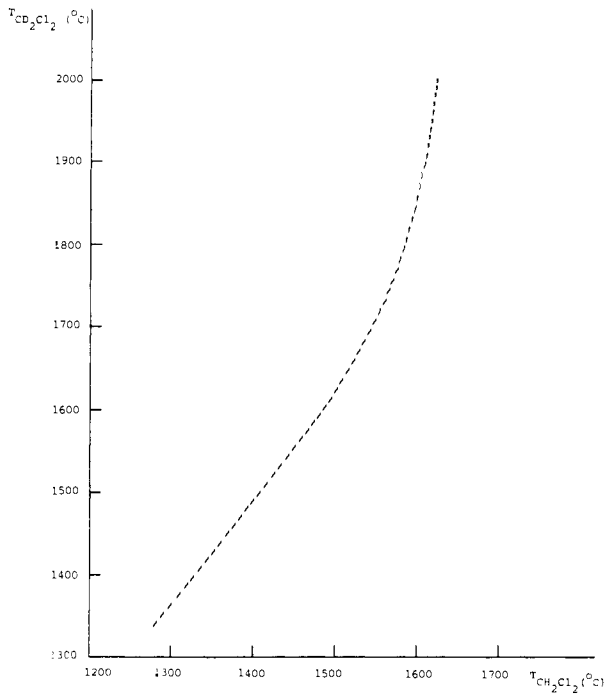

Figure 3. Plot of vibrational temperatures of CD_2Cl_2 ($T_{CD_2Cl_2}$) vs. various predicted vibrational temperatures of CH_2Cl_2 ($T_{CH_2Cl_2}$).

It is clear from these results that selectivity was attained only when ir pulses with sufficient intensity and having durations shorter than the overall relaxation time were applied. By increasing either the pressure or the light intensity of the ir pulsed laser, experiment breakdown occurs. Under these conditions, most of the methylene chloride was decomposed after 100 pulses; the isotope ratio, however, remained constant.

As in our previous study,[1] the energy of activation (65 kcal/mol) was much higher than the energy per Einstein of the laser light. The detailed mechanism of energy accumulation was not studied. However, the reaction could not occur by single photon absorption. Multiphoton absorption, cascade excitation, or combination of the two mechanisms may be responsible for the observed reaction.

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Received November 12, 1974

The Stereochemically Correct Catalytic Site on Cyclodextrin Resulting in a Better Enzyme Model

Sir:

Cyclodextrin reactions show roughly the same rate enhancement with respect to hydroxide ion reactions as do chymotrypsin reactions.[1,2] There is one proviso, however. The rate constant for the chymotrypsin reaction was determined at pH 7.9, its maximum, whereas the rate constant for the cyclodextrin reaction was determined at pH 13, its maximum. If one can reduce the pH at which cyclodextrin operates, for example by introduction of an imidazole group into the molecule, its rate enhancement might truly parallel the reaction with chymotrypsin.[3]

The first such attempt by Cramer and Mackensen at introducing an imidazole group as a catalytic site shows only a slight rate enhancement over a combination of cyclodextrin and imidazole.[4] In their compounds, however, substitution occurs preferentially at carbon atom 6 of the glucose unit in the cyclodextrin. Namely the catalytic site (imidazole group) is attached to a *primary* alcohol group on the essentially closed face of the toroidal cyclodextrin molecule where the substrate could not be catalyzed in the cavity. The specific stereochemical relationship between the cyclodextrin and their substances in the cleavage of phenyl esters in alkaline solution is due to a nucleophilic reaction of an alkoxide ion derived from the *secondary* hydroxyl group at carbon atom 2 and 3, of the glucose unit in a cyclodextrin.[2]

Another attempt by Breslow and Overman, who prepared cyclodextrin with a metal ion coordinated nucleophile, showed that the reaction occurred at pH 5, the same general magnitude as Cramer's compounds.[5]

We wish to report here the preparation of an α-cyclodextrinhistamine compound (IV) in which the histamine group is attached to a *secondary* alcohol group on the more open face of the toroidal α-cyclodextrin. We wish also to demonstrate that this compound will accelerate the hydrolysis of *p*-nitrophenyl acetate by attack of the imidazole group on a substrate molecule bound in the cavity because of the appropriate position of the catalytic functionality (imidazole group) on the cyclodextrin.

The α-cyclodextrinhistamine compound (IV) was synthesized through the *p*-tosyl ester (I) and then the iodide (II) of α-cyclodextrin (α-CD). α-CD was tosylated with 10 equiv of *p*-toluenesulfonyl chloride in buffer solution of pH 11 at 25° for 1 hr, followed by ion-exchange chromatography using Amberlite MB-3, giving α-CD monotosylate (I), whose structure was confirmed by uv absorption (λ_{max} 263 nm, log ϵ 2.78, referred to ethyl tosylate, λ_{max} 262, log ϵ 2.75) together with proton, NMR spectra in D_2O which showed an absorption at δ 7.48 (ppm) due to the benzene ring hydrogens and 4.76 assigned to C_1H of the glucose ring. The relative areas of these peaks were 2:3, demonstrating monosubstitution on α-CD. Iodination of tosyl α-CD with NaI was carried out in water at 80° for 1 hr, then in situ free histamine was added and the mixture was kept at 80° for 48 hr. The product was purified by chromatography using Amberlite IR-120 and Sephadex G-15 columns, and gave IV in overall yield ca. 10%. The presence of an imidazole ring in IV was ascertained by the Pauly test. When histamine was replaced with ammonium hydroxide in the above treatment, the reaction was carried out in an autoclave (130° for 48 hr), followed by chromatography on Amberlite and Sephadex G-15, which then gave amino-α-CD (III). The intermediate iodo-α-CD (II) was also purified on Amberlite and Sephadex and was identified.

The products (I-IV) were found to be pure and free from cross-contamination as judged by paper chromatography

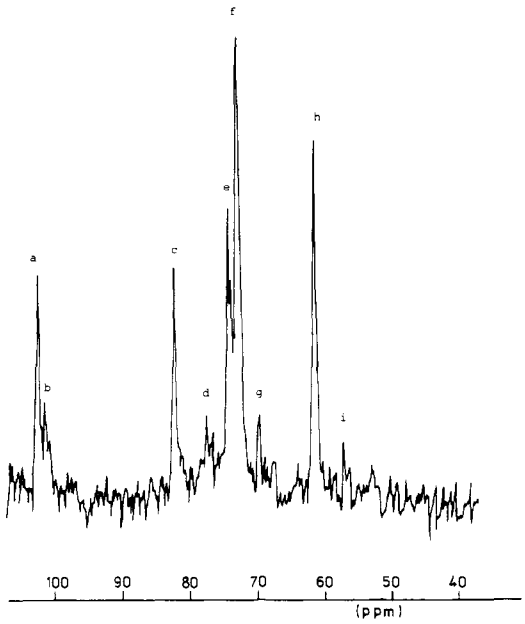
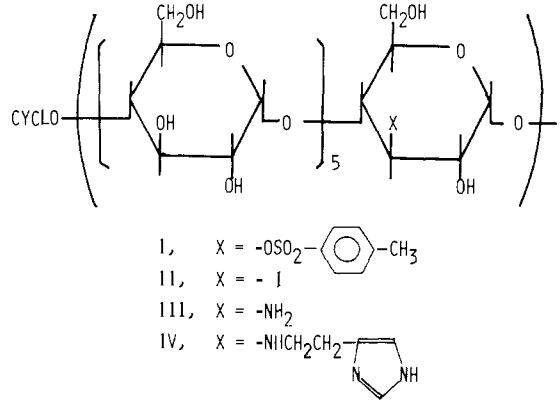


Figure 1. ^{13}C NMR spectrum of amino-α-CD (III). Spectrum was measured at 25.1 MHz with a JEOL PS-100 spectrometer. All protons were decoupled. III was measured in aqueous (ca. 2 *M*) solution. Chemical shifts were measured by using dioxane as external standard, and referred to Me_4Si. Spectra were determined by scanning 6000 times by use of a time-averaging device.

(solvent: 1-butanol–dimethylformamide–water 2:1:1 v/v/v) using iodine vapor as a detecting reagent. The R_f values were 0.21 for α-CD, 0.28 for I, 0.45 for II, 0.41 for III, and 0.39 for IV.



The ^{13}C NMR spectrum of III clearly established that tosylation had occurred at the C-3 position on a glucose residue of cyclodextrin (Table I, Figure 1). The changes of the chemical shift due to the substitution by the amino group on a glucose ring were reasonable values: Δδ 17.01 (ppm) for C-3′, 4.31 for C-2′, and 4.76 for C-4′. These values correlated well with the change of the values between glucose and glucosamine hydrochloride; Δδ 17.1 (ppm) for C-2, 3.1 for C-1, and 3.8 for C-3.[6] No peak appeared in the ^{13}C NMR spectrum, which might be due to 6-amino-6-deoxyglucose which was reported previously to occur at 46.0–46.1 ppm.[7]

Preliminary kinetic results concerning the pseudo-first-order rate constants for deacylation of *p*-nitrophenyl acetate in the presence of added catalysts are shown in Table II. It shows that the stereochemically correct active site attached to α-CD involving histamine accelerates *p*-nitrophenyl hydrolysis 60–80 times more than does α-CD itself, and 5.5–6.3 times more than the 1:1 mixture of α-CD and histamine at these pH's.

It is suggested that this enhanced reactivity of deacylation of *p*-nitrophenyl acetate in the presence of IV is a result of binding and reaction within the IV-*p*-nitrophenyl acetate complex. The observation both of competitive inhibition and of substrate specificity is consistent with this interpretation. The results are shown in Table III. The hydrolysis of *p*-nitrophenyl acetate in the presence of IV was competitively inhibited by the addition of cyclohexanol while the rate of hydrolysis catalyzed by added histamine was not affected by added cyclohexanol. Other evidence for the mode of the catalysis is that 8-acetoxy-5-quinoline sulfonate, a molecule which does not fit into the CD cavity,[5] is only 76% as reactive toward IV as toward a 1:1 mixture of CD and histamine. The evidence that 8-acetoxy-5-quinoline sulfonate does not complex in the cavity is shown by the

Table I. ^{13}C NMR Chemical Shifts of Amino-α-cyclodextrin (III)

δ (ppm) of III	δ (ppm) of α-CD[a]	Assignment
a 102.35	102.9	C-1
b 101.34		C-1′ (1.01)[b]
c 82.02	82.8	C-4
d 77.26		C-4′ (4.76)[b]
e 74.08	75.4	C-3
f 72.68	73.5	C-5
	73.7	C-2
g 69.42		C-2′ (4.31)[b]
h 61.34	62.3	C-6
i 57.07		C-3′ (17.01)[b]

[a] Data cited from T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, 2425 (1973). Chemical shifts in this table were taken from the NMR spectrum in Figure 1. [b] Change of the chemical shift (ppm) due to the substitution by the amino group on a glucose ring.

Table II. Pseudo-First-Order Rate Constants[a] for the Deacetylation of *p*-Nitrophenyl Acetate in the Presence of Added Catalysts

	10^4k (sec^{-1})[b]	
Catalyst	pH 8.02 ± 0.03	pH 8.37 ± 0.03
None	0.441 ± 0.001	0.789 ± 0.023
α-CD	0.886 ± 0.009	1.27 ± 0.01
Histamine	7.30 ± 0.06	12.1 ± 0.2
α-CD + histamine (1:1 mixture)	9.67 ± 0.04	16.0 ± 0.8
α-CD–histamine (IV)	53.6 ± 0.5	101 ± 2

[a] Rate of release of *p*-nitrophenol determined spectrometrically at 400 nm in 0.2 *M* Tris–HCl buffer, I = 0.200 (KCl), [catalyst] = 1.00×10^{-2} *M*, [*p*-nitrophenyl acetate] = 1.00×10^{-4} *M*, with 0.50% (v/v) added acetonitrile at 25.0 ± 0.1°. [b] Average of two or more runs. Error values reflect reproducibility between separate runs. Individual rate constants followed pseudo-first-order kinetics.

Table III. Rate Constant[a] for the Reaction of *p*-Nitrophenyl Acetate (pNPA) and 8-Acetoxyquinoline 5-Sulfate (AQS) in the Presence of Added Catalyst and Cyclohexanol

Catalyst[b]	Substrate[c]	Cyclohexanol (*M*)	10^4k (sec^{-1})[d]
IV	pNPA	0	7.07 ± 0.03
IV	pNPA	0.205	3.56 ± 0.02
Histamine	pNPA	0	1.76 ± 0.01
Histamine	pNPA	0.205	1.62 ± 0.01
IV	AQS	0	0.779 ± 0.062
CD + histamine (1:1 mixture)	AQS	0	1.03 ± 0.02
IV	AQS	0.205	1.50 ± 0.13

[a] The pseudo-first-order rate constant of pNPA and AQS determined spectrometrically at 400 nm in 0.10 *M* Tris–HCl buffer, pH 8.03 ± 0.03, I = 0.200 (KCl) at 25.0 ± 0.1°. [b] [catalyst] = 1.67×10^{-3} *M*. [c] pNPA (15 μl) in acetonitrile was added to 3.00 ml of buffer containing catalyst. [pNPA] = 1.00×10^{-4} *M*. AQS (45 μl) in distilled water was prepared before use and added to 3.00 ml of buffer containing catalyst. [AQS] = 3.01×10^{-4} *M*. [d] Average of two or more runs. Error values reflect reproducibility between separate runs. Individual rate constants followed first-order kinetics.

fact that cyclohexanol cannot inhibit the hydrolysis of 8-acetoxy-5-quinoline sulfonate with IV.

A scale molecular model of a complex of *p*-nitrophenyl acetate with IV can result in fixing the position of the ester group in close proximity to the secondary hydroxyl groups and imidazole group.

It should be pointed out that this newly prepared catalyst showed its rate acceleration around the same pH and possibly has the same mode of kinetics as chymotrypsin during the release of phenol (the acylation step) in the hydrolysis of ester. The present catalyst contains imidazole and hydroxyl groups in the same stereochemically correct manner as chymotrypsin does in its active site. This fact thus furnishes a better enzyme model for chymotrypsin catalysis.

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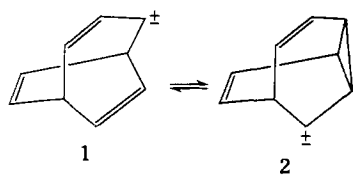
Received November 4, 1974

Degeneracy and Stability of the Homobullvalenyl Cation

Sir:

Winstein's concept of homoaromaticity[1] and the refining applications of MO theory by Goldstein and Hoffmann[2] to longicyclic systems of potential bicycloaromaticity[3] have led to considerable effort directed toward experimental tests of theory. In at least formal agreement with prediction the ion $\mathbf{1}^-$ has been found to be delocalized where $\mathbf{1}^+$ rearranges to $\mathbf{2}^+$.[4]



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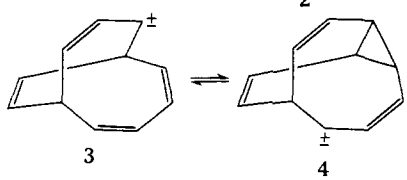
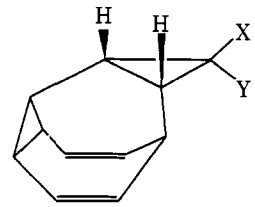


(2)

More recently approaches to precursors of **3** and **4** have appeared[5,6] since, in principle, electronic factors present in **1** should be reversed **3**. We report here the first experimental verification of this prediction and at the same time apply the first limits to the extent of homoaromatic or longicyclic stabilization in $\mathbf{3}^+$ and $\mathbf{4}^+$.



5, X = $OOCCF_3$; Y = Br
6, X = H; Y = OH
7, X = H; Y = OTf
7-*d*, X = D; Y = OTf

Our approach to **3** and **4** has been based upon functional group manipulation of the dibromocarbene adduct of bullvalene. The discovery that α-bromocyclopropyl trifluoroacetates can be reduced with sodium borohydride to cyclopropanols[7] has now afforded an entry to the parent $[CH]_{11}$ system. Thus, treatment of the α-bromotrifluoroacetate **5** with sodium borohydride in THF afforded[8] a 77% yield of the cyclopropanol **6**.[9] The NMR spectrum of **6** was similar to that of **5**[10] with an additional triplet (δ 3.17, J = 7.5 Hz) signaling the *cis*-cyclopropane vicinal coupling. Treatment of **7** with trifluoromethyl sulfonic anhydride in pyridine for 2 hr produced a 99% yield of the corresponding triflate **7**.

The solvolysis of **7** proceeded smoothly in 40% aqueous acetone (3 hr at 95°, 2,6-lutidine buffer) to give a mixture of two alcohols, **8** and **9**, which, upon Sarett oxidation,[11] afforded a 65% isolated yield of the known ketones **10**[6b] and **11**[5b] (relative amounts 17:83) (Scheme I). That the origin of **9** is due to a subsequent *thermal* process is confirmed by appropriate variations in the **8**/**9** ratio with time and temperature and the known parallel chemistry of **10**.[5b]

Formally, the conversion of **7** to **8** requries only the opening of two cyclopropane rings leading first to $\mathbf{4}^+$ and then to $\mathbf{3}^+$. Solvolysis of specifically labeled **7-*d***[12] has revealed far greater complexity. While the proton NMR spectra of **8**, **9**, and **10** are complex, that of **11** is nearly first order, and all

Scheme I