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(32) NIH Postdoctoral Fellow, 1974–1975.

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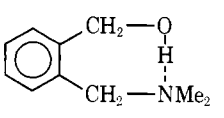
o-(Dimethylaminomethyl)benzyl Alcohol. An Improved Model for Serine Esterases That Is Internally Hydrogen Bonded in Aqueous Solution[1]

Sir:

The action of acetylcholine esterase, α-chymotrypsin, and several other enzymes apparently involves attack on a substrate carbonyl group by a serine hydroxyl group that is hydrogen bonded to an imidazole nitrogen atom from a histidine residue.[2-6] Similar mechanisms have been suggested for cleavage of *p*-nitrophenyl acetate[7,8] and other active esters[8] in the presence of various amino alcohols. These amino alcohols are better catalysts than they would be expected to be as amines and give esters as reaction intermediates. Similar evidence for 3-quinuclidinol, where internal hydrogen bonding between hydroxy and amino groups is sterically impossible, shows that attack by the zwitterion form of the amino alcohol can be important.[9] However, catalytic activities of several tertiary-amino alcohols were estimated to be 1.2–6 times as large as expected from a zwitterion mechanism.[8] We have now found that *o*-(dimethylaminomethyl)benzyl alcohol (**1**) is internally hydrogen bonded in aqueous solution and is particularly effective, for its basicity, at cleaving *p*-nitrophenyl acetate via internal amine-assisted attack by its hydroxylic oxygen atom.

o-(Dimethylaminomethyl)benzylamine, a bifunctional catalyst for dedeuteration of acetone-d_6,[10] is about 15 times as basic as would be expected from data on benzylamine, *N,N*-dimethylbenzylamine, and other reference compounds.[11] This increased basicity is evidence for stabilization of the monoprotonated form of the amine (**2**) by internal hydrogen



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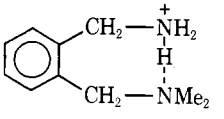

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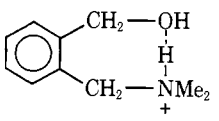
bonding. Such internal hydrogen bonding in **1** should decrease its basicity, but this decrease could be offset by stabilization of the conjugate acid of **1** (**3**) by internal hydrogen bonding. The internal hydrogen bonding suggested for **1** would be impossible for the corresponding methyl ether, but hydrogen bonding of the type shown for **3** would be possible for the protonated methyl ether. The decreased basicity of alcohol **1** relative to its methyl ether (see Table I) is therefore evidence for internal hydrogen bonding in **1**.[12]



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N,N-Dimethylbenzylamine and especially *o*-(dimethylaminomethyl)benzyl methyl ether were taken as models for the catalytic activity to be expected from the tertiary amino group of **1**. Table I shows that at ionic strength 0.20, where the greater solubility of the amines permits more reliable rate constants to be obtained, **1** is more than 100 times as good a catalyst for the decomposition of *p*-nitrophenyl acetate as either of the two models (in spite of the greater basicity of the models).

Table I. Catalysis of the Decomposition of *p*-Nitrophenyl Acetate in Water at 25 °C

Catalyst	Ionic strength	$100k$,[a] $M^{-1}\ s^{-1}$	pK
o-$Me_2NCH_2C_6H_4CH_2O$-H	1.00	4.90 (0.06)	8.77
	0.20	5.05 (0.08)	
o-$Me_2NCH_2C_6H_4CH_2O$-Me	1.00	<0.1[b]	9.09
	0.20	<0.03[b]	
$C_6H_5CH_2NMe_2$	1.00	<0.03[b]	9.03
	0.20	0.034 (0.019)	
o-$Me_3NCH_2C_6H_4CH_2O$-H^+ + OH^-	1.00	10200 (900)[c]	

[a] The parenthesized numbers are estimated standard deviations. In each case the reaction was shown to be first order in catalyst by varying the catalyst concentration by at least tenfold. [b] These are estimated upper limits; the least-squares values were slightly negative. [c] $M^{-2}\ s^{-1}$.

Studies using *o*-$HOCH_2C_6H_4CH_2NMe_3^+Br^-$ (**4**) showed that the catalytic activity of **1** is also much greater than would be expected for attack by its zwitterion form. We assume that the acidity of the hydroxylic proton in **4** is the same as that in the conjugate acid of **1** and that the rate constants for attack of zwitterion forms of **1** and **4** on *p*-nitrophenyl acetate are the same. We define K_w as the autoprotolysis constant of water, K_{1H} as the acidity constant of protonated **1**, k_n as the total catalysis constant for electrically neutral forms of **1**, k_z as that part of k_n that arises from attack by the zwitterion, and k_{qb} as the rate constant for the term of the form k_{qb}[**4**][OH^-][pNPA] observed when *p*-nitrophenyl acetate is hydrolyzed in the presence of **4**. It follows that

$$k_z = k_{qb}K_w/K_{1H}$$

From the values of K_w, K_{1H}, and k_{qb} (Table I) a value of 0.0012 $M^{-1}\ s^{-1}$ is obtained for k_z. The k_n value for **1** listed in Table I is 40 times this large. This is much larger than the factors estimated for other amino alcohols[8] and hence much more likely to be larger than the errors in the estimate of k_z.

The necessity for having the alcohol and amine groups in the same molecule is shown by the observation that 0.035 M benzyl alcohol has no detectable effect on the rates of reaction in the presence of 0.01–0.05 M *N,N*-dimethylbenzylamine.

The hydrolysis of *p*-nitrophenyl acetate in the presence of **1** gave the acetate of **1** as a reaction intermediate. This was shown by a ferric acetohydroxamate analysis[15] and by the infrared spectra of cyclohexane extracts of the reaction solutions.

Other amino alcohols that are significantly less basic than their methyl ethers will be attractive possible models for serine enzymes if their hydroxy groups are not too hindered sterically.

Supplementary Material Available: A table of pK values for corresponding methoxy and hydroxy amines (2 pages). Ordering information is given on any current masthead page.

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(12) In the absence of internal hydrogen bonding the methoxy substituent should decrease basicity as much as or more than a hydroxy substituent does. The respective values of σ^* for methoxymethyl and hydroxymethyl substituents are 0.64 and 0.56; the σ_I constants for methoxy and hydroxy are 0.27 and 0.25.[13] We have found pK_a values in aqueous solution for nine amines with methoxy substituents attached to saturated carbon. In only one case is the amine more basic than the corresponding hydroxyamine and in that case the difference in pK_a values is only 0.05.[14]
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(14) Data given in the supplementary material.
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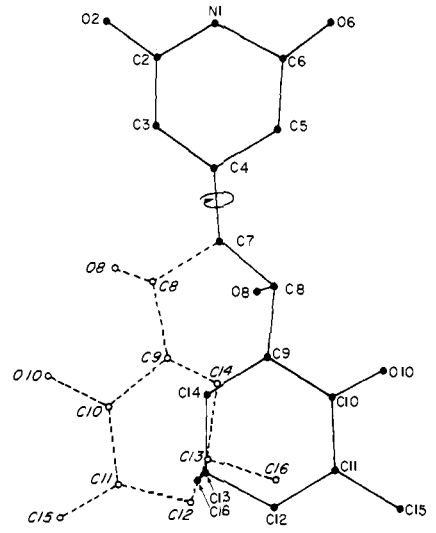

Figure 1. Superposition of molecules A (solid bonds, block lettering) and B (dashed bonds, italic lettering) over their geometrically common portions showing the striking difference in the orientation around the exocyclic bond C(4)-C(7). View is parallel to the c^* axis.

Simultaneous Occurrence of a Chair and a Boat Conformation in Crystalline Cycloheximide

Sir:

As part of our investigation of protein synthesis inhibitors, we have determined the crystal structure of the antibiotic cycloheximide (4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione) (Figure 1). Cycloheximide inhibits the translocation step of eucaryotic ribosomal protein synthesis, possibly by blocking the release of deacylated tRNA from the ribosome.[1] The molecule consists of the two saturated rings, glutarimide and dimethylcyclohexanone, connected by a two carbon chain. There are two crystallographically independent cycloheximide molecules in the asymmetric part of the unit cell and these are found to have markedly different conformations. The most interesting difference occurs in the cyclohexanone moiety which exhibits the chair conformation in one molecule and the twist boat conformation in the other. As far as we are aware, this is the first observation of the coexistence of a chair and boat conformation in a crystal. Another unusual feature of this structure is that the hydroxyl group of one of the molecules is not involved in hydrogen bonding.

Crystals of cycloheximide ($C_{15}H_{23}NO_4$) are monoclinic, with space group $P2_1$, and cell dimensions a = 15.151 (5) Å, b = 7.761 (2) Å, c = 14.012 (4) Å, and β = 113.56 (5)°. The observed density of 1.24 g cm^{-3} indicated the presence of two crystallographically independent molecules in the asymmetric unit. The structure was solved by direct methods[2] and refined by full-matrix least-squares methods to a final R value of 3.5% using 2325 unique reflections collected on a Picker FACS-I diffractometer. All 46 hydrogen atoms were located by difference electron density synthesis. A table of atomic coordinates is available; see paragraph at end of paper regarding supplementary material. The details of the crystallographic work will be reported elsewhere.

The cycloheximide molecule could be expected to have considerable conformational freedom because of the presence of three carbon-carbon single bonds linking the glutarimide and dimethylcyclohexanone rings. The crystal structure analysis has shown that the two molecules have very different overall conformations, resulting not only from the rotations of the linkage bonds, but also from differences in the puckering of the dimethylcyclohexanone rings. The exocyclic torsion angle C(5)-C(4)-C(7)-C(8) is gauche (−63°) in molecule A and trans (174°) in molecule B, so that the glutarimide and dimethylcyclohexanone rings have different orientations in the two molecules (Figure 1). Despite this very pronounced difference, the torsion around the central C(7)-C(8) bond is in the apparently preferred trans conformation in both molecules (Table I), thereby forcing them into an extended rather than a folded configuration. Likewise, the torsion angle around the third exocyclic bond (C(8)-C(9)) is similar in both molecules.

The dimethylcyclohexanone rings differ in the two molecules in that the familiar chair conformation is adopted in molecule A and the twist boat conformation in molecule B (Figure 2). A comparison of their endocyclic torsion angles is given in Table I. In the chair form, one of the methyl groups is axial and the other equatorial resulting in some unfavorable 1,3 nonbonded interactions between the axial methyl group and the axial hydrogen atoms at C(9) and C(11). These interactions

Table I. Torsion Angles in Cycloheximide

Atoms	Exocyclic torsions Molecule A	Molecule B
C(5)-C(4)-C(7)-C(8)	−62.8 (3)	174.1 (2)
C(3)-C(4)-C(7)-C(8)	175.5 (2)	52.9 (3)
C(4)-C(7)-C(8)-C(9)	179.0 (2)	160.1 (2)
C(7)-C(8)-C(9)-C(10)	−164.7 (2)	174.8 (2)
C(7)-C(8)-C(9)-C(14)	70.1 (3)	49.9 (3)

Atoms	Endocyclic torsions (cyclohexanone) Molecule A chair	Molecule B twist boat	(Ideal) twist boat[3]
C(9)-C(10)-C(11)-C(12)	46.9 (3)	24.5 (3)	33.2
C(10)-C(11)-C(12)-C(13)	−50.6 (4)	38.6 (3)	33.2
C(11)-C(12)-C(13)-C(14)	55.0 (4)	−67.7 (3)	−70.6
C(12)-C(13)-C(14)-C(9)	−55.9 (4)	30.8 (3)	33.2
C(13)-C(14)-C(9)-C(10)	52.4 (4)	28.9 (3)	33.2
C(14)-C(9)-C(10)-C(11)	−48.1 (3)	−59.7 (3)	−70.6