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.

Acknowledgment. We are grateful to Mr. H. N. Blount for obtaining the polarographic results. This work was supported by AFOSR(SRC)-OAR U.S.A.F. Grant No. 1069-66.

(15) R. L. Ward, *J. Chem. Phys.*, **38**, 2588 (1963).

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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 ± 18 M^{-1} sec^{-1} at 21° in carbon tetrachloride solution. The result from the line-broadening experiment was k = 330 ± 23 M^{-1} sec^{-1}. The small deuterium isotope effect observed in the earlier experiments was verified by direct measurement: k_{OH}/k_{OD} = 1.24.

A difference of 100 M^{-1} 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 × 10^{-3} M. It is likely that 100 M^{-1} 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} 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.

Acknowledgment. This work was supported by The National Science Foundation, The National Institutes of Health, and the Petroleum Research Fund of the American Chemical Society.

(1) R. Krelick and S. I. Weissman, *J. Am. Chem. Soc.*, **88**, 2645 (1966).

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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]

(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}).

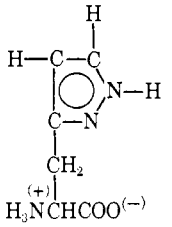
(4) A. C. M. Paiva and T. B. Paiva, *Biochim. Biophys. Acta*, **48**, 412 (1961).

(5) E. Schroder, *Ann. Chem.*, **680**, 142 (1964).

(6) E. Schroder and R. Hempel, *ibid.*, **684**, 243 (1965).

Table I. Biological Activity of [5-Valine]-angiotensin II Analogs

Analog	Rat pressor, %: Pithed	Rat pressor, %: Nephrectomized	Guinea pig myotropic, %
H-Asn-Arg-Val-Tyr-Val-His-Pro-Phe-OH ([Asn¹-Val⁵]-angiotensin II)	100	100	100
H-Asn-Arg-Val-Tyr-Val-Phe-Pro-Phe-OH ([Asn¹-Val⁵-Phe⁶]angiotensin II)		1	
H-Asn-Arg-Val-Tyr-Val-Lys-Pro-Phe-OH ([Asn¹-Val⁵-Lys⁶]-angiotensin II)		0.1	
H-Asp-Arg-Val-Tyr-Val-Pyr(3)ala-Pro-Phe-OH ([Val⁵-Pyr(3)Ala⁶]-angiotensin II)	78.9 ± 1.33	56.6 ± 2.6	52.0 ± 0.98



β-(pyrazolyl-3)-alanine

We find that [Val⁵-Pyr(3)Ala⁶]-angiotensin II exhibits surprisingly high pressor and myotropic activities (Table I).

The pressor activity of the pyrazole analog was assayed in two rat preparations against [Asn¹-Val⁵]-angiotensin II (angiotensinamide Ciba). In the pithed preparation the entire central nervous system was destroyed and the animal was maintained on artificial respiration. With this preparation the shape of the pressor response was almost identical with that of the reference compound. With bilaterally nephrectomized rats anesthetized with pentobarbital and pretreated with "pentolinium tartrate" the shapes of the pressor response curves were identical, but the dose–response curve did not parallel that obtained with the reference standard. The value recorded in Table I is an average of the per cent response at four dose levels. Myotropic activity was assayed on the isolated ileum of the guinea pig, and here again the dose–response curve of the analog was shifted to the right. Detailed accounts of the biological properties of [Val⁵-Pyr(3)Ala⁶]-angiotensin II, including its ability to elicit aldosterone release, will be presented elsewhere.

For the synthesis of [Val⁵-Pyr(3)Ala⁶]-angiotensin II, the azide of benzyloxycarbonylaspartylarginylvalyltyrosine was coupled with *t*-butyl valyl-β-(pyrazolyl-3)-alanylprolylphenylalaninate (*Anal.* Found for hemihydrate: C, 62.2; H, 7.5; N, 14.7; O, 15.6; $[\alpha]^{26}D$ −52.81° (*c* 1.352, water); single chlorine- and ninhydrin-positive spot with R_f^{I} 0.76; R_f^{III} 0.69; amino acid ratios in acid hydrolysate $Val_{1.01}Pyr(3)Ala_{0.97}Pro_{1.01}Phe_{1.02}$) to give *t*-butyl benzyloxycarbonylaspartylarginylvalyltyrosylvalyl-β-(pyrazolyl-3)-alanylprolylphenylalaninate which was partially deblocked by exposure to TFA. The ensuing crude benzyloxycarbonyl octapeptide was purified by chromatography on the ion exchanger AG-1 X2. Hydrogenolysis of the homogeneous benzyloxycarbonyl octapeptide afforded [Val⁵-Pyr(3)Ala⁶]-angiotensin II ($[\alpha]^{27}D$ −47.5° (*c* 0.29, 20% aqueous dioxane); amino acid ratios in AP-M digest $Asp_{1.04}Arg_{1.04}Val_{1.95}Tyr_{1.04}Pyr(3)Ala_{0.99}Pro_{0.99}Phe_{0.94}$; single ninhydrin-, Sakaguchi-, and chlorine-positive spot with R_f^{I} 0.49; R_f^{III} 0.55; peptide content 93% based on amino acid analysis).

(7) K. Hofmann and H. Bohn, *J. Am. Chem. Soc.*, **88**, 5914 (1966).

The results which are presented in this communication demonstrate conclusively that the pressor and myotropic activities of angiotensin do not depend on the characteristic acid–base properties of the imidazole ring. In conjunction with the experiments of Paiva and Paiva[4] and Schröder,[5,6] they indicate further that the stereo structure of the five-membered heterocyclic ring of histidine and not its charge is of crucial significance for high-level angiotensin activity.

To date, we have explored the effect on biological activity of imidazole–pyrazole replacements with three peptides, *i.e.*, S-peptide,[8] [Gln⁵]-β-corticotropin$_{1-20}$ amide,[1] and [Val⁵]-angiotensin II. The results demonstrate different roles for histidine residues in biologically active peptides. The acid–base character of imidazole appears to be of key significance in those situations where this ring system plays a direct role in a catalytic event. This fact was demonstrated unequivocally for histidine-12 in pancreatic ribonuclease S′ by the observation[8] that [Pyr(3)Ala¹²]-S-peptide$_{1-14}$ competes with S-peptide for S-protein to form an inactive S-protein-[Pyr(3)Ala¹²]-S-peptide$_{1-14}$ complex. The role of histidine in the corticotropins and angiotensin is different. In these molecules the stereochemistry of the five-membered heterocyclic ring and very likely its aromatic character appear to contribute to the binding between peptide and receptor. The results to date indicate that the charge difference between imidazole and pyrazole does not influence significantly the forces which are responsible for this binding.

Acknowledgment. The skillful technical assistance of Miss Judy Montibeller and Miss Jean Yevick is gratefully acknowledged.

(8) F. M. Finn and K. Hofmann, *ibid.*, **89**, 5298 (1967).
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Norbornadien-7-oneiron Tricarbonyl

Sir:

Recent interest in the elusive norbornadien-7-one system (**1**)[1] prompts us to record the successful synthesis

(1) S. Yankelevich and B. Fuchs, *Tetrahedron Letters*, 4945 (1967), and references cited therein.