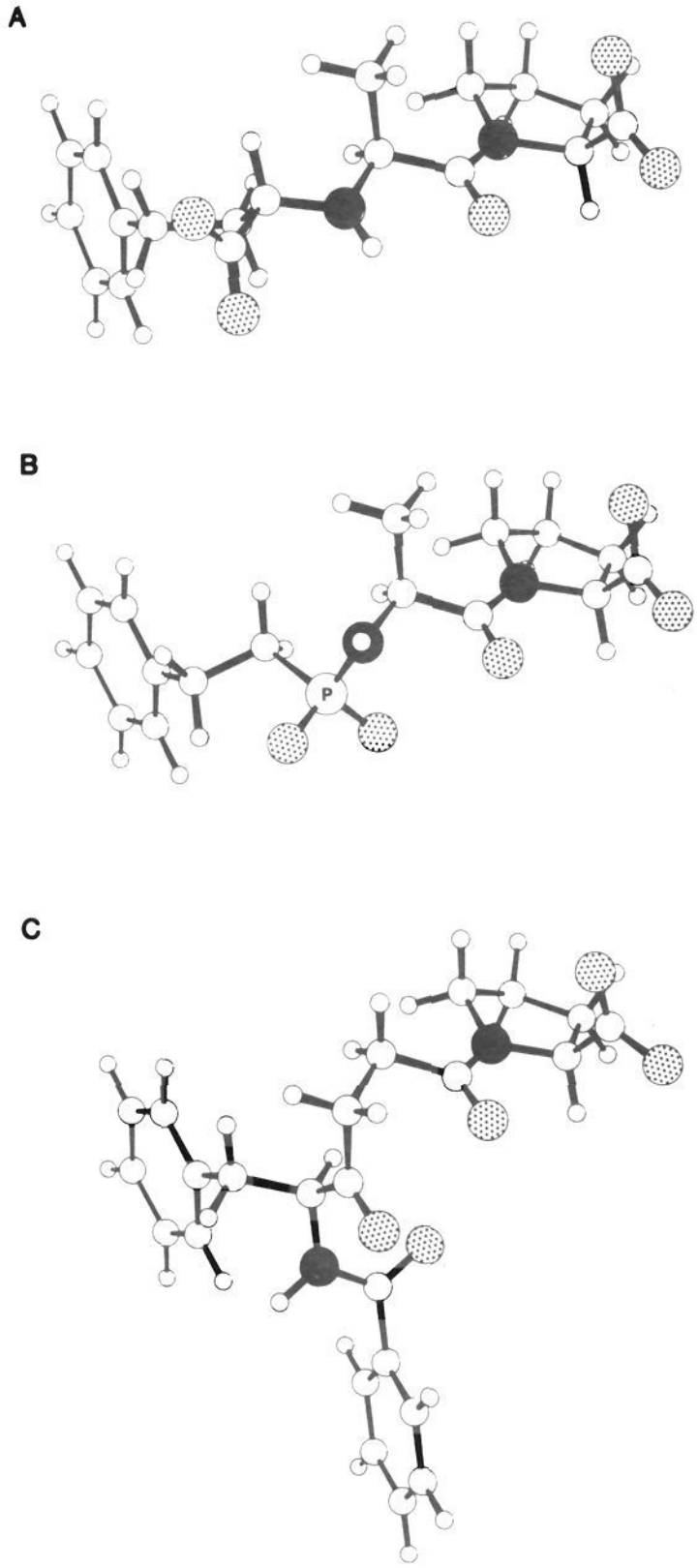


Figure 6. The proposed biologically active conformations of (a) MK422 (**7**), (b) **8**, (c) **9**. Light and dark shadings represent oxygen and nitrogen atoms, respectively.

conformation of captopril pins down the likely position of the zinc atom in the active site, as shown in Figure 5. The geometric variables defining the zinc atom with respect to each of the binding groups are given in Table III. As is evident from the figure, the range of torsion angles indicates that the orientation of the zinc-binding function differs somewhat in the various inhibitors. This is consistent with the situation observed crystallographically in the related enzyme thermolysin, where zinc-binding ligands form both tetracoordinate and pentacoordinate complexes

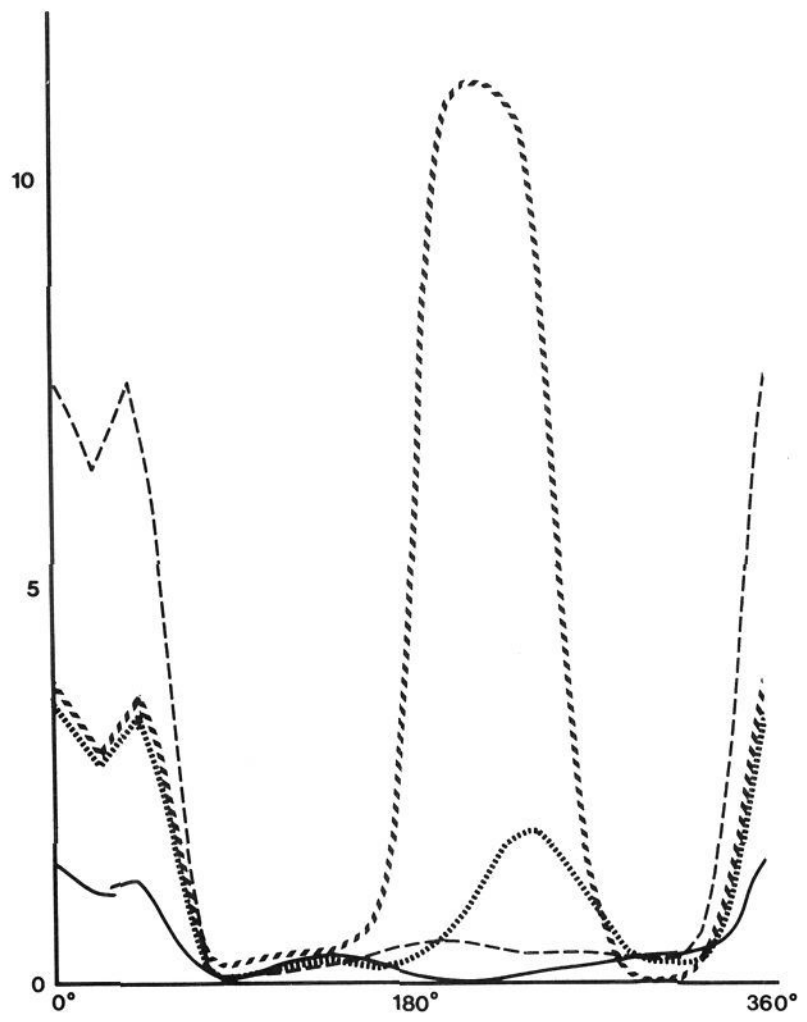


Figure 7. Potential energy curves (in kcal/mol) calculated for rotation of the amide carbonyl binding site around the C=O axis in representative inhibitors: **1** (■ ■ ■), **7** (- - -), **8** (–), **9** (//).

to zinc over a similar range of approach angles.[22] The bond lengths and bond angles associated with the interactions are also reasonably consistent with those expected for zinc–ligand complexes, although the Zn–O–C angle in **9** is unusually acute. We would anticipate that further improvements in these interactions would follow relatively minor changes in the active site conformation to accommodate individual inhibitors.

Aromatic Binding Site (τ_2, τ_3, τ_4). The conformation of the phenethyl group in compounds **7** and **8** is defined by a broad potential energy minimum centered on τ_2 = 180–240°, τ_3 = 180°, and τ_4 = ±90°. For compound **9** the benzoylamino group, which favors the trans configuration, limits the phenethyl group to a narrower conformational range with τ_2 near ±90°, τ_3 = 60–180°, and τ_4 = ±90°.

Superimposition of these various low-energy conformations of **7**–**9** leads to a unique arrangement of the phenyl ring which is common to all three molecules. This common model thus defines the probable biologically active conformations for each of the major classes of angiotensin-converting enzyme inhibitors, as illustrated in Figures 3 and 6.

(22) M. A. Holmes and B. W. Matthews, *Biochemistry*, **20**, 6912 (1981).

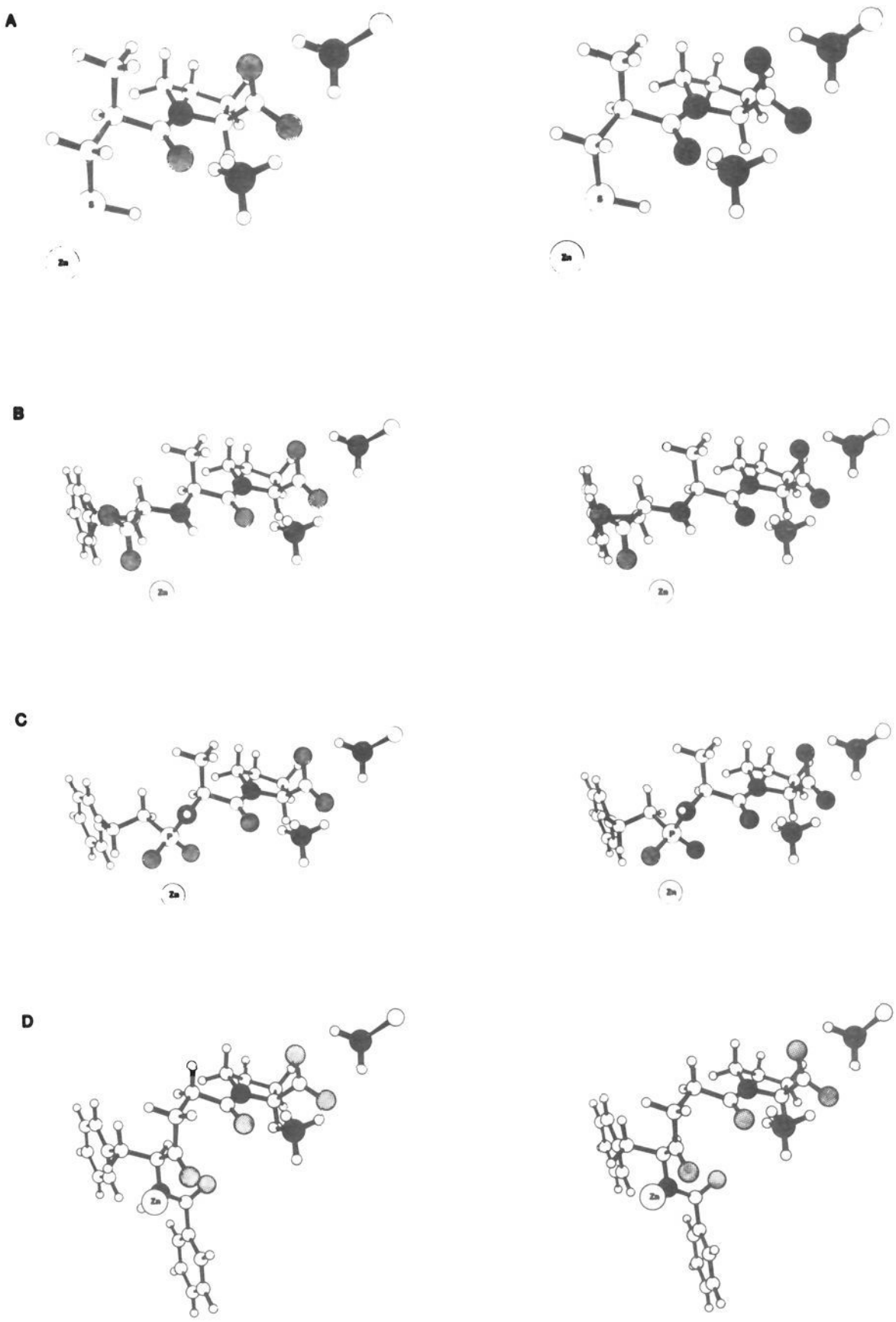


Figure 8. Stereoscopic views of the representative ACE inhibitors binding in the postulated active site structure (a) **1**, (b) **7**, (c) **8**, (d) **9**. The active site is represented by an onium group (top right foreground) which interacts with the carboxyl terminal, a hydrogen bonding group (lower center foreground) which interacts with the carbonyl group and the zinc atom. The aromatic binding site is not shown but implied by the common location of the phenethyl groups in **7**, **8**, and **9**.

Active-Site Mapping. Identification of the precise location of the terminal carboxyl binding group is hampered by the lack of any significant conformational restriction on rotation of the terminal carboxyl group and the lack of any other groups which might sterically interfere with its placement. This means that the carboxyl binding group, which is probably an arginine guanidinium ion,[4] could lie almost anywhere on a roughly hemispherical surface, placing a protonated nitrogen approximately 2.8 Å from either or both carboxyl oxygen atoms.

The binding site for the amide carbonyl is more restricted; the results of potential energy calculations on the placement of this group in **1**, **7**, **8**, and **9**, assuming an O···X distance of 2.8 Å and C=O···X angle of 150°, are summarized in Figure 7. They show that the only regions which accommodate all the inhibitors are those with N—C=O···X torsion angles near 100° or 300°. Of these, the region near 100°, which lies on the same side of the proline ring as the carboxyl binding group and is less spatially restricted, seems more probable.

The remaining two binding groups are the zinc atom, whose location is reasonably tightly specified by the data above (Figure 5, Table III), and the aromatic binding site. As is evident from Figure 6, the regions on either side of the aromatic rings in inhibitors **7**–**9** are relatively free of potential steric hindrance. This suggests that the aromatic groups of these inhibitors may be accommodated in a hydrophobic binding pocket similar to that observed by X-ray crystallography[23,24] in the related enzymes thermo-

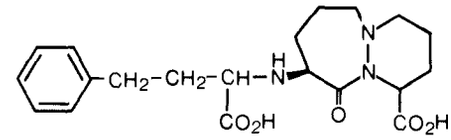
(23) W. R. Kester and B. W. Matthews, *J. Biol. Chem.*, **252**, 7704 (1977).
(24) A. F. Monzingo and B. W. Matthews, *Biochemistry* **21**, 3390 (1982).

Table III. Geometric Parameters Defining Position of Zinc Atom Relative to Zinc-Binding Functions

compd	bond length Zn–O,S, Å	bond angle, deg	torsion angle, deg
1, 3–5	2.0	124 (Zn, S, C)	180 (Zn, S, C, C)
6, 8	1.8	111 (Zn, O, P)	−96 (Zn, O, P, N)
7	2.0	138 (Zn, O, C)	17 (Zn, O, C, C)
9	1.7	97 (Zn, O, C)	120 (Zn, O, C, C)

lysin and carboxypeptidase A.

These features are summarized in Figure 8, which shows the four structurally distinct ACE inhibitors, 1, 7, 8, and 9, binding to a three-dimensional model of the active site. It should be stressed that this model is not a totally unique interpretation of the data: in particular, the alternative values of ϕ_1 in captopril (60°, 180°) have been excluded on the basis of relatively small energy differences, and the locations of the carboxyl and carbonyl binding groups are not yet tightly defined. We believe, however, that the obvious agreement between the conformational and orientational requirements for the four major binding groups in these different classes of ACE inhibitors, as illustrated in Figure 8, supports the proposed model, which provides a simple template for the design of further conformationally restricted analogues. A similar conclusion has been reached by Hassall and co-workers,[25,26] who have used the activities of a series of bicyclic analogues related to **12** to design the potent new inhibitor **13** ($I_{50} = 6 \times 10^{-10}$ M), and



13

by Tute, who has derived a very similar model[27] by fitting captopril and related inhibitors to the observed crystal structure of carboxypeptidase A.

(25) M. R. Attwood, R. J. Francis, C. H. Hassall, A. Krohn, G. Lawton, I. L. Natoff, J. S. Nixon, S. Redshaw, and W. A. Thomas, *FEBS Lett.*, **165**, 201 (1984).
(26) C. H. Hassall, A. Krohn, C. J. Moody, and W. A. Thomas, *J. Chem. Soc., Perkin Trans. 1*, 155 (1984).
(27) M. S. Tute, private communication.
(28) M. Fujinaga and M. N. G. James, *Acta Crystallogr., Sect. B*, **B36**, 3196 (1980).

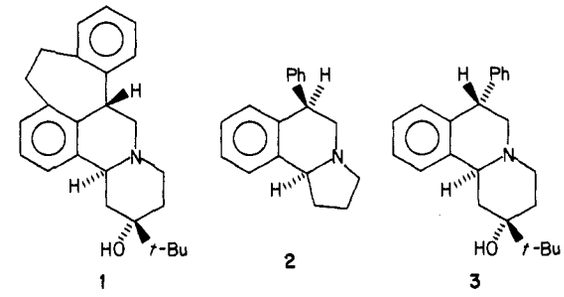
The pK_a of Butaclamol and the Mode of Butaclamol Binding to Central Dopamine Receptors

Frank A. Chrzanowski, Beth A. McGrogan, and Bruce E. Maryanoff*

Department of Chemical Research and Section of Basic Pharmaceutics, McNeil Pharmaceutical, Spring House, Pennsylvania 19477. *Received June 4, 1984*

The pK_a values for butaclamol (1), 1,2,3,5,6,10bβ-hexahydro-6α-phenylpyrrolo[2,1-*a*]isoquinoline (2, McN-4612-Y), and 2-*tert*-butyl-1,3,4,6,7,11bβ-hexahydro-7β-phenyl-2*H*-benzo[*a*]quinolizin-2α-ol (3, McN-4171) were determined to be 7.2, 9.1, and 7.0, respectively. The values for 1 and 3 are anomalous; however, the value for 1 (7.2) is not as low as the one reported in the literature (pK_a = 5.9). We also determined pK_a values for apomorphine, chlorpromazine, and lidocaine, for reference purposes (7.6, 9.2, and 7.9, respectively). The results indicate that 1 would not be predominantly unprotonated under the physiological conditions of receptor binding, rather it would be about 50% protonated. This fact may contravene a suggested binding model used to map the central dopamine receptor (viz., ref 3).

Butaclamol (1) is a dopamine receptor antagonist and a potent antipsychotic agent,[1] which has shown clinical activity.[2] The compound possesses a rather rigid molecular geometry and exhibits high, enantioselective affinity for central dopamine receptors.[1] The limited conformational flexibility of 1 has established it and its analogues as agents for the "mapping" of dopamine receptors in the central nervous system (CNS).[3] In interacting with the dopamine receptor, it is presumed that the nitrogen atom would be a relatively important binding site. Indeed, the nitrogen has been viewed[3] as the primary site for 1 and its congeners. However, one needs to ask a question that can be critical to specification of receptor geometry: Is the nitrogen more likely to be in the free-base or protonated state?



1 **2** **3**

In considering this key question, Philipp et al.[3b] concluded that since butaclamol has a pK_a of 5.9, compared to a pH of 7.3 for homogenized rat caudate nucleus, the butaclamol ligand exists almost exclusively in the free-base (unprotonated) form during the binding event. Thus, they excluded ligand–receptor binding based on ionic interaction via a charged, protonated species in favor of binding via a hydrogen bond between the nitrogen lone pair of electrons and the receptor. This selection was a crucial one in their establishment of geometric parameters for receptor mapping.

More recently, Froimowitz and Matthysse have tried to rationalize the "anomalously low pK_a for butaclamol" on the basis of unfavorable geometries for ion solvation with protonated butaclamol.[4] Specifically, it was suggested that the strong preference of protonated 1 for a cis conformation (i.e., a cis D–E ring fusion) gives rise to its high acidity,

(1) (a) Voith, K.; Herr, F. *Psychopharmacologia* **1975**, *42*, 11. (b) Bruderlein, F. T.; Humber, L. G.; Voith, K. *J. Med. Chem.* **1975**, *18*, 185. (c) Seeman, P. *Pharmacol. Res.* **1980**, *32*, 230. (d) Kukla, M. J.; Bloss, J. L.; Brougham, L. R. *J. Med. Chem.* **1979**, *22*, 401 and ref 5–12 cited therein.
(2) (a) Inaz, F.; Ban, T. A.; Lehmann, H. E. *Psychopharmacol. Bull.* **1976**, *12*, 31. (b) Nestoros, J. N.; Lehmann, H. E.; Ban, T. A. *Int. Pharmacopsychiatry* **1978**, *13*, 138. (c) Clark, M. L.; Paredes, A.; Costiloe, J. P.; Wood, F. *J. Clin. Pharmacol.* **1977**, *17*, 529.
(3) (a) Humber, L. G.; Bruderlein, F. T.; Philipp, A. H.; Götz, M.; Voith, K. *J. Med. Chem.* **1979**, *22*, 761. (b) Philipp, A. H.; Humber, L. G.; Voith, K. *Ibid.* **1979**, *22*, 768.

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