Boron Fluoride–Alcohol Alkylations. III. Stereochemistry of Alkylation of Benzene with 2-Propanol-1-d_3[1]

Sir:

A knowledge of the stereochemical consequence of a Friedel–Crafts alkylation of an aromatic hydrocarbon is an essential component of any theory of reaction mechanism, yet no unambiguous study has been reported heretofore.[2] Alkylations with alcohols and boron fluoride are advantageous because starting material and product are not affected by the catalyst.[1b] The reaction of optically active 2-butanol with benzene and boron fluoride has been shown to give *sec*-butylbenzene with 99% racemization during the alkylation step.[3] However, the significance of this result is clouded by the known rapid rearrangements of secondary systems in this reaction[1b]; thus, even an encumbered 2-butyl cation could give the appearance of racemization by rapid equilibration of the cationic charge between the 2- and 3-positions.[2]

This ambiguity is avoided with the isopropyl system which is known not to rearrange—only one secondary cation position is available. Mislow, O'Brien, and Schaefer's[4] preparation of optically active 2-propanol-1-d_3, $\alpha^{21.6}D$ +0.280 ± 0.007° (*l* 1, neat), was repeated. The expected alkylation product was synthesized from optically pure (−)-3-phenylbutanoic acid. The α-hydrogens were exchanged for deuterium by treatment of the methyl ester with sodium methoxide in methanol-*d*. Reaction of the acid with iodine and lead tetraacetate[5] gave 1-iodo-2-phenylpropane-1-d_2, which on reduction with lithium aluminum deuteride, gave 2-phenylpropane-1-d_3, $\alpha^{22}D$ +0.48 ± 0.02° (*l* 1, neat). From the known stereochemistry of the starting acid,[6] the (+)-hydrocarbon is assigned the *R* configuration.

Reaction of the (+)-(*S*)-2-propanol-1-d_3 with benzene and boron fluoride at 5° gave 2-phenylpropane-1-d_3, $\alpha^{25}D$ +0.009 ± 0.005° (*l* 1, neat). A similar alkylation in a 60:40 benzene–nitromethane solvent at 50° gave the hydrocarbon with $\alpha^{25}D$ +0.033 ± 0.005° (*l* 1, neat). Hence, even in the absence of rearrangement, alkylation proceeds with >93% racemization and very little net inversion of configuration. This racemization is not due to prior racemization of alcohol or to subsequent racemization of product or to an equilibration with olefin. We conclude that the isopropyl cation intermediate in the alkylation is a largely free and unencumbered cation. The reaction is much like an SN1 solvolysis and has little of the character of a direct displacement reaction or of a π-complex with benzene.[1b,2]

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The Kinetics of the Trypsin-Catalyzed Hydrolysis of *p*-Nitrophenyl α-N-Benzyloxycarbonyl-L-lysinate Hydrochloride[1]

Sir:

Although the kinetics of the presteady state and steady state of the hydrolysis of the nonspecific substrate, *p*-nitrophenyl acetate, by α-chymotrypsin[2,3] and trypsin[4] have been delineated, a complete kinetic analysis of this kind had not been carried out for the corresponding hydrolysis of a specific substrate of these enzymes. This paper reports the first such reaction, the trypsin-catalyzed hydrolysis of *p*-nitrophenyl α-N-benzyloxycarbonyl-L-lysinate hydrochloride, which can be analyzed in terms of eq. 1 whose symbols

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k_2} ES' \xrightarrow[+\ P_1]{k_3} E + P_2 \qquad (1)$$

have been defined previously.[3]

Previous work with α-chymotrypsin reactions[5] indicated that both the presteady-state and steady-state reactions with trypsin were probably dependent on a basic group of $pK_a \sim 7$. Therefore the present kinetic analysis was carried out at pH 2.66. At this low pH, the rate of the presteady-state reaction was indeed slow, slow enough in fact to be measured on a Cary Model 14 spectrophotometer. Using these conditions we were able to observe the major part of the presteady-state liberation of *p*-nitrophenol (occurring in about the first 30 sec. of reaction), followed by a slow, steady-state (zero-order) liberation of *p*-nitrophenol (Figure 1). Presteady-state rate constants at different initial substrate concentrations were calculated by graphically extrapolating the steady-state straight line of a *p*-nitrophenol *vs.* time curve and plotting the logarithm of the difference between this extrapolated line and the experimental curve as a function of time. The experimental points give good straight lines, the slope of which yields the first-order rate constant, *b* (Table I). The definition of *b*, based on eq. 1 and $S_0 \gg E_0$, is[3]

$$b = \frac{(k_2 + k_3)S_0 + k_3K_s}{S_0 + K_s} \qquad (2)$$

A plot of $1/b$ *vs.* $1/S_0$, which yields a straight line in the α-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl acetate, gives instead for the present data a very definite curvature. The obvious explanation in terms of eq. 2 is that the condition $(k_2 + k_3)S_0 \gg k_3K_s$ is not satis-

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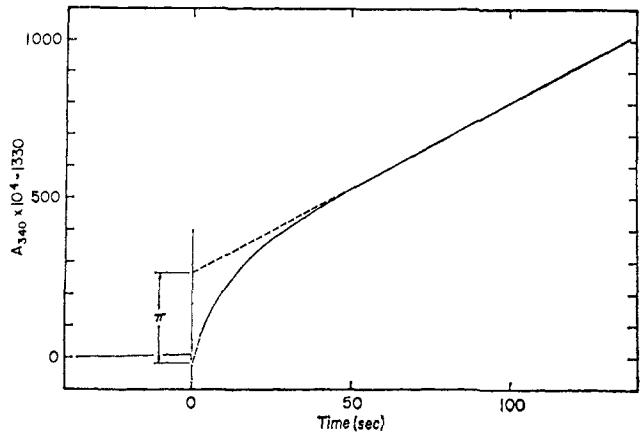

Figure 1. The trypsin-catalyzed hydrolysis of *p*-nitrophenyl α-N-benzyloxycarbonyl-L-lysinate at pH 2.66, 0.05 *M* citrate buffer; $E_0 = 7.40 \times 10^{-6}$ *M*; $S_0 = 2.03 \times 10^{-4}$ *M*, 1.29% (v./v.) acetonitrile–water, 25°. Cary Model 14 spectrophotometer, 8 in./min. recording chart speed, noise level = 5×10^{-4} absorbance unit.

fied in this system. Therefore, for the calculation of the individual rate constants, we have used a combination of the presteady-state data together with data from the steady state, determined under identical conditions.

Table I. The Presteady State of the Trypsin-Catalyzed Hydrolysis of *p*-Nitrophenyl α-N-Benzyloxycarbonyl-L-lysinate Hydrochloride[a]

$S_0 \times 10^5$, *M*	$b \times 10^2$, sec.$^{-1}$	$\pi \times 10^6$, *M*
56.5	18.2	5.36
38.5	13.4	4.74
20.3	9.44	4.57
13.7	7.88	4.03
9.78	5.71	3.62

[a] $E_0 = 7.40 \times 10^{-6}$ *M*. For other conditions, see Table II.

From the Lineweaver–Burk plot for the steady state, $k_{cat}E_0$ and K_s/k_2E_0 were determined from the intercept and the slope, respectively. Equation 2 may be rearranged to eq. 3. The quotient of K_s/k_2E_0 divided by

$$\left(\frac{S_0}{k_{cat}E_0} + \frac{K_s}{k_2E_0}\right)/b = \frac{K_s}{k_2k_3E_0} + \frac{S_0}{k_2k_3E_0} \qquad (3)$$

the intercept of a plot of the left side of eq. 3 *vs.* S_0, $K_s/k_2k_3E_0$, yields k_3. The product of the slope of this plot ($1/k_2k_3E_0$) and $k_{cat}E_0$ ($=k_2k_3E_0/(k_2 + k_3)$) yields ($k_2 + k_3$) and thus k_2. Knowledge of the k_2/k_3 ratio (27.6) then permits the calculation of K_s from $K_m(app) = K_s/(1 + (k_2/k_3))$. The rate constants are summarized in Table II.

Table II. The Trypsin-Catalyzed Hydrolysis of *p*-Nitrophenyl α-N-Benzyloxycarbonyl-L-lysinate Hydrochloride[a-c]

k_2	0.395 sec.$^{-1}$
k_3	1.43×10^{-2} sec.$^{-1}$
K_s	7.95×10^{-4} *M*

[a] 25.0°, 1.29% (v./v.) acetonitrile–water, pH 2.66, 0.05 *M* citrate. [b] Worthington 2× crystallized, lyophilized bovine trypsin (TRL 6256); age of solution: >20 min. and <4 hr. [c] The substrate was a Cyclo Chemical Corp. product, m.p. 151°, $[\alpha]^{20}D$ 21.6° (*c* 2, dimethylformamide).[6]

Knowledge of the rate and equilibrium constants of the reaction permits the calculation of the enzyme concentration from π, the "initial burst" of *p*-nitrophenol, as a function of the substrate concentration. Values of π were determined by graphical extrapolation, to $t = 0$, of the differences between the steady-state extrapolated lines and the absorbances at time *t* (Table I). By plotting $1/\sqrt{\pi}$ as a function of $1/S_0$ we obtain a straight line (using the value of $1/K_m(app)$ from the steady-state kinetics as an additional point). The intercept of this plot on the ordinate gives[6] $\pi_0 = E_0/(1 + (k_3/k_2))^2$, and thus $E_0 = 6.45 \times 10^{-6}$ *M*, indicating a purity of the enzyme, by weight, of 50%. Since our system of equations is redundant, we can calculate E_0 in an independent way: from k_2 and k_3, we can calculate k_{cat}, and then from the experimental value of $k_{cat}E_0$, $E_0 = 7.40 \times 10^{-6}$ *M* (57% purity by weight). The agreement of the two E_0 values seems reasonable, considering the experimental difficulties and the complexity of the calculations.[7]

The observation of "initial burst" of *p*-nitrophenol and of the kinetics of both the presteady-state and steady-state reactions are satisfactorily described by the three-step mechanism of eq. 1. These observations are consistent with the conclusion that, within experimental error, the totality of the tryptic hydrolysis of *p*-nitrophenyl α-N-benzyloxycarbonyl-L-lysinate hydrochloride involves a single reaction pathway with the formation of an α-N-benzyloxycarbonyl-L-lysyl-trypsin intermediate. This substrate is the most specific substrate of trypsin, based on its $k_{cat}(lim) = 170$ sec.$^{-1}$, the fastest known trypsin catalysis. Studies of the pH dependence of this reaction show that k_{cat} is dependent, as usual, on a single basic group of pK_a 6.80 ($I = 0.05$) from pH 2 to pH 7.4, indicating that the results obtained here at pH 2.66 may be reasonably extrapolated to neutral pH. Thus, the present observations must be pertinent to the pathway of trypsin catalysis.

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The Trypsin-Catalyzed Hydrolysis of the *p*-Nitrophenyl, Methyl, and Benzyl Esters of α-N-Benzyloxycarbonyl-L-lysine[1]

Sir:

The first step in the elucidation of the mechanism of any reaction is the establishment of the reaction pathway, that is, the characterization of intermediates formed in the reaction. Several pieces of evidence indicate that trypsin-catalyzed reactions proceed through an acyl-enzyme intermediate. The most important experimental indications are: (1) the methyl, ethyl, isopropyl, benzyl, and cyclohexyl esters of α-N-benzoyl-L-arginine are hydrolyzed with identical rate constants by trypsin[2]; (2) acetyl-trypsin is formed in

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