Like nitrobenzene,[15] CF_3NO_2 can be photolyzed in tetrahydrofuran to produce the hydrogen atom adduct, $CF_3N(O\cdot)OH$: $A_N = 22.75$ gauss and $A_F = 6.85$ gauss. Commercial CF_3NO (Peninsular Chemresearch Inc.) which may contain a trace of CF_3NO_2 was used.

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Direct Measurement of the Rate of Hydrogen-Atom Exchange between a Phenol and Its Phenoxy Radical

Sir:

A recent determination of the rate of hydroxyl-hydrogen-atom exchange between 2,4,6-tris-*t*-butylphenol and its phenoxy radical by nuclear magnetic resonance methods[1] may be subject to a significant systematic error. The contribution of the chemical reaction to the measured total rate of relaxation of nuclear magnetization was obtained by subtraction of an estimated dipolar rate. In the case under consideration, the latter was an uncomfortably large fraction of the total rate.

We have now measured the rate of the reaction by a direct kinetic method. Solutions of 2,4,6-tris-*t*-butylphenoxy radical and of 3,5-dideuterio-2,4,6-tris-*t*-butylphenol were mixed rapidly by a standard stopped-flow method, and the subsequent exchange reaction was observed by measurement of the time dependence of the intensity of the esr absorption at a convenient point in the spectrum. During the course of the reaction, the esr spectrum changes from one characteristic of the protonated radical with its 1–2–1 pattern with 1.67-gauss splitting to a superposition of the spectra of the protonated and deuterated radicals present in the equilibrium mixture. The latter has a 1–2–3–2–1 pattern with deuterium splitting of 0.25 gauss. For the purpose of our measurement, it was advantageous to work at moderate resolution so that neither the proton splittings of the *t*-butyl groups nor the deuterium splittings were resolved. Because of the poor signal-to-noise ratio at the short integrating times required for faithful recordings of the kinetic curves, it was necessary to sum between 20 and 30 repetitions in a multichannel analyzer.

Our data yielded a second-order rate law with $k = 219 \pm 18\ M^{-1}\ \mathrm{sec}^{-1}$ at 21° in carbon tetrachloride solution. The result from the line-broadening experiment was $k = 330 \pm 23\ M^{-1}\ \mathrm{sec}^{-1}$. The small deuterium isotope effect observed in the earlier experiments was verified by direct measurement: $k_{\mathrm{OH}}/k_{\mathrm{OD}} = 1.24$.

A difference of $100\ M^{-1}\ \mathrm{sec}^{-1}$ between the rate constants obtained by the two methods exists. Part of the discrepancy may be accounted for by the difference in concentration of phenol at which the two experiments were carried out. In the nuclear magnetic resonance experiment the concentrations were in the neighborhood of 1 *M;* in the stopped-flow experiment they were near $5 \times 10^{-3}\ M$. It is likely that $100\ M^{-1}\ \mathrm{sec}^{-1}$ is the maximum error in the rate constants reported in the earlier work. The constants for the more rapid reaction ($k \sim 10^3\ M^{-1}\ \mathrm{sec}^{-1}$) are thus probably correct to 10%.

A more complete analysis of the interpretation of the nuclear magnetic resonance data as well as a detailed description of the fully automated stopped-flow apparatus will be presented in a subsequent publication.

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Studies on Polypeptides. XXXIX. Elimination of the Imidazole Portion of Histidine as an Essential Site for Biological Function of Angiotensin[1-3]

Sir:

Paiva and Paiva[4] photolyzed [Asn^1-Val^5]-angiotensin II in an atmosphere of oxygen in presence of methylene blue and observed that the decrease in biological activity (pressor, oxytocic, and myotropic) paralleled the destruction of the imidazole portion of the histidine residue. They concluded from this experiment that the imidazole ring was essential for biological activity. Schröder replaced the histidine in [Asn^1-Val^5]-angiotensin II by phenylalanine ([Asn^1-Val^5-Phe^6]-angiotensin II)[5] and lysine ([Asn^1-Val^5-Lys^6]-angiotensin II)[6] and found the corresponding analogs to possess a very low order of biological activity (Table I). These findings appeared to support the conclusions of Paiva, *et al.*[4]

In order to assess the importance for angiotensin activity of the acid–base characteristics of the imidazole portion of histidine, we synthesized [Val^5-Pyr(3)Ala^6]-angiotensin II and evaluated some of its biological properties. In this peptide the histidine residue of [Val^5]-angiotensin II is replaced by the isosteric β-(pyrazolyl-3)-L-alanine.

The advantages of Pyr(3)Ala for evaluating the role for biological activity of the acid–base properties of the imidazole portion of histidine have been discussed.[7]

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(1) See K. Hofmann, H. Bohn, and R. Andreatta, *J. Am. Chem. Soc.*, **89**, 7126 (1967), for paper XXXVIII in this series.

(2) The authors wish to express their appreciation to the U. S. Public Health Service for generous support of this investigation.

(3) The amino acid residues are of the L configuration. The following abbreviations are used: β-(pyrazolyl-3)-alanine = Pyr(3)Ala; TFA = trifluoroacetic acid; AP-M = aminopeptidase M (G. Pfleiderer, P. G. Celliers, M. Stanulovic, E. D. Wachsmuth, H. Determann, and G. Braunitzer, *Biochem. Z.*, **340**, 552 (1964)). Thin layer chromatograms were performed in the systems 1-butanol–acetic acid–water 60:20:20 (R_f) and 1-butanol–pyridine–acetic acid–water 30:20:6:24 (R_f^{III}).

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