the filtrate after evaporation upon trituration with ether (17.7 mg, 90%, mp 163–170° dec).

The product was characterized as follows: the NH_2-terminal residue was determined by the dansyl chloride method[12] to be glutamic acid, without a trace of the previous leucine amino terminus; thin layer chromatography gave a single spot (R_f 0.1),[13] and high-voltage electrophoresis gave single spots in pyridinium acetate buffer pH 3.6 (R_f 0.78) and at pH 6.5 (R_f 0.61), with L-lysine as reference (R_f 1.0); digestion with aminopeptidase M[14] (AP-M) was complete as judged by paper and thin layer chromatography and gave ratios upon amino acid analysis of Glu 0.96, Lys 1.66, Ser 1.00, Leu 1.0, Pro 0.86, confirmed by acid hydrolysis (6 *N* HCl, sealed evacuated tube, 110°, 20 hr) and consistent with 100% coupling efficiency without racemization at the carboxyl-terminal serine residue. Coupling conditions with NEPIS, in which reaction time was held to 2 hr with fourfold excess of soluble tetrapeptide and in which only twofold excess was used for 6- to 48-hr reaction time, gave incomplete reactions.

The same peptide coupling reaction was carried out with DCC plus hydroxysuccinimide (HOSu) as the coupling agent.[11] Complete coupling without detectable racemization was obtained in 2 hr at room temperature. Dansylation showed NH_2-terminal glutamic acid only and complete digestion with AP-M yielded the following amino acid ratios: Glu 1.06, Lys 1.99, Ser 1.01, Leu 1.0, Pro 0.98. Reaction for 4 hr at 0°, or reaction at room temperature using DCC without HOSu, gave a product in which dansyl analysis demonstrated a small proportion (<10%) of amino-terminal leucine and electrophoresis gave an additional minor spot.

The tripeptide *t*-BOC-L-Leu-L-Ala-L-Tyr-OH (0.19 mmol, 89 mg) in dimethylformamide was activated with equimolar NEPIS and triethylamine at 0° and added in fourfold excess to H_2N-(ε-trifluoroacetyl-L-lysyl)$_5$ polymer, shaken for 2 days at room temperature, washed, and cleaved with HBr–TFA to yield the partially protected octapeptide H_2N-Leu-Ala-Tyr-(ε-TFA-Lys)$_5$-OH as the HBr salt. Thin layer chromatography gave a single spot in three systems.[15] After removal of the ε-TFA groups with piperidine at 0° for 1 hr, electrophoresis gave a single spot with R_f 1.35 (Lys, R_f 1.0) at pH 3.6; dansyl analysis showed only leucine as the amino terminus. Digestion with aminopeptidase M gave Leu 1.09, Ala 0.94, Tyr 1.01, Lys 5.0. For comparison, the same octapeptide product was prepared from *t*-BOC-L-Leu-L-Ala-L-Tyr-$NHNH_2$ (0.10 mmol, 48 mg) by conversion at −15° with 4 equiv of HCl in dioxane and 4.4 equiv of freshly prepared $NaNO_2$ to the azide and prompt addition to the penta-ε-TFA-lysyl polymer at 0–4° for 20 hr. The crude product obtained upon cleavage gave R_f values identical with those above in chromatographic and electrophoretic systems, but contained about one-third unreacted pentalysine as estimated from amino acid ratios after acid hydrolyses and enzyme digestions.

(12) W. R. Gray in "Methods in Enzymology," Vol. XI, C. H. W. Hirs, Ed., Academic Press, New York, N. Y., 1967, p 139.

(13) 1-Butanol–acetic acid–pyridine–water (4:1:1:2).

(14) 2% AP-M by weight in 0.07 *M* phosphate buffer, pH 7.8; volume 0.3 ml; 37° overnight. (AP-M obtained from Henley and Co., New York, N. Y.).

(15) (a) R_f 0.86;[13] (b) 1-butanol–acetic acid–water (4:1:5), R_1 0.71; (c) pyridine–water (80:20), R_f 0.67.

Although several model peptides have been coupled successfully to peptide polymers, our preliminary experience in coupling sequences of the staphylococcal nuclease indicates that refinement of conditions may be required when using longer peptides or when coupling certain pairs of carboxyl- and amino-terminal residues. For example, coupling the hydrophobic octapeptide or tetrapeptide sequences (both containing COOH-terminal valine) corresponding to residues 32–39 or 36–39 of staphylococcal nuclease[7] to the nonapeptide polymer containing residues 40–48, with NH_2-terminal aspartic acid, gave only 20–30% coupling with either NEPIS or DCC. The aspartic acid residue may offer special difficulty. Thus, the dipeptide Z-L-Phe-L-Tyr-OH was coupled readily with DCC-HOSu to Leu-Pro polymer (aminopeptidase digestion gave Phe 1.03, Tyr 0.98, Leu 1.0, Pro 1.03; dansyl showed all Phe; thin layer chromatography and electrophoresis systems all gave a single spot), but under identical conditions was coupled only to the extent of 40% to the β-benzyl-L-aspartyl-terminal nonapeptide polymer.

Nevertheless, the modification of the solid-phase synthetic approach described here offers the advantages of fewer steps in a long sequence and of convenience in substituting residues in structural analog studies.

Furthermore, coupling peptide fragments rather than individual amino acid monomers should give a major advantage in the purification of the polypeptide product. The peptides with incomplete sequences that accumulate from less than quantitative coupling at any step might be removed if they differ from the completed peptide by the several residues constituting any fragment rather than by single residues.

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On the Mechanism of Oxidative Decarboxylation. The Potassium Persulfate Promoted Decarboxylation of Substituted Phenylacetic Acids

Sir:

Conflicting opinion on the detailed steps of the mechanism for the anodic oxidation of the salts of carboxylic acids has raised the question as to whether a stepwise process (A) is involved in the electrochemical reaction[1,2] or whether the rate-determining step is a concerted process[3] (B).

$$RCO_2^- \longrightarrow [RCO_2\text{---e (anode)}]^{\ddagger} \longrightarrow RCO_2\cdot + e \quad \text{(A)}$$
$$RCO_2\cdot \longrightarrow R\cdot + CO_2$$
$$RCO_2^- \longrightarrow [R\text{---}CO_2\text{---e (anode)}]^{\ddagger} \longrightarrow R\cdot + CO_2 + e \quad \text{(B)}$$

Mechanistically analogous reactions carried out in homogeneous solution which formally lead to carboxylate radicals have been discussed in terms of one-bond or multibond scission. The classic work of

(1) P. H. Reichenbacker, M. Y. Liu, and P. S. Skell, *J. Am. Chem. Soc.*, **90**, 1816 (1968), and the references cited therein.

(2) B. E. Conway, "Theory and Principles of Electrode Processes," The Ronald Press, New York, N. Y., 1965, pp 136, 166 ff, 244 ff.

(3) L. Eberson, *Acta Chem. Scand.*, 17, 2004 (1963).

Bartlett[4] on the mode of decomposition of *t*-butyl peresters gives a precedented example for an analogous reaction.

We wish to report here the preliminary results of our work on the mechanism of the potassium persulfate promoted decarboxylation of substituted phenylacetic acids which we feel may be relevant to the resolution of the conflicting opinions as to the nature of the transition state during one-electron-transfer processes from carboxylic acids or the aforementioned analogous reactions. Persulfate oxidation appears to be a promising method for a study of these reactions since Fichter and his coworkers[5-8] and Russell and Thomson[9] have shown that the products obtained on persulfate oxidation of aliphatic and aromatic acids are similar to those formed by anodic oxidation, although some caution must be exercised in the comparison of a heterogeneous reaction involving an electrode surface and a homogeneous chemical method for decarboxylation.

The decarboxylation by persulfate of salts of carboxylic acids has been previously described,[5-10] and the absolute kinetics for the oxidative decarboxylation of oxalic acid has been reported.[11] The partial mechanism for the decarboxylation in concentrated aqueous base (eq 1–4) is consistent with the reported results of

$$S_2O_8^{2-} \xrightarrow{k_1} 2SO_4\cdot^- \quad (1)$$

$$SO_4\cdot^- + H_2O \xrightarrow{k_2} HSO_4^- + \cdot OH \quad (2)$$

$$SO_4\cdot^- \text{ or } \cdot OH + RCO_2^- \xrightarrow{k_3} RCO_2\cdot + SO_4^{2-} \quad (3)$$

$$RCO_2\cdot \xrightarrow{k_4} R\cdot + CO_2 \quad (4)$$

these investigations. If a steady-state assumption is made for the concentration of $RCO_2\cdot$, then the rate of formation of carbon dioxide is equal to the rate of disappearance of the carboxylate anion. If two different

$$\frac{d(CO_2)}{dt} = \frac{-d(RCO_2^-)}{dt} = k_3(SO_4\cdot^-)(RCO_2^-)$$

carboxylic acids are run in the same solution, division and integration of the two resulting simultaneous expressions for the disappearance of the carboxylate anion from each of the carboxylic acids allows the direct computation of the relative rates of electron transfer from the pair of anions.

Competitive decarboxylation reactions between phenylacetic acid-1-C^{14} and nonradioactive substituted phenylacetic acids were carried out in degassed systems. Measurement of the total amounts of carbon dioxide formed from the acidified reactions of aqueous mixtures of phenylacetic acid-1-C^{14} (0.0132–0.0148 *M*), substituted phenylacetic acids (0.0132–0.0148 *M*), potassium hydroxide (0.26 *M*), and potassium persulfate (0.057 *M*) gave quantitative yields of labeled and unlabeled carbon dioxide at 20 kinetic half-lives for the decarboxylation reaction (20 hr). Quantitative determination of the amount of carbon dioxide formed during the course of the competitive decarboxylation reactions was carried out on acidified reaction mixtures using standard vacuum-line procedures.[12] The measured carbon dioxide was absorbed quantitatively in a mixture of ethanolamine–ethylene glycol monomethyl ether on the vacuum line, and the amount of radioactive carbon dioxide was determined using liquid scintillation counting techniques.[13]

A minimum of four or five determinations of each ratio of the relative rate constants at 74.3° was determined at varying percentages of completion of the reaction. The largest deviation for the separate determinations was 3.4%. The ratios of rate constants for the oxidative decarboxylation of various substituted phenylacetic acids relative to phenylacetic acid-1-C^{14} are listed in Table I.

Table I. Relative Rate Constants for the Potassium Persulfate Promoted Decarboxylation of Substituted Phenylacetic Acid at 74.3°

Substituent	Rel rate[a]
p-Methoxy	2.51 ± 0.09
p-Phenoxy	2.18 ± 0.02
p-Methyl	1.46 ± 0.03
H	1.00
p-Bromo	0.958 ± 0.008
p-Chloro	0.919 ± 0.007
m-fluoro	0.875 ± 0.010
m-Bromo	0.815 ± 0.006
m-Chloro	0.784 ± 0.020

[a] The values given are averages of four or five independent determinations and the errors reported are average deviations from the mean value given.

The rate of electron transfer was found to decrease with the decreasing electron density at the benzylic carbon. This trend is opposite to that observed in the effects of substituents on the dissociation constants for substituted phenylacetic acids[14] and is consistent with substituent stabilization leading to the electron transfer from a carboxylate anion.

The data in Table I were fitted to the Hammett equation[15] and the following linear free energy correlations were obtained.

$$\log(k/k_0) = -0.600\sigma + 0.12 \qquad s = 0.081,\ r = 0.919$$

$$\log(k/k_0) = -0.436\sigma^+ + 0.06 \qquad s = 0.035,\ r = 0.983$$

The obvious good correlation with σ^+ values in comparison with the lack of correlation with σ substituent constants, using Jaffé's criteria for correlation (*r*, the correlation coefficient, and *s*, the standard deviation),[14b] militates for an interpretation of the electron transfer from the carboxylate anion as being a process concerted to some extent with the loss of carbon dioxide during the rate-determining step. The cor-

(4) (a) P. D. Bartlett and R. Hiatt, *J. Am. Chem. Soc.*, **80**, 1398 (1958); (b) P. D. Bartlett and R. E. Pincock, *ibid.*, **82**, 1764 (1960); (c) P. D. Bartlett and C. Rüchardt, *ibid.*, **82**, 1756 (1960); (d) P. D. Bartlett and D. M. Simons, *ibid.*, **82**, 1753 (1960).

(5) Fr. Fichter and H. Lapin, *Helv. Chim. Acta.*, 12, 993 (1929).

(6) Fr. Fichter and H. E. Suenderhauf, *ibid.*, **16**, 338 (1933).

(7) Fr. Fichter and J. Hees, *ibid.*, **18**, 704 (1935); **19**, 149 (1936).

(8) Fr. Fichter and L. Panizzon, *ibid.*, **15**, 996 (1932).

(9) J. Russell and R. H. Thomson, *J. Chem. Soc.*, 3379 (1962).

(10) R. H. Thomson and A. G. Wylie, *Proc. Chem. Soc.*, 65 (1963).

(11) For a review of this topic, see D. A. House, *Chem. Rev.*, **61**, 198 (1961).

(12) R. T. Sanderson, "Vacuum Manipulation of Volatile Compounds," John Wiley & Sons, Inc., New York, N. Y., 1948.

(13) H. H. Jeffay and J. Alvarez, *Anal. Chem.*, **33**, 612 (1961).

(14) (a) J. F. J. Dippy and H. B. Watson, *J. Chem. Soc.*, **161**, 1888 (1934); (b) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

(15) The σ constants used were taken from D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 3309 (1958); σ^+ constants were taken from the work of Y. Okamoto and H. C. Brown, *J. Am. Chem. Soc.*, **80**, 4979 (1958), except for the *p*-phenoxy substituent constant for which the value was taken from ref 16.

relation with σ^+ infers a direct resonance interaction of the substituent with the incipient benzyl radical, influencing to some extent resonance structure I of the proposed transition state for electron transfer.[16-19]

$$[ArCH_2CO_2^-SO_4\cdot^- \longleftrightarrow \underset{I}{ArCH_2^+CO_2\cdot^-SO_4^{2-}} \longleftrightarrow ArCH_2CO_2\cdot SO_4^{2-}]$$

As yet incomplete results from our laboratory on alkyl-, diaryl-, and triaryl-substituted acetic acids indicate a duality of mechanism, or a continuum for the degree of bond breaking during the electron-transfer process, which seems to correlate with the stability of the radical (R·) formed. The results of this work will be reported at a later date.

Acknowledgment. We are indebted to the National Research Council of Canada and the University of Alberta for their generous support of this work.

(16) G. A. Russell and R. C. Williamson, Jr., *J. Am. Chem. Soc.*, **86**, 2357 (1964).
(17) C. Walling and B. Miller, *ibid.*, **79**, 4181 (1957).
(18) B. R. Kennedy and K. U. Ingold, *Can. J. Chem.*, **44**, 2381 (1966).
(19) G. A. Russell, *J. Org. Chem.*, **23**, 1407 (1958).

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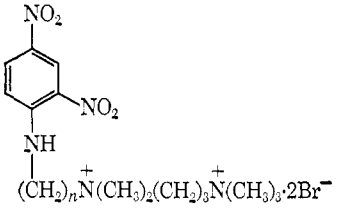
Topography of Nucleic Acid Helices in Solutions. XI. A Novel Method of Distinguishing between Ribo- and Deoxyribonucleic Acids by the Use of Reporter Molecules[1]

Sir:

We report the results of a preliminary study of the effect of double-stranded native and denatured ribo- and deoxyribonucleic acid helices on the uv and induced CD of the reporter molecules **1** and **2**. In line with our previous report on single- and double-stranded homo-



1, $n = 2$
2, $n = 3$

polymers,[1] substantial hypochromism in the near-uv absorption spectrum and an induced CD of the reporter molecules are observed on binding to nucleic acids. The effect of various polynucleotide systems on the absorption spectra of **1** and **2** is summarized in Table I. Several interesting points may be made. (1) The transition in the 350-mμ region is probably a charge-transfer $\pi^* \leftarrow \pi$ type since it has an extinction coefficient, ϵ_{max}, of 16,000, characteristic of an allowed transition, and a red shift in the maximum is observed in going from 95% ethanol to 0.01 *M* sodium phosphate buffer (338 → 350 mμ and 343 → 356 mμ for $n = 2$ and 3, respectively).[1,2] (2) Interaction of the reporter molecule with all the nucleic acids studied leads to a complex in which the environment of the chromophore is more polar than that of water since a further shift to the red is observed (350 → 355 mμ and 356 → 362 mμ for $n = 2$ and 3, respectively). (3) Substantial hypochromism is observed when the reporter molecule is bound to the polynucleotides. The extent of hypochromism appears to depend on the nucleic acid system and not on the number of methylene groups, n, between the ring nitrogen and quaternary nitrogen of the reporter molecule. The per cent hypochromicity of the reporter molecule is greater upon binding deoxyribonucleic as compared with ribonucleic acid (Table I). Moreover, there is a slight decrease in the hypochromism of **1** and **2** upon denaturing the nucleic acid (at least in the case of DNA).

Table I. The Effect of Ribo- and Deoxyribonucleic Acids on the Absorption Spectra of Reporter Molecules **1** and **2**[a]

	Reporter molecule							
	1, $n = 2$				**2**, $n = 3$			
Conditions	λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]	λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]
95% EtOH	338	16,600			343	16,050		
H_2O–buffer[d]	350	16,600			356	16,160		
Salmon testes								
DNA (N)[e]	355	10,060	65	80	362	10,340	56	80
DNA (D)[f]	355	10,780	53	80	361	10,300	57	80
Calf thymus								
DNA (N)[e]	355	10,000	66	74	362	9,680	67	74
DNA (D)[f]	355	10,860	52	74	361	10,660	52	74
Yeast								
RNA (N)[e]	354	12,300	35	80	361	11,100	46	80
RNA (D)[f]	355	11,860	40	80	361	11,295	43	80
Torula								
RNA (N)[e]	355	11,800	41	80	361	12,330	31	80
RNA (D)[f]	355	12,130	36	80	361	11,800	37	80

[a] At 25.0 ± 0.2° in 0.01 *M* sodium phosphate buffer, pH 6.40–6.50 (0.01 *M* in Na^+). All uv spectra were taken in 10-mm cells using a Cary 14 spectrometer at 25.0 ± 0.2°. Values of λ_{max} and ϵ_{max} in the presence of nucleic acid reported in this table are limiting values, *i.e.*, additional change in spectra is not observed at further excess of nucleic acid. [b] % hypochromicity (% H) = $[\epsilon_{max}(H_2O)/\epsilon_{max}(p) - 1.0]100$, where $\epsilon_{max}(H_2O)$ and $\epsilon_{max}(p)$ are the extinction coefficients in the absence and presence of the polynucleotides. [c] P/R indicates the ratio polynucleotide phosphate/reporter molecule. In all cases reported above, 5×10^{-5} *M* **1** or **2** was used. [d] In 0.01 *M* sodium phosphate buffer (0.01 *M* in Na^+), pH 6.50. [e] Native (N) calf thymus and salmon testes DNA are Worthington products Lot No. 642 and 6CFA, respectively. Yeast RNA (Lot 6234) and torula RNA (Lot 55711) were obtained from Worthington and Calbiochem, respectively. [f] Denatured (D) nucleic acids were obtained by heating in a boiling water bath for 15 min and then immediate quenching in ice water.

The dramatic effect, however, arises from the induced circular dichroism of **1** and **2** upon binding to RNA and DNA. Figure 1 shows the CD results of **1** and **2** bound to native calf thymus and salmon testes DNA and to yeast and torula RNA. A summary of the results including the molar ellipticities, $[\theta]$, of the (positive) peaks and (negative) troughs together with the associated wavelengths for the complexes formed with native and denatured DNA and RNA are shown in Table II.

(1) Part X: E. J. Gabbay and J. Mitschele, submitted for publication.
(2) The large red shifts observed on changing solvents (95% EtOH to H_2O) which correspond to 2.9 and 3.0 kcal for **1** and **2**, respectively, are indicative of an intramolecular charge-transfer transition: J. N. Murrell, "The Theory of Electronic Spectra of Organic Molecules," John Wiley & Sons, Inc., New York, N. Y., 1963, p 305.