Relative Influences of Electron-Withdrawing Functional Groups on Basicities of Amino Acid Derivatives[1]

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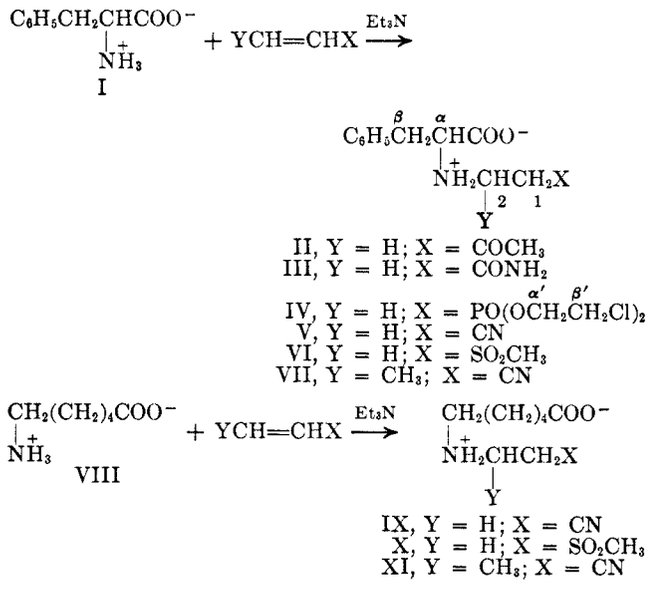
A series of phenylalanine and ε-aminocaproic acid derivatives were prepared by treating the amino groups with α,β-unsaturated compounds. The pK_2 values of the modified amino groups were determined at 30°. The decrease in basicities of the amino groups in the derivatives is presumably due to inductive and field effects of electron-withdrawing functional groups. A procedure to determine these effects is described. Basicities of amino groups in amino acids modified with vinyl compounds appear to be linearly related, with one striking exception, to reaction rates of amino groups with the same vinyl compounds. The results are useful for predictions of basicities, reaction rates, and of physicochemical properties of the substituted vinyl adducts of amino acids, peptides, and proteins.

The present investigation was initiated to establish whether acid–base equilibria of amino groups in amino acids may be modified in a systematic manner. The procedure used is offered as a convenient system to determine inductive and field effects of electron-withdrawing functional groups. The results are related to previously determined reactivities of amino groups in amino acids and peptides with vinyl compounds and should be of value for predictions of physicochemical properties of similarly modified proteins.

Results

Amino groups of phenylalanine, chosen as a model for α-amino acids, and of ε-aminocaproic acid, a model for ε-amino groups of lysine side chains, were treated with α,β-unsaturated compounds. Products II–VII and IX–XI were isolated and characterized (Scheme I).

SCHEME I



Acid–base equilibrium constants (pK_2 values) and ΔpK_2 values are summarized in Table I. A comparison of reaction rates to ΔpK_2 values is given in Table II.

(1) This is part IX in a series on Reactions of Amino Acids, Peptides, and Proteins with α,β-Unsaturated Compounds. For part VIII, see M. Friedman, *J. Am. Chem. Soc.*, **89**, 4709 (1967).

(2) Worked under summer program for graduate students during 1965 and 1966.

(3) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

TABLE I

pK_2 VALUES OF AMINO GROUPS IN AMINO ACIDS AND DERIVATIVES AT 30° (μ = 0.15)

Compound	pK_2	ΔpK_2
DL-Phenylalanine (I)	9.00	
N-Methylcarbonylethyl-DL-phenylalanine (II)	8.60	0.40
N-Carbamidoethyl-DL-phenylalanine (III)	8.10	0.90
N-2-Bis(β′-chloroethyl)phosphonylethyl-DL-phenylalanine (IV)	7.30	1.70
N-Cyanoethyl-DL-phenylalanine (V)	6.60	2.40
N-Methylsulfonylethyl-DL-phenylalanine (VI)	5.87	3.13
N-2-Cyanopropyl-DL-phenylalanine (VII)	6.35	2.65
ε-Aminocaproic acid (VIII)	10.62	
N-Cyanoethyl-ε-aminocaproic acid (IX)	8.15	2.47
N-Methylsulfonylethyl-ε-aminocaproic acid (X)	7.80	2.82
N-2-Cyanopropyl-ε-aminocaproic acid (XI)	8.25	2.37
β-Alanine[a] (XII)	10.06	
N-Cyanoethyl-β-alanine[a] (XIII)	7.85	2.21
Asparagine[a] (XIV)	8.72	
α-N-Cyanoethylasparagine[a] (XV)	6.46	2.26
Tyrosine[b] (XVI)	9.00	
N-Cyanoethyltyrosine[b] (XVII)	6.50	2.50
N,N-Dicyanoethyltyrosine[b] (XVIII)	4.13	4.87

[a] Data from M. Friedman and J. S. Wall, *J. Am. Chem. Soc.*, **86**, 3735 (1964). [b] Data from M. Friedman, *Biochem. Biophys. Res. Commun.*, **23**, 626 (1966).

TABLE II

RELATIONSHIP BETWEEN SECOND-ORDER ANION RATE CONSTANTS[a] (k_A⁻) FOR THE REACTION OF THE AMINO GROUP IN GLYCINE WITH CH_2=CH—X, ΔpK_2 VALUES FOR $C_6H_5CH_2CH(CO_2^-)NH_2^+CH_2CH_2X$, AND σ_I CONSTANTS[b] FOR X

X	$k_A^- \times 10^4$, l./mole/sec^{-1}	ΔpK_2	σ_I
1. $CONH_2$	6.30	0.90	0.21
2. $PO(OCH_2CH_2Cl)_2$	20.9	1.70	
3. CN	50.0	2.40	0.53
4. SO_2CH_3	306.0	3.13	0.62
5. $COCH_3$	4000.0	0.40	0.23

[a] M. Friedman and J. S. Wall, *J. Org. Chem.*, **31**, 2888 (1966). [b] R. W. Taft, E. Price, I. R. Fox, I. C. Lewis, K. K. Anderson, and G. T. Davis, *J. Am. Chem. Soc.*, **85**, 709 (1963).

Discussion

Basicities of Amino Groups.—Data in Table I indicate that secondary amino groups of amino acid derivatives are less basic than primary amino groups of starting materials. The decrease in basicities (ΔpK_2 values) in the phenylalanine series ranges from 0.4 pK_2 units for the methyl vinyl ketone derivative II to 3.13 pK_2 units for the methyl vinyl sulfone derivative VI. The average ΔpK_2 value for the five N-

cyanoethyl derivatives V, IX, XIII, XV, and XVIII is 2.37 ± 0.128. This result demonstrates that the nature of side chain R does not profoundly influence the pK_2 value of $R(CO_2^-)NH_2^+CH_2CH_2CN$.

These observations suggest a procedure to determine these effects which involves modification of the primary amino group of phenylalanine with an α,β-unsaturated compound to an N-monoalkyl derivative, followed by titration of the amino groups at the same temperature.

The procedure complements a number of other methods to determine inductive effects of various functional groups and has advantages which include (a) compounds are prepared in a one-step synthesis, (b) only one measurement is required, (c) resonance effects are absent, and (d) the magnitude of the effects is large.[4-6]

Phenylalanine is proposed as the standard amino compound because it uniformly gave crystalline products. With a number of other amino acids some of the products were oils which did not crystallize readily.

Correlation between Basicities and Reactivities.—Relative reactivities of amino and thiol groups in amino acids, aminothiol acids, and peptides with α,β-unsaturated compounds were previously correlated by linear free-energy relationships.[1,7-9]

To further assess the nature of the parameters that govern reactivities of amino groups in amino acids and peptides with α,β-unsaturated compounds, reaction rates of the amino group of glycine with several vinyl compounds are compared with ΔpK_2 values of phenylanine derivatives modified with the same vinyl compounds (Table II). A plot of log k_{A^-} *vs.* ΔpK_2 values for the first four compounds listed in the table is linear (Figure 1). The straight line is described by

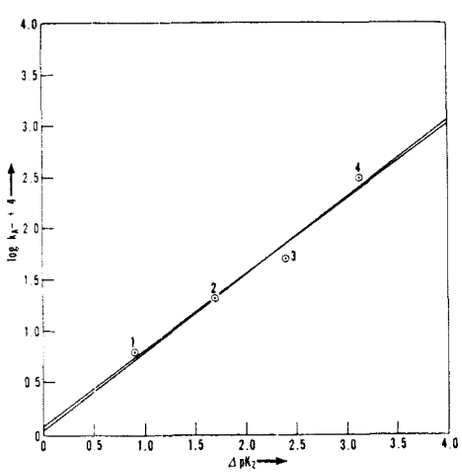


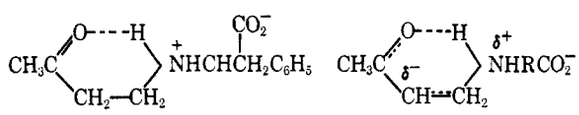
Figure 1.—Plot of log k_{A^-} for the reaction of the amino group in glycine with CH_2=CHX *vs.* ΔpK_2 values of $C_6H_5CH_2CH(CO_2^-)NH_2^+CH_2CH_2X$ where X equals either 1, $CONH_2$; 2, $PO(OCH_2CH_2Cl)_2$; 3, CN; or 4, SO_2CH_3.

eq 1 with a correlation coefficient $r = 0.988$. The cal-

$$\log (k_{A^-} + 4) \pm 0.094 = 0.735\ \Delta pK_2 - 0.082 \qquad (1)$$

culations and plotting were performed with the aid of a computer.[1] This relationship indicates that the influence of inductive effects of electron-withdrawing functional groups on reaction rates of amino groups parallels the corresponding influence on basicities of the resulting amino acid derivatives. Equation 1 may be used to calculate predicted rates or acid–base equilibrium constants.

Reaction rates of CH_2=CHX with amino groups, except the rate for methyl vinyl ketone, are directly proportional to the ΔpK_2 values. It is striking that methyl vinyl ketone is the fastest reacting vinyl compound, whereas the corresponding phenylalanine derivative II has the smallest ΔpK_2 value. Internal hydrogen bonding, as illustrated in IIa, should stabilize the protonated form and account for the small ΔpK_2 value for this amino acid derivative. The low frequency absorption of the carbonyl group of II in the infrared (1690 cm^{-1}) can be explained as being due to such hydrogen-bonding interactions since carbonyl stretching frequencies of normal aliphatic ketones fall within the range of 1705–1725 cm^{-1}.[10] Analogous hydrogen bonding, as illustrated in IIb, should favor the transition state for the reaction of $R(CO_2^-)NH_2$ with methyl vinyl ketone, accounting for the fast rate of this vinyl compound.



IIa IIb

A possible reason why the acrylamide derivative III fits the linear plot may be that intermolecular bonding between hydrogens on the amino or amide groups, or both, and the carbonyl or carboxylate groups, or both, predominates over intramolecular bonding between hydrogens on the amino and the carbonyl groups.

In a previous study it was shown that plots of log k_{A^-} for the reaction of amino groups with CH_2=CHX against ultraviolet maxima of *p*-XArOH and XAr, $E_{1/2}$ values of CH_2=CHX, $\sigma-\sigma^\circ$, and σ_R values were linear.[9] Since these parameters are presumably related to resonance effects, it was suggested that resonance stabilization of transition states by electron-withdrawing functional groups appears to be the major factor which determines relative electrophilic reactivities of α,β-unsaturated compounds.

As was pointed out, however, the spread in the resonance parameters is quite narrow and may be within experimental error. Thus, the set of four substituents which show a linear correlation have an average value of 0.10 ± 0.02 and is constant.[11,12] For this reason, σ_I or some parameter related to it, such as ΔpK_2, gives a linear correlation as expected.

An additional factor that probably contributes to the deviation of the acetyl group from the linear correlations might be attributed to the σ_R value for this

(4) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).
(5) H. K. Hall, Jr., *J. Am. Chem. Soc.*, **79**, 5441 (1957).
(6) See Table II, footnote *b*.
(7) M. Friedman and J. S. Wall, *J. Am. Chem. Soc.*, **86**, 3735 (1964).
(8) M. Friedman, J. F. Cavins, and J. S. Wall, *ibid.*, **87**, 3672 (1965).
(9) See Table II, footnote *a*.
(10) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co. Ltd., London, 1958.
(11) For reviews on linear free-energy correlations, see (a) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953); (b) H. Van Bekkum, B. E. Verkade, and B. M. Wepster, *Rec. Trav. Chim.*, **78**, 815 (1959); (c) R. W. Taft, *J. Am. Chem. Soc.*, **81**, 5353 (1959); (d) P. A. Wells, *Chem. Rev.*, **63**, 171 (1963); (e) C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.*, **2**, 323 (1964).
(12) A σ_R value of 0.08 is calculated for the bis-β'-chloroethylphosphonyl group from the equation $\sigma_R = {}^3/_2(\sigma_p - \sigma_m)$ by assuming that the resonance parameter of this group equals that of the bisethylphosphonyl group. We thank one of the referees for valuable suggestions on this and related points.

functional group which is about twice the average value for the other functional groups.

A plot of ΔpK_2 *vs.* σ_I values for $CONH_2$, CN, and SO_2CH_3 is linear. The σ_I values used (Table II) are those obtained by Taft and coworkers from studies of F^{19} nmr shielding parameters of *meta*-substituted fluorobenzenes in weakly protonic solvents.[6] The linear function is described by eq 2. The question

$$\sigma_I \pm 0.02 = 0.188\Delta pK_2 + 0.05 \quad (2)$$

naturally arises whether eq 2 correlates a wider range of substituents, including electron-donating groups. If such a correlation exists, then σ_I may be used to define new ΔpK_2 values.

In conclusion, results of the present study on relative influences of electron-withdrawing functional groups on acid–base equilibria of amino groups in amino acid derivatives demonstrate that the introduced side chains cause a decrease in basicities covering a wide range. It may be predicted that analogous derivatizations of amino groups in proteins should alter the acid–base equilibria, charge distribution, and other electrochemical properties of the modified proteins. The extent of such changes with a series of vinyl compounds should parallel the ΔpK_2 values.

In a relevant discussion on the role of inductive effects in cooperative phenomena in proteins, Ling[13] suggested that inductive effects of protein side chains influence hydrogen-bond strengths of polypeptide amide groups, the α-helical content, and other properties of proteins. Therefore, proteins derivatized with vinyl compounds should possess conformations that differ from those of the starting materials. These differences should also be related to the ΔpK_2 values.

Experimental Section

Melting points were taken on a Fisher-Jones apparatus and are not corrected. Infrared spectra were determined as KBr pellets on a Perkin-Elmer Model 421 spectrophotometer.[14] The units are in cm^{-1}. Acrylamide, acrylonitrile, crotononitrile, and methyl vinyl ketone were obtained from Matheson; bis(β-chloroethyl) vinyl phosphate was obtained from Stauffer and methyl vinyl sulfone from K & K Laboratories. The liquid reagents were distilled before use. DL-Phenylalanine and ϵ-aminocaproic acid were purchased from Nutritional Biochemicals and the other compounds were synthesized as follows.

N-Methylcarbonylethyl-DL-phenylalanine (II).—To a solution of 2 g (0.0121 mole) of phenylalanine dissolved in 95 ml of H_2O and 1.80 ml of triethylamine (0.0135 mole) was added 1.2 ml (0.0140 mole) of methyl vinyl ketone. The reaction mixture was left standing for 7 days at room temperature and the volume was reduced to one-half the original. A solid precipitate was collected and recrystallized from aqueous ethanol. Additional crops were obtained by reducing the volume to give a total yield of 1.93 g (0.086 mole, 71.5%), mp 190–191°. The infrared spectrum gave signals at 1570 (COO^-) and 1690 (C=O-unsymmetrical peak).

Anal. Calcd for $C_{13}H_{17}NO_3$ (235.29): C, 66.36; H, 7.28; N, 5.95. Found: C, 66.08; H, 7.42; N, 6.23.

N-Carbamidoethyl-DL-phenylalanine (III).—Acrylamide (0.89 g, 0.0127 mole) was added to 2 g (0.012 mole) of a phenylalanine solution containing 1.8 ml (0.0135 mole) of triethylamine. The reaction mixture was stirred for 5 days, the volume was reduced under vacuum, and the precipitated solid was filtered off, washed with water, dried, and recrystallized from ethanol–water to yield 2.25 g (0.0096 mole, 80.0%), mp 250–253°. The infrared spectrum gave signals at 3210 and 3410 (NH_2), 1570 (COO^-), 1650 ($CONH_2$).

Anal. Calcd for $C_{12}H_{16}N_2O_3$ (236.27): C, 61.00; H, 6.82; N, 11.85. Found: C, 61.06; H, 6.93; N, 12.08.

N-2-Bis(β'-chloroethyl)phosphonylethyl-DL-phenylalanine (IV).—To a solution of 2 g of phenylalanine (0.0121 mole) dissolved in 90 ml of H_2O and 1.8 ml (0.0135 mole) of triethylamine was added 2 ml (0.0140 mole) of bis(β-chloroethyl)vinyl phosphonate. The reaction mixture was stirred magnetically and eventually became homogeneous. Stirring was continued for 4 days when the volume was reduced nearly to dryness. A solid was collected and recrystallized from a small amount of water as long needles. The yield was nearly quantitative, mp 154°. The infrared spectrum gave signals at 1230 (P=O), 1625 (NH_2^+, strong peak with a shoulder at 1575 due to COO^-).

Anal. Calcd for $C_{15}H_{22}Cl_2NO_5P$ (398.23): C, 45.24; H, 5.57; N, 3.52; Cl, 17.80; P, 7.78. Found: C, 45.54; H, 5.66; N, 3.52; Cl, 17.42; P, 7.90.

N-Cyanoethyl-DL-phenylalanine (V).—To a solution of 2 g (0.012 mole) of phenylalanine in 125 ml of H_2O and 1.75 ml of triethylamine (pH 10.1) was added 0.9 ml (0.015 mole) of acrylonitrile. The solution was shaken and left standing at room temperature for 2 weeks. The reaction mixture was evaporated to dryness under vacuum. The product was washed with ethanol–water and recrystallized from aqueous ethanol as white needles to yield 2.2 g (0.0103 mole, 84.5%), mp 240–242°. The infrared spectrum gave signal at 1570 (COO^-) and 2245 (CN).

Anal. Calcd for $C_{12}H_{14}N_2O_2$ (218.26): C, 66.04; H, 6.46; N, 12.83. Found: C, 66.33; H, 6.36; N, 12.58.

N-Methylsulfonylethyl-DL-phenylalanine (VI).—To a solution of 3 g (0.0182 mole) of phenylalanine dissolved in 150 ml of H_2O and 2.7 ml (0.0191 mole) of triethylamine was added 1.68 ml (0.0191 mole) of methyl vinyl sulfone. The reaction mixture was shaken and left standing for 11 days. After the volume was reduced to one-half of the original, crystals were collected (1.5 g). The volume was further reduced and additional crops of crystals were collected to yield 4.0 g (0.0149 mole, 80%), mp 235–236°. The infrared spectrum gave signals at 1120, 1325 (SO_2), and 1570 (COO^-).

Anal. Calcd for $C_{12}H_{17}NO_4S$ (271.36): C, 52.11; H, 6.31; N, 5.16; S, 11.82. Found: C, 53.11; H, 6.40; N, 5.16; S, 12.01.

N-2-Cyanopropyl-DL-phenylalanine (VII).—To a solution of 2 g (0.0121 mole) of phenylalanine in 125 ml of H_2O and 1.75 ml of triethylamine (0.0123 mole) which had a pH of 10.1 was added 1.03 ml of *trans*-crotononitrile. The reaction mixture was shaken and then kept with occasional shaking for 2 weeks. The reaction mixture was then evaporated to dryness; the solid residue was recrystallized from ethanol–water as fluffy white needles. The yield was nearly quantitative, mp 208–210°. The infrared spectrum gave a signal at 2245 (CN). The compound crystallized with 1 molecule of water of crystallization which could be partially removed on heating under high vacuum at 100°.

Anal. Calcd for $C_{13}H_{16}N_2O_2 \cdot H_2O$ (250.30): C, 62.38; H, 7.25; N, 11.19. Found: C, 62.42; H, 7.29; N, 11.33.

N-Cyanoethyl-ϵ-aminocaproic Acid (IX).—To a solution of 5 g (0.0382 mole) of ϵ-aminocaproic acid in 30 ml of water and 5.32 ml (0.0386 mole) of triethylamine was added 2.9 ml (0.045 mole) of acrylonitrile dropwise with stirring over a period of 10 min. The reaction mixture was stirred for 20 hr and evaporated to dryness; the residue was redissolved in water and evaporated again to dryness. The residue was suspended in hot ethanol and dissolved by adding water dropwise. Ether was then added until the solution turned cloudy. The flask was placed in a refrigerator and the product crystallized as white needles to give a nearly quantitative yield, mp 130° (lit.[15] mp 131°). The infrared spectrum gave a signal at 2250 (CN).

N-Methylsulfonylethyl-ϵ-aminocaproic Acid (X).—To a solution of 1 g (7.64 mmoles) of ϵ-aminocaproic acid in 6 ml of water and 1.1 ml (7.7 mmoles) of triethylamine (pH of solution, 11.0) was added 0.69 ml (7.7 mmoles) of methyl vinyl sulfone. The reaction mixture was stirred for 21 hr and evaporated to dryness. The yellow oil solidified after a few days and the solid was crystallized from ethanol to yield 780 mg (3.3 mmoles, 43.5%), mp 161–162°. The infrared spectrum gave a signal at 1138 (S=O).

Anal. Calcd for $C_9H_{19}NO_4S$ (237.32): C, 45.55; H, 8.07;

(13) G. N. Ling, *Biopolymers, Symp.*, **1**, 91 (1964).

(14) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

(15) P. F. Butskus, N. V. Raguomene, G. I. Denis, and A. I. Butskene, *Zh. Obshch. Khim.*, **32**, 738 (1962).

N, 5.90; S, 13.51. Found: C, 45.57; H, 8.11; N, 5.67; S, 13.50.

N-2-Cyanopropyl-ε-aminocaproic Acid (XI).—To a solution of 2 g (0.0153 mole) of ε-aminocaproic acid in 17 ml of water and 2.3 ml of triethylamine (0.0165 mole) was added 1.3 ml (0.0160 mole) of *trans*-crotononitrile. The solution was stirred magnetically for 18 days, evaporated until nearly dry, washed with water, and evaporated. The residue consisted of a moist mass. It was spread out on filter paper and air dried for 20 hr. The powdery white solid was collected and recrystallized from ethanol–water as white needles. The yield of the first crop was around 1 g (28%), mp 125°. The infrared spectrum gave a signal at 2247 (CN).

Anal. Calcd for $C_{10}H_{18}N_2O_2$ (198.27): C, 60.57; H, 9.15; N, 14.13. Found: C, 60.51; H, 9.19; N, 14.10.

Determination of pK_2 Values of Amino Groups.—Automatic titrations were carried out in a TTT1C titrator with Titrigraph (Radiometer-Copenhagen) standardized with NBS as previously described.[6,7] The pK_2 values were determined graphically and the accuracy is estimated to be ±0.05 pK_2 units.

Registry No.—II, 15095-71-3; III, 15095-72-4; IV, 15095-73-5; V, 15095-74-6; VI, 15095-75-7; VII, 15095-76-8; IX, 15095-77-9; X, 15206-35-6; XI, 15095-78-0.

Model Reactions for the Biosynthesis of Thyroxine. XI. The Nature of a Free Radical Formed in the Autoxidation of 4-Hydroxy-3,5-diiodophenylpyruvic Acid[1]

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The structures of various free radicals formed by oxidation of 4-hydroxy-3,5-diiodophenylpyruvic acid (DIHPPA) have been determined by means of electron spin resonance. Two short-lived radicals were identified as the enol and keto tautomers, respectively, of the phenoxyl radical of DIHPPA. The previously reported long-lived radical, formed in the autoxidation of DIHPPA, which had been suspected to be an intermediate in the synthesis of thyroxine from DIHPPA and 3,5-diiodotyrosine, has now been identified as 2,6-diiodobenzosemiquinone (DISQ). DISQ does not react with 3,5-diiodotyrosine to form thyroxine. ^{17}O labeling was used in the structure determination of the long-lived radical by means of electron spin resonance.

In the von Mutzenbecher reaction,[4] in which a slightly alkaline solution of 3,5-diiodotyrosine is exposed to air for several days, a slow self-coupling process takes place which leads to the formation of thyroxine. Hillmann[5] suggested that in the biosynthesis of thyroxine DIHPPA[6], the keto acid analog of diiodotyrosine, might be an intermediate. Meltzer and Stanaback[7] found that in the presence of oxygen DIHPPA reacts indeed with diiodotyrosine to form thyroxine in far better yield than that obtained by von Mutzenbecher. Subsequently this model reaction was investigated in greater detail.[8]

Johnson and Tewkesbury[9] offered a hypothesis for the mechanism by which diiodotyrosine is converted to thyroxine in the von Mutzenbecher reaction. According to this hypothesis diiodotyrosine is first oxidized to its phenoxyl radical. Then two molecules of the phenoxyl radical combine to form thyroxine with the concomitant loss of one aliphatic side chain. If one applies this kind of mechanism to the model reaction in which thyroxine is formed from DIHPPA and diiodotyrosine, one will have to assume that both DIHPPA and diiodotyrosine are oxidized to their radicals which then couple.

A few years ago, we reported the formation of a relatively stable free radical when oxygen is bubbled through a buffered solution (pH 7.8) of DIHPPA.[10] This radical gives rise to an electron spin resonance (esr) signal consisting of three bands with an intensity ratio of 1:2:1. We described some of the properties of the radical and suggested that it might be DIHPPA·.[6] Since, however, various characteristics of the observed radical cannot easily be reconciled with the structure of DIHPPA·, a new effort was made to identify the radical. This reinvestigation showed that the radical is 2,6-diiodobenzosemiquinone (DISQ)[6] and that it is not an intermediate in the formation of thyroxine from DIHPPA and diiodotyrosine. Oxidation of DIHPPA with permanganate or ceric sulfate led to the formation of other radicals which have all the characteristics predicted for the enol and keto tautomer respectively of DIHPPA·.

Results and Discussion

Formation of the Stable Radical.—The extent to which the previously described stable radical[10] is formed in the autoxidation of DIHPPA depends very much on the pH, the temperature, the nature of the buffer salt used, the salt concentration, and the presence or absence of certain additives. When oxygen is bubbled at room temperature through a 10^{-3} *M* solution of DIHPPA in 0.2 *M* borate buffer, pH 7.5, for 10 min, and this is followed by nitrogen bubbling, an esr signal can be observed. The size of the signal increases considerably when the pH is raised to 8.5. Upon raising the pH further, the signal still increases, but undergoes rapid deformation. Deformation is slower

(1) Paper X: K. Toi, G. Salvatore, and H. J. Cahnmann, *Biochim. Biophys. Acta*, **97**, 523 (1965).

(2) Visiting Scientist from Kyoto University, Japan.

(3) Recipient of U. S. Public Health Service Grant AM 07955 from the National Institute of Arthritis and Metabolic Diseases.

(4) P. von Mutzenbecher, *Z. Physiol. Chem.*, **261**, 253 (1939).

(5) G. Hillmann, *Z. Naturforsch.*, **11b**, 424 (1956).

(6) Abbreviations: DIHPPA, 4-hydroxy-3,5-diiodophenylpyruvic acid; DIHPPA·, the phenoxyl radical of DIHPPA; DISQ, 2,6-diiodobenzosemiquinone.

(7) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961).

(8) *Cf.* papers V through X of this series and H. J. Cohnmann and T. Shiba, *Biochem. Prep.*, **10**, 171, 176 (1963).

(9) T. B. Johnson and L. B. Tewkesbury, Jr., *Proc. Natl. Acad. Sci. U. S.*, **28**, 73 (1942).

(10) T. Matsuura, H. Kon, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3058 (1964).