terms on the right of eq 3 are equivalent to log ${}^{An}\gamma^{W}_{Ag^+}$ − log ${}^{An}\gamma^{W}_{Ph_4As^+}$ or log K^{An}/K^{W}, where K is the equilibrium constant for (1). It will be noted that eq 3 is independent of the solubility product of $AgBPh_4$ and Ph_4AsBPh_4 and does not require corrections for Debye–Hückel salt effects. The value of log ${}^{An}\gamma^{W}_{Ag^+}$ = 2.8 from this cation–molecule assumption compares unfavorably with our value of 6.1[1] by applying Strehlow's ferricinium cation, ferrocene molecule assumption to polarographic data. Table I encourages us to accept a value of between 3 and 4 for log ${}^{An}\gamma^{W}_{Ag^+}$ and to recommend the value of 3.9, which follows from the cation–anion assumption that ${}^{An}\gamma^{W}_{Ph_4B^-}$ = ${}^{An}\gamma^{W}_{Ph_4As^+}$. Thus we are now in close agreement with Kolthoff[3] and Popovych[6] as to an acceptable assumption for single ion medium activity coefficients for transfer from water.

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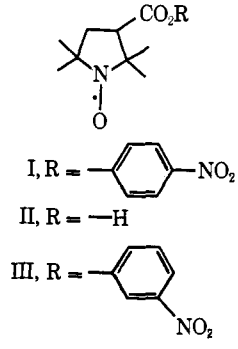
Enantiomeric Specificity in the Chymotrypsin-Catalyzed Hydrolysis of 3-Carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxy *p*-Nitrophenyl Ester

Sir:

Enantiomeric specificity is an important property of enzyme-catalyzed reactions which has been studied extensively in the chymotrypsin-catalyzed reactions of many substrates. However, there is a dearth of quantitative information comparing the relative magnitudes of the individual rate parameters for both enantiomers of asymmetric substrates which are hydrolyzed by chymotrypsin according to the general scheme of eq 1. We wish now to report our measurements of these kinetic parameters for the chymotrypsin-catalyzed hydrolysis of both enantiomers of the spin-labeled chiral nonspecific ester substrate 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxy *p*-nitrophenyl ester.[1-3]

$$E + S \underset{K_s}{\rightleftharpoons} E\cdot S \xrightarrow{k_2} ES' + P_1 \xrightarrow{k_3} E + P_2 \qquad (1)^{4,5}$$



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The racemic acid II was resolved using α-methylbenzylamine in acetone. The dextrorotatory isomer of II, prepared using L(−)-α-methylbenzylamine, has mp 205–207°, $[\alpha]^{25}D$ +79 ± 1° (EtOH). The levorotatory isomer of II, prepared using (+)-α-methylbenzylamine, has mp 203–205°, $[\alpha]^{25}D$ −81 ± 2° (EtOH). The (+) and (−) isomers of ester I were prepared by coupling the corresponding isomers of the acid II to *p*-nitrophenol with dicyclohexylcarbodiimide.[1]

From visible absorption measurements of the rate of release of *p*-nitrophenolate from (+)- or (−)-I at 25° in pH 7.0 phosphate buffer, μ = 0.5, in 1% CH_3CN, with varying amounts of excess chymotrypsin, values of $K_s = (5.1 \pm 1.2) \times 10^{-4}$ M and $k_2 = (4.1 \pm 0.5) \times 10^{-2}$ sec^{-1} were calculated for the acylation of the enzyme by (−)-I, and values of $K_s = (4.1 \pm 0.4) \times 10^{-4}$ M and $k_2 = 0.37 \pm 0.02$ sec^{-1} were calculated for the reaction of (+)-I. From similar measurements of rates of *p*-nitrophenolate release in the presence of excess (+)- or (−)-I, values of $k_3 = (2.5 \pm 0.3) \times 10^{-4}$ sec^{-1} for the acyl enzyme derived from the reaction of (−)-I with chymotrypsin, and $k_3 = (5.2 \pm 0.2) \times 10^{-3}$ sec^{-1} for the acyl enzyme derived from (+)-I and chymotrypsin, were calculated. Thus, the enantiomeric specificity of the k_3 step of the chymotrypsin-catalyzed hydrolysis of I is greater than that of the k_2 step.

Solutions of the acyl enzymes derived from (+)- or (−)-I and chymotrypsin were prepared in analogy with the method of Berliner and McConnell[1] by allowing the *p*-nitrophenyl ester to react with chymotrypsin in pH 4.8 acetate buffer. The acyl chymotrypsin was separated from acid II, *p*-nitrophenol, and unreacted ester by filtration through G-15 Sephadex using pH 2.4 phosphate buffer, μ = 0.3. The esr spectra of solutions of the two diastereomeric acyl enzymes prepared in this way are similar and characteristic of "strongly immobilized" spin labels. By raising the pH of each acyl enzyme solution to 6.9–7.3 and following the increase in height of the high-field line of the esr spectrum of acid II with time, values of $k_3 = (2.3 \pm 0.2) \times 10^{-4}$ sec^{-1} for release of (−)-II and $k_3 = (4.5 \pm 1.0) \times 10^{-3}$ sec^{-1} for release of (+)-II were determined. These values do not differ significantly from the values for k_3 determined by visible absorption spectroscopy.

In an attempt to examine the nature of the noncovalent binding of (+)- and (−)-I to the enzyme, the interaction of the two isomers of I with chymotrypsin at pH 2.3, where the chymotrypsin-catalyzed release of *p*-nitrophenol from the esters is very slow, was also studied. Measurements of K_s by the method of equilibrium dialysis at 0 and 15°, followed by extrapolation to 25°, gave values of $K_s = 4 \times 10^{-3}$ M for either isomer of I at pH 2.3 in HCl–KCl solution, μ = 0.08, in 1% CH_3CN. Addition of 1.1×10^{-3} M chymotrypsin to a 5×10^{-5} M solution of either isomer of I in the pH 2.3 HCl–KCl mixture caused a small change in the shape of the solution's esr spectrum, corresponding to an approximately twofold increase in average rotational correlation time.[6] The small increase in correlation time cannot be accounted for by the increase in viscosity on addition of the enzyme, and, presumably, is due to

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the presence of a small amount of ester–enzyme complex in which the spin label is more immobilized than in the free ester but less immobilized than in the acyl enzyme.

The K_s values for the nonspecific substrates (+)- and (−)-I at pH 7.0 are lower than the K_s values for many specific substrates of chymotrypsin.[7] The low K_s values may reflect a large amount of "wrong-way"[8] or nonproductive binding of the substrates to the enzyme. In the chymotrypsin-catalyzed hydrolysis of ester I, as in many chymotrypsin-catalyzed reactions,[9] greater enantiomeric specificity is found in the catalytic steps of the reaction than in the binding step. However, the stereospecificity observed for the catalytic steps in the case of this nonspecific ester substrate is much lower than that found in the reactions of specific substrates with the enzyme.[10-12] Indeed, our results lead to the interesting conclusion that *the enantiomeric specificity in the chymotrypsin-catalyzed reaction of the nonspecific ester substrate I is only slightly greater than that found in the hydrolysis of the closely related ester III catalyzed by the model enzyme, cyclohexaamylose.*[13]

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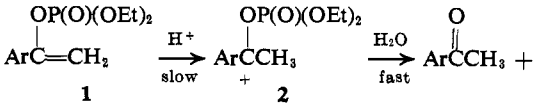
Bond Cleavage in Acid-Catalyzed Hydrolysis of Vinyl Phosphates[1]

Sir:

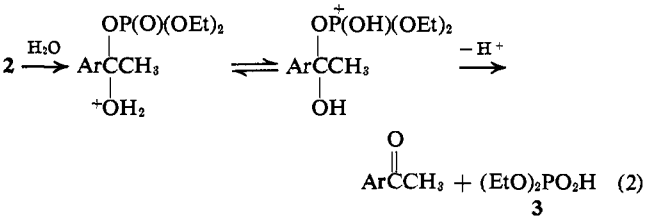
Results reported herein reveal that, contrary to prior belief, the acid-catalyzed hydrolysis of simple vinyl phosphates proceeds with C–O, rather than P–O, bond cleavage.

The acid-catalyzed hydrolysis of diethyl α-arylvinyl phosphates has been shown to proceed by the $A_{SE}2$ mechanism involving rate-determining proton transfer to the double bond (eq 1).[2,3] However, these kinetic studies are not informative as to the mechanism of the conversion of the cation intermediate **2** to the acetophenone and diethylphosphoric acid (**3**).



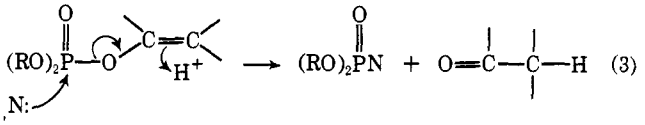
(EtO)$_2$PO$_2$H (1)
3

One possibility would be attack of water at carbon and eventual C–O bond cleavage (eq 2). This mechanism is similar to that for the acid-catalyzed hydration of olefins[4] and an analogous bond cleavage route was assumed to be operative in the hydrolysis of α-arylvinyl acetates.[5]



3 + (EtO)$_2$PO$_2$H (2)

However, for the acid-catalyzed hydrolysis of vinyl phosphates a different mechanism has been proposed. This was originally suggested by Cramer and Lichtenthaler,[6] and involves nucleophilic attack on phosphorus with P–O bond cleavage (eq 3). Variations of this mechanism have been utilized by research workers in the field[3] and incorporated in reviews on the subject.[7,8] These mechanisms differ as to the nature of the nucleophile and the timing of the steps, but agree on P–O bond cleavage.



The evidence on which this mechanism was proposed[6] consisted primarily of rate comparisons and product formation from the attack of nucleophiles besides water such as dialkylphosphoric acids and iodide ion. Since the kinetic arguments are clearly invalid for deductions regarding the steps after the rate-determining step, and the results involving nucleophiles other than water need not be applicable to the hydrolysis reaction, it appeared desirable to establish the position of bond cleavage in the hydrolysis reaction using isotopic labeling.[9]

The incorporation of ^{18}O label in the product diethylphosphoric acid (**3**) from hydrolysis of diethyl α-phenylvinyl phosphate (**1a**, Ar = Ph) was examined for 310 min reaction time in 1:1 mixtures of 0.25 *M* aqueous HCl and dioxane at 70°. The kinetics of the reaction of **1a** have been determined from 25 to 70° in 0.01–4.5 *M* acid solutions in water, and found to proceed by the $A_{SE}2$ pathway (eq 1).[2,3] Dioxane was added in

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