Table I. Peptide Syntheses with EEDQ (I)

Starting acid and amine	Peptide	Reaction solvent[a]	Reaction time, hr	Yield, %
Benzoic + aniline	Benzanilide	B	12[b]	85
L-Bz-Leu + Gly-OEt	L-Bz-Leu-Gly-OEt	THF; B or B–EtOH (4:1)	6–7	95
Bz-Gly + Gly-OEt	Bz-Gly-Gly-OEt	B–EtOH (1:1)	2	99
Bz-Gly + aniline	Bz-Gly-anilide	B	2	90
CBZ-L-Ala + L-Ala-OEt	CBZ-L-Ala-L-Ala-OEt	B–EtOH (1:1)	1	98
CBZ-D-Ala + Gly-OEt	CBZ-D-Ala-Gly-OEt	B	3	90
CBZ-L-Ala + Gly-OEt	CBZ-L-Ala-Gly-OEt	B	3	90
p-Nitrobenzoic + DL-Ser-OEt	*p*-Nitro-Bz-Ser-OEt	B–EtOH (4:1)	3	60[c]
Cinnamic + imidazole	Cinnamoylimidazole	B	12[b]	60[c]

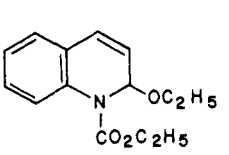
[a] B = benzene; THF = tetrahydrofuran. [b] A shorter reaction time was not tried. [c] Optimum conditions were not determined.

(I, EEDQ) was recently disclosed.[1] An investigation of the chemical behavior of this compound showed that it can readily induce the formation of peptide linkages. The new reagent allows the coupling in high yield of acylamino acids with amino acid esters in a single operation and without racemization. The coupling reaction was carried out at 30–35° in benzene, ethanol, or tetrahydrofuran (THF). In order to detect the possible occurrence of racemization, the supersensitive Young test,[2-4] involving the synthesis of Bz-Leu-Gly-OEt, was applied.

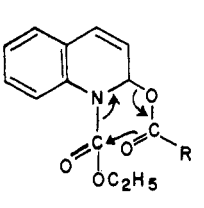
A typical procedure was as follows. To a solution of 0.003 mole of Bz-Leu and 0.003 mole of Gly-OEt in 25 ml of THF was added 0.0032 mole of I and the mixture was stirred at room temperature for 6–7 hr. After evaporation, the residue was crystallized from ethyl acetate–petroleum ether (bp 30–60°) to give Bz-Leu-Gly-OEt (95% yield), mp 157–158°, $[\alpha]^{25}D$ −33.5 ± 0.5° (*c* 3, EtOH); washing the crude material with warm absolute ether followed by evaporation of the ether and fractional crystallization of the residue from EtOH–H_2O gave no detectable quantity of racemic peptide.[4] Similar results were obtained using benzene or benzene–EtOH mixtures. Additional examples of peptide bond syntheses using I as the coupling agent are assembled in Table I.

Experimental evidence was obtained that the mechanism of carboxyl group activation by I involves the transient formation of a mixed carbonic anhydride intermediate. Whereas I fails to react with amines under conditions of ready peptide synthesis, its 2-ethoxy substituent is readily displaced by alcohols and thiols in the presence of an acid catalyst.[1] However, with carboxylic acids such as benzoic and cinnamic acids, the expected product of exchange II cannot be isolated (even at low temperatures) presumably because of rapid breakdown by way of a six-membered transition state (II, arrows) to quinoline and the mixed carbonic anhydride III. In fact, the mixed anhydrides of benzoic and cinnamic acids could be readily detected and characterized (ir, nmr, mass spectrometry) when the reaction was carried out in the absence of amines. The selective activation by I of carboxyl functions in the presence of other nucleophiles and the absence of racemization in the product peptide may offer special advantages over other well-known coupling reagents.[5] The use of I as a substitute in the classical mixed carbonic anhydride method[3-5] is indicated because the slow formation and rapid consumption of intermediate III precludes its accumulation and thus minimizes the possibility of side reactions (such as racemization). The reaction of I with various enzymes will be reported separately.

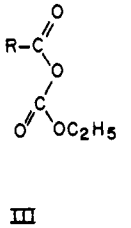
Acknowledgment. The authors are indebted to the National Research Council of Canada for the financial support of this work.



I



II



III

(1) B. Belleau, R. Martel, G. Lacasse, M. Ménard, N. L. Weinberg, and Y. G. Perron, *J. Am. Chem. Soc.*, 90, 823 (1968).
(2) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).
(3) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, 89, 5012 (1967).
(4) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, 88, 1338 (1966).
(5) M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience Publishers, Inc., New York, N. Y., 1966.

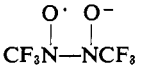
B. Belleau, G. Malek
Department of Chemistry, University of Ottawa
Ottawa, Ontario, Canada

Received January 10, 1968

Electron Spin Resonance Study of the Electrolysis of Trifluoronitrosomethane and Trifluoronitromethane. Bis(trifluoromethyl) Semidiazoxide

Sir:

Various perfluoro nitroxides have been studied by electron spin resonance.[1] We report here the electron spin resonance of bis(trifluoromethyl) semidiazoxide[2]



(1) W. D. Blackley and R. R. Reinhard, *J. Am. Chem. Soc.*, 87, 802 (1965); W. D. Blackley, *ibid.*, 88, 480 (1966); E. T. Strom and A. L. Bluhm, *Chem. Commun.*, 115 (1966).

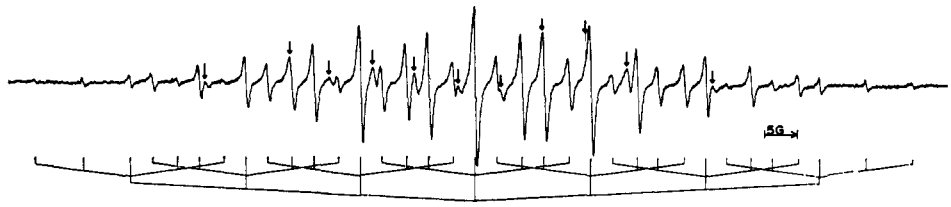


Figure 1. Spectrum of bis(trifluoromethyl) semidiazoxide radical anion. Arrows indicate the positions of trifluoromethylnitromethane radical anion peaks.

Electrolytic reduction of trifluoronitrosomethane (CF_3NO) at its half-wave potential, -0.25 V *vs.* saturated aqueous calomel electrode, in either N,N-dimethylformamide, acetonitrile, or dimethyl sulfoxide, produces the spectrum in Figure 1. The spectrum is interpreted in terms of a heptet due to six equivalent fluorine nuclei ($I = {}^1/_2$) split into pentets of intensity 1:2:3:2:1 due to two equivalent nitrogen nuclei ($I = 1$). The most reasonable structure for the radical is the semidiazoxide radical.[3] Coupling constants are given in Table I.

Table I. Coupling Constants for $CF_3\overset{O\cdot}{N}\!-\!\overset{O^-}{N}CF_3$ and $CF_3NO_2\cdot^-$ [a]

		CH_3CN	CH_3CN[b]	DMSO	DMSO[b]	DMF	DMF[b]
$CF_3\overset{O\cdot}{N}\!-\!\overset{O^-}{N}CF_3$	A_N	17.74	16.85	18.04	17.83	18.26	17.92
	A_F	7.38	7.58	7.38	7.36	7.36	7.45
$CF_3NO_2\cdot^-$	A_N	20.20	20.94	19.99	20.30	20.04	20.65
	A_F	12.71	11.33	13.26	12.75	13.26	12.32

[a] In gauss at room temperature; coupling constants were calculated utilizing the Varian Fieldial scan calibrations. [b] ~6% water by volume.

The vertical arrows in Figure 1 indicate a second radical species of slightly higher g value. The underlying spectrum is enhanced by electrolysis at potentials greater than -0.25 V and consists of a 1:1:1 triplet split into quartets. This spectrum is not due to CF_3NO but instead has proved to be trifluoronitromethane radical anion ($CF_3NO_2\cdot^-$) since electrolytic reduction of CF_3NO_2 gives a spectrum identical with the underlying spectrum in Figure 1.[4]

The trifluoronitrosomethane radical anion has not been detected to date, only the semidiazoxide, even in extremely dilute solutions. The strong acceptor properties of the trifluoromethyl group probably account for the unusual stability of this radical anion. Semidiazoxide formation is not hindered by the presence of oxygen or the addition of nitrosobenzene, benzaldehyde, anisaldehyde, or hexafluoroacetone.

Two of the more probable routes for semidiazoxide radical formation are given by eq 1, 2a, and 2b.[6] Russell has concluded that azoxybenzene formation in

$$CF_3NO \xrightarrow{e} CF_3NO\cdot^- \quad (1)$$

$$CF_3NO\cdot^- \xrightarrow{CF_3NO} CF_3\overset{O\cdot}{N}\!-\!\overset{O^-}{N}CF_3 \quad (2a)$$

$$2CF_3NO\cdot^- \longrightarrow CF_3\overset{O^-}{N}\!-\!\overset{O^-}{N}CF_3 \xrightarrow{CF_3NO} CF_3\overset{O\cdot}{N}\!-\!\overset{O^-}{N}CF_3 \quad (2b)$$

basic solutions of nitrobenzene proceeds *via* dimerization of the nitrosobenzene anion radical.[6] Diols and diol dianions of the type $RN(O^-)N(O^-)R$ have been proposed as intermediates in the formation of azoxy compounds from aromatic nitroso compounds and arylhydroxylamines.[7,8] Like nitrosobenzene,[7] CF_3NO reacts with hydroxide ions[9] in the presence of air to give $CF_3NO_2\cdot^-$ but with potassium *t*-butoxide in DMSO the semidiazoxide radical is produced. In aqueous sodium hydroxide CF_3NO is known to produce CF_3NO_2 and hexafluoroazoxymethane ($CF_3N{=}N^+(O^-)CF_3$).[10] The systems are thus analogous. The addition of water to $CF_3NO_2\cdot^-$ leads to an increase in the nitrogen coupling.[11,12] In both radicals the change in fluorine coupling with addition of water is opposite in direction to the change in nitrogen coupling. Although the mechanism whereby the nitrogen coupling is increased by protic solvents is still open to question,[13] it appears that the data illustrate another case of spin transfer to fluorine *via* a 1,3 p-π interaction.[14]

(2) This nomenclature is derived from considering the reduction of a diazoxide as analogous to the reduction of quinones and α-diketones which produces semiquinones and semidiones.

$$RN(O^-)\!=\!N(O^-)R \xrightarrow{e} RN(O\cdot)\!-\!N(O^-)R$$

(3) A spectrum of six lines ($A_F \simeq A_N$ = 10.8–12.5 gauss) partially resolved into doublets (2.8 gauss) has been previously assigned to this radical (V. A. Ginsburg, A. N. Medvedev, S. S. Dubov, and M. F. Lebedeva, *Dokl. Akad. Nauk SSSR*, 167, 1083 (1966); *Chem. Abstr.*, 65, 598 (1966); V. A. Ginsburg, A. N. Medvedev, M. F. Lebedeva, S. S. Dubov, and A. Ya. Yakubovich, *Zh. Obshch. Khim.*, 35, 1418 (1965); *Chem. Abstr.*, 63, 14672 (1965)). We have also observed this spectrum under various reaction conditions with a variety of reagents but are unable to make a structural assignment for the radical at this time. The previous assignment of this spectrum to the bis(trifluoromethyl) semidiazoxide radical anion becomes untenable in light of the spectrum described here. We thank a reviewer for pointing out this work.

(4) Trifluoronitromethane was synthesized by hydrogen peroxide oxidation of trifluoronitrosomethane.[5] The half-wave reduction potential of CF_3NO_2 is -0.72 V in MeCN.

(5) J. Jander and R. N. Haszeldine, *J. Chem. Soc.*, 912 (1954).

(6) CF_3NO is known to be monomeric.[5] CF_3NO is blue whereas CF_3NO_2, $CF_3NO_2\cdot^-$, and the semidiazoxide are colorless.

(7) G. A. Russell and E. J. Geels, *J. Am. Chem. Soc.*, 87, 122 (1965); G. A. Russell, E. J. Geels, F. J. Smentowski, K.-Y. Chang, J. Reynolds, and G. Kaupp, *ibid.*, 89, 3821 (1967).

(8) M. M. Shemyakin, V. I. Maimind, and B. K. Vaichunaite, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1260 (1957); *Chem. Abstr.*, 52, 6231 (1958); L. A. Neiman, V. I. Maimind, and M. M. Shemyakin, *Tetrahedron Letters*, 3157 (1965).

(9) Small quantities of a commercial solution of benzyltrimethylammonium hydroxide in methanol were used in tetrahydrofuran.

(10) J. Jander and R. N. Haszeldine, *J. Chem. Soc.*, 919 (1954).

(11) The same effect is found in nitrobenzene radical anions: W. M. Gulick, Jr., and D. H. Geske, *J. Am. Chem. Soc.*, 87, 4049 (1965), and references therein. For trifluoromethylnitrobenzene radical anions see E. G. Janzen and J. L. Gerlock, *ibid.*, 89, 4902 (1967).

(12) A solvent effect in nitroalkane radical anions has not been found: L. H. Piette, P. Ludwig, and R. N. Adams, *ibid.*, 83, 3909 (1961); E. W. Stone and A. H. Maki, *J. Chem. Phys.*, 37, 1326 (1962).

(13) Compare J. M. Gross and M. C. R. Symons, *J. Chem. Soc., Sect. A*, 451 (1966), and P. Ludwig, T. Layloff, and R. N. Adams, *J. Am. Chem. Soc.*, 86, 4568 (1964), with P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, 39, 609 (1963).

(14) P. J. Scheidler and J. R. Bolton, *J. Am. Chem. Soc.*, 88, 372 (1966).

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).

John L. Gerlock, Edward G. Janzen

Department of Chemistry, The University of Georgia
Athens, Georgia 30601

Received December 9, 1967

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).

Martin R. Arick, S. I. Weissman

Department of Chemistry, Washington University
St. Louis, Missouri 63130

Received January 26, 1968

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).