Figure 1. First derivative e.s.r. spectrum of phenylnitroxide in dimethyl sulfoxide (80%)-t-butyl alcohol (20%). The synthetic absorption spectrum is based on the following h.f.s.c.: $a^N = 9.10$, $a_{o,o,p}{}^H = 3.0$, $a_{NH}{}^H = 11.90$, and $a_m{}^H = 1.14$ gauss.

In ethanol or t-butyl alcohol **1** apparently undergoes protonation followed by the irreversable loss of hydroxide ion from **2** to yield azoxybenzene.[6] Apparently in dimethyl sulfoxide (80%)-t-butyl alcohol (20%) containing potassium t-butoxide the low reactivity of protons and a value of K_N that greatly favors $C_6H_5NO^{\cdot-}$ prevents the last step of the condensation from occurring. Although we have found no evidence for the reversal of the condensation reaction in dimethyl sulfoxide (80%)-t-butyl alcohol (20%) at 23°, a solution of azoxybenzene (0.05 M) in pure dimethyl sulfoxide 50% saturated with potassium hydroxide slowly forms the nitrosobenzene radical anion, evidently via

$$OH^- + C_6H_5N(O){=}NC_6H_5 \; \rightleftharpoons \; 2 \longrightarrow 1$$
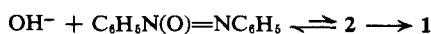
Exposure of such a solution to oxygen after a 3-hr. reaction period (1 × 10⁻³ M nitrosobenzene radical anion) destroyed the e.s.r. signal. After exposure to oxygen a new e.s.r. signal slowly developed which was recognized as that of nitrobenzene radical anion.

The present results explain why mixed condensations between XC_6H_4NO and YC_6H_4NHOH yield all possible azoxybenzenes, $XC_6H_4N(O){=}NC_6H_4X$, $XC_6H_4N(O){=}$

p-$RC_6H_4NO^{-2}$ 2Na⁺, and p-$RC_6H_4N[O^-Na^+]$-$N[O^-Na^+]C_6H_4R$-p [T. Kauffmann and S. M. Hage, $Angew. Chem. Intern. Ed. Engl.$, 2, 156 (1963)]. Acidification yields the azoxybenzene derivative.

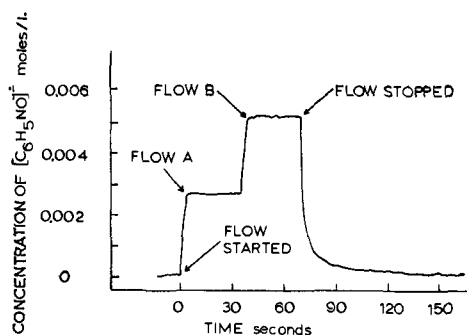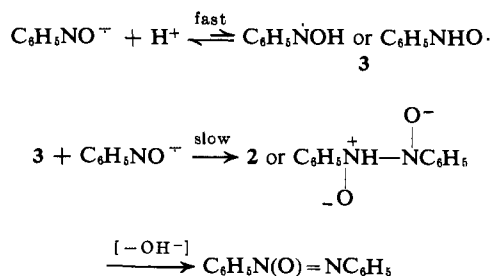(6) Another possible interpretation is that the nitrosobenzene radical anion is first protonated, $e.g.$

$$C_6H_5NO^{\cdot-} + H^+ \xrightarrow[\text{fast}]{} C_6H_5\dot{N}OH \text{ or } C_6H_5NHO\cdot$$
$$\textbf{3}$$

$$3 + C_6H_5NO^{\cdot-} \xrightarrow{\text{slow}} 2 \text{ or } C_6H_5\overset{+}{N}H{-}N\overset{|}{\underset{-O}{C_6H_5}}$$

$$\overset{[-OH^-]}{\longrightarrow} C_6H_5N(O) = NC_6H_5$$

Figure 2. Concentration of nitrosobenzene radical anion measured in ethanol at 23 ± 1° from the reaction of a solution initially 0.010 M in nitrosobenzene with a solution 0.010 M in phenylhydroxylamine and 0.10 M in sodium hydroxide; flow A, radical anion detected 3 sec. after mixing; flow B, radical anion detected 0.5 sec. after mixing.

NC_6H_4Y, $YC_6H_4N(O){=}NC_6H_4Y$, and $YC_6H_4N(O){=}$ NC_6H_4X.[7,8]
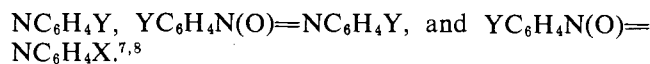
(7) E. Bamberger and E. Renauld, $Ber.$, **30**, 2278 (1897); V. O. Lukashevich, $Compt. rend. Acad. Sci.$, $USSR$, **21**, 376 (1938); Y. Ogata, M. Tsuchida, and Y. Takagi, $J. Am. Chem. Soc.$, **79**, 3397 (1957).

(8) Various experiments with ¹⁵N- and ¹⁸O-labeled nitrosobenzene or phenylhydroxylamine [M. M. Shemyakin, V. I. Maimind, and B. K. Vaichunaite, $Izv. Akad. Nauk SSSR, Otd. Khim. Nauk$, 1260 (1957); S. Oae, T. Fukumoto, and M. Yamagami, $Bull. Chem. Soc. Japan$, **36**, 728 (1963)] are consistent with the proposed condensation mechanism.

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Inhibition of an Ester Hydrolysis by Imidazole

$Sir:$

Since the reports of Bender and Turnquest[1a] and Bruice and Schmir[1b] on the imidazole-catalyzed hydrolysis of p-nitrophenyl acetate, many studies have been made of the catalytic properties of imidazole.[1b-3] It is generally recognized that imidazole can enhance markedly the rate of hydrolysis (via a nucleophilic catalysis) of phenyl esters and thiolesters, and that it may have a moderate general base catalytic effect on the hydrolysis of aliphatic esters. In this paper we report an instance of ester hydrolysis significantly $inhibited$ by imidazole.

Aqueous solutions containing known concentrations of imidazole and sodium hydroxide were equilibrated at 25.0°. A suitable aliquot of a stock solution of methyl $trans$-cinnamate in acetonitrile was added, and the absorbance of the solution was monitored at 295 mμ. From plots of log $(A_t - A_\infty)$ $vs.$ time the apparent first-order rate constants were evaluated. A progressive decrease in this rate constant was observed with increase in imidazole concentration. Some typical data follow (at 25.0°, in 0.0181 N NaOH containing 0.4% acetonitrile, with ionic strength 1.0): at molar imidazole concentrations of 0.00, 0.08, 0.16, 0.32, and 0.40, the observed first-order rate constants

(1) (a) M. L. Bender and B. W. Turnquest, $J. Am. Chem. Soc.$, **79**, 1652 (1957); (b) T. C. Bruice and G. L. Schmir, $ibid.$, **79**, 1663 (1957).

(2) M. L. Bender and B. W. Turnquest, $ibid.$, **79**, 1656 (1957); M. L. Bender, $Chem. Rev.$, **60**, 53 (1960); W. P. Jencks and J. Carriuolo, $J. Am. Chem. Soc.$, **82**, 675 (1960); W. P. Jencks and J. Carriuolo, $ibid.$, **83**, 1743 (1961); T. C. Bruice and S. J. Benkovic, $ibid.$, **86**, 418 (1964).

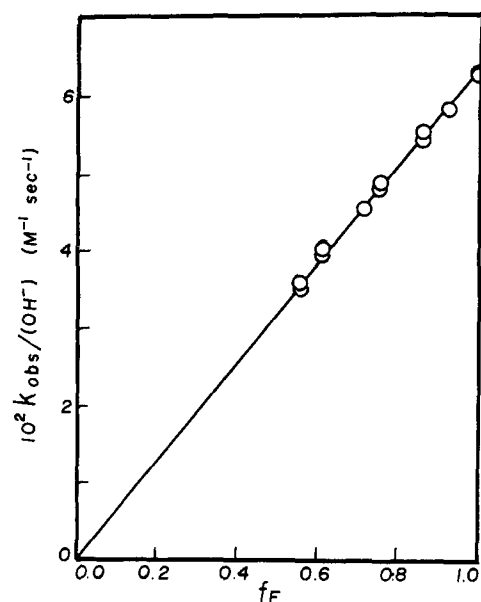(3) J. F. Kirsch and W. P. Jencks, $ibid.$, **86**, 837 (1964).

Figure 1. Plot of the equation $k_{obsd}/(OH^-) = kf_E$ for the imidazole-inhibited hydrolysis of methyl cinnamate.

were (in sec.$^{-1}$), respectively, 0.00112, 0.00105, 0.00098, 0.00086, and 0.00082.

The inhibitory effect of the imidazole, though small, is real. The decrease in rate of hydrolysis of methyl cinnamate caused by imidazole is more pronounced than would be expected from a nonspecific solvent effect. (Addition of 0.4 M acetonitrile changes the rate constant from 0.00112 to 0.00108 sec.$^{-1}$.) A very pertinent comparison to be made is between these data and similar studies carried out with ethyl acetate[3]; in the latter case a slight acceleration of the rate was observed. An interpretation of the results reported here, which is consistent with all available information, postulates an equilibrium interaction of the ester (E) and the imidazole (I) to form a 1:1 complex (C) that is relatively unreactive toward hydroxide ion.[4]

$$E + I \underset{}{\overset{K}{\rightleftarrows}} C$$

$$E + OH^- \xrightarrow{k} products$$
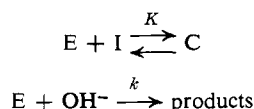
With this reaction scheme, the velocity may be written $v = k(OH^-)f_E(E_t)$, where (E_t) is the sum of concentrations of free and complexed ester and f_E is the fraction of ester in the free form. The experimental rate equation is $v = k_{obsd}(E_t)$. Combining these relations gives $k_{obsd}/(OH^-) = kf_E$. A plot of $k_{obsd}/(OH^-)$ vs. f_E should give a straight line passing through the origin; the slope should equal k, which of course can be independently determined.

The calculation of f_E requires knowledge of K, the complex formation constant. This was obtained at 25.0° and at several pH's using the solubility techniques.[5] As expected, evidence of interaction was obtained, and K was estimated to be 1.0 ± 0.1. K was independent of pH in the range 7.2–8.3. The plot of $k_{obsd}/(OH^-)$ against f_E is shown in Figure 1

(4) This mechanism ignores some possible reactions, such as imidazole-catalyzed hydroxide ion attack of the ester, but the data may be accounted for with the postulated scheme.

(5) T. Higuchi and K. A. Connors, Advan. Anal. Chem. Instrumentation, in press.

(which includes more data than are reported above). Evidently the postulated scheme is consistent with the data. This interpretation of the results follows closely the analysis of Higuchi and his co-workers in their studies of the inhibition of hydrolysis of local anesthetic esters by xanthine derivatives.[6,7]

This communication is, we believe, the first report of an inhibitory effect of imidazole in a nonenzymatic homogeneous system. The results may be of significant aid in suggesting mechanisms for the specificity behavior of enzymes and of drug–receptor interactions. It is particularly interesting that a functional entity (i.e., imidazole) that has been widely implicated as a catalytic function in proteolytic enzymes may also act to prevent attack on a substrate by a nucleophilic reagent (hydroxide ion in the present case). This observation leads to the idea that enzyme specificity could, in part, be a result of a functional unit in the active site interacting to a greater or lesser extent with the substrate, thus (in one conceivable mode) preventing attack by a catalytic function in the active site; a range of reactivities would be possible, with the rate of reaction dependent upon the extent of complexation between the substrate and the interacting function of the active site. Other mechanisms could be postulated. We are presently extending this study to consider the effects that the structures of the substrate, inhibitor, and attacking agent have on the inhibition phenomenon.

(6) T. Higuchi and L. Lachman, J. Am. Pharm. Assoc., 44, 521 (1955); L. Lachman, L. J. Ravin, and T. Higuchi, ibid., 45, 290 (1956).

(7) An alternate interpretation may be given in terms of the effect of imidazole on the activity coefficients of the reactants. In this interpretation f_E becomes an activity coefficient, and the solubility study provides an estimate of this quantity. In a sense this hypothesis is equivalent to the complex formation hypothesis, with the latter interpretation furnishing a mechanism for the activity coefficient effect. It is interesting that T. C. Bruice and R. N. Topping, J. Am. Chem. Soc., 85, 1488 (1963), have demonstrated complex formation between imidazole species and α-aminophenylacetic acid.

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Batrachotoxin. The Active Principle of the Colombian Arrow Poison Frog, Phyllobates bicolor

Sir:

The venom obtained by Märki and Witkop[1] from the skin of the Colombian arrow poison frog, Phyllobates bicolor, is the most active venom so far known (Table I). Our recent expedition (December 1963–January 1964) to the Choco rain forest of Western Colombia[2] netted 2400 frogs whose skin extracts yielded a total of 30 mg. of the crystalline major active principle which we name batrachotoxin.

(1) F. Märki and B. Witkop, Experientia, 19, 239 (1963).

(2) We are greatly indebted to Mrs. Martè Latham, who organized and guided this difficult and dangerous venture. A colorful report on this expedition is scheduled to appear in the National Geographic Magazine.