mole) of sodium dicyanamide, 500 ml. of butyl alcohol and 40 ml. of water was heated under reflux for 7.5 hours. The formed sodium chloride was separated and the filtrate concentrated to a thick sirup under vacuum (50 mm.). Upon treatment with water the product granulated to a white solid which was dried and recrystallized (ethyl acetate). There was obtained 78.5 g. (83%), m.p. 114–115°.

*Anal.* Calcd. for $C_{10}H_{12}N_4$: C, 63.8; H, 6.4; N, 29.8. Found: C, 63.8; H, 6.5; N, 29.8.

**([N-β-Phenethyl]-amidino)-urea Nitrate Monohydrate.**—A mixture of 4.7 g. (0.025 mole) of β-phenethyldicyan diamide in 12 ml. of isopropyl alcohol and 4 ml. of hydrochloric acid was heated under reflux for 2.5 hours. The hot solution was poured into 70 ml. of water, filtered (carbon), neutralized (methyl red) with 40% sodium hydroxide and filtered (carbon). After addition of 25.0 g. of sodium nitrate and storage at 10° for 6 hours, 4.4 g. (61%) of product was obtained, m.p. 155–158°; recrystallized (ethanol–hexane) m.p., 162–164°.

*Anal.* Calcd. for $C_{10}H_{15}N_5O_4 \cdot H_2O$: C, 42.0; H, 6.0; N, 24.4. Found: C, 41.9; H, 5.9; N, 25.1.

The picrate melted at 195–197° (ethanol–hexane).

*Anal.* Calcd. for $C_{16}H_{17}N_7O_8$: C, 44.2; H, 3.9; N, 22.5. Found: C, 44.1; H, 3.9; N, 22.9.

**Ultraviolet Absorption Spectra.**—The absorption spectra were determined in a DK-1 Beckman recording spectrophotometer using 1-cm. cells. The pertinent data are recorded for the compounds examined solvent, $\lambda_{max}$ mμ, ε × $10^{-3}$.

β-Phenethylbiguanide hydrochloride:water, 233, 14.5; 1 × $10^{-3}$ *N* HCl, 233, 11.2; 1 × $10^{-2}$ *N* HCl, non-specific absorption; 1 × $10^{-4}$ *N* NaOH, 233, 14.5; 1 × $10^{-1}$ *N* NaOH, 232, 12.7; 1 *N* NaOH, 225–228 (plateau), 12.0; methanol, 234, 17.7.

β-Phenethylbiguanide dihydrochloride:water, 233, 14.3.

Phenylbiguanide hydrochloride:water, 242, 14.6; 5 × $10^{-3}$ *N* HCl, 242, 11.8; 1 × $10^{-2}$ *N* NaOH, 223–230 (plateau), 12.4.

**Acknowledgment.**—The authors are grateful to Dr. K. Geiger for making available generous supplies of β-phenethylbiguanide hydrochloride, and to Mr. M. Blitz for the ultraviolet absorption data.

YONKERS 1, N. Y.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

# Oxidative Cleavage of Amides. A Method for Selective Chemical Degradation of Peptides[1,2]

BY E. J. COREY AND L. F. HAEFELE

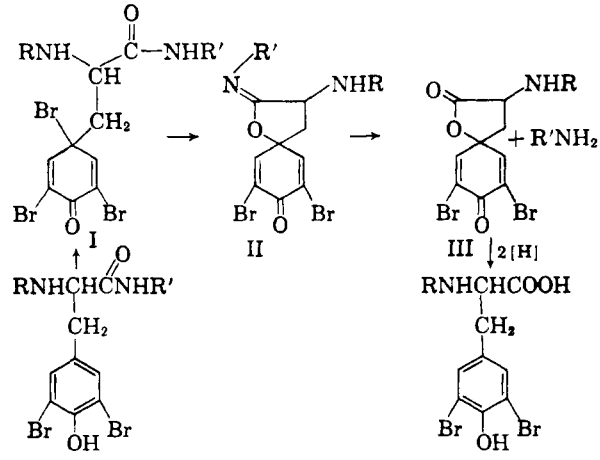
RECEIVED OCTOBER 2, 1958

It has been demonstrated that the carbonyl–nitrogen fission of tyrosine amides by bromine, first observed by du Vigneaud and co-workers with oxytocin and vasopressin, occurs with simple tyrosine amide and phloretamide derivatives as well. The cleavage reaction has been shown to be an oxidative process in which the phenolic ring is converted to a dienone system, and not simple hydrolysis. The studies indicate that the reaction is general for this type of amide and is useful for the selective chemical degradation of peptides.

Du Vigneaud and co-workers, in the course of their important studies on the structures of oxytocin and vasopressin, made the interesting discovery that treatment with aqueous bromine causes cleavage of these polypeptides selectively at the amide linkage between the carbonyl of tyrosine and the nitrogen of the attached amino acid unit.[3–6] Thus in the case of oxytocin fission occurs between tyrosine and isoleucine units and in the case of vasopressin between tyrosine and phenylalanine units. A striking feature of this cleavage is the speed with which it occurs even under very mild conditions. In a typical experiment cleavage was effected by treatment of the polypeptide with bromine in aqueous methanol containing 0.1 *N* hydrogen chloride at −10 to −15° for one hour. Under these conditions the degree of simple acid-catalyzed hydrolysis is negligible.

It was also reported by du Vigneaud and Ressler[6] that with a solution of 1 *N* hydrogen bromide or glacial acetic acid containing bromine, cleavage did not occur although the tyrosine unit underwent dibromination to a 3,5-dibromotyrosyl residue. The cleavage reaction could also be prevented by prior conversion of the phenolic hydroxyl in the tyrosine unit to an ether function.

All these phenomena can be interpreted reasonably in terms of the intermediacy of the species I, II and III, which implies that the fission observed by du Vigneaud and co-workers might be a general and useful reaction. The intermediate I is closely analogous to the perbromophenols, *e.g.*, phenol tetrabromide, and the remaining steps have ample precedence.[7] The intermediate II might also be formed directly without the intervention of I by a concerted reaction. If a mech-




(1) Taken from the Ph.D. thesis of L. Haefele, University of Illinois, July, 1958.

(2) This work was generously supported by the Alfred P. Sloan Foundation.

(3) J. M. Mueller, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **204**, 857 (1953).

(4) C. Ressler, S. Tripett and V. du Vigneaud, *ibid.*, **204**, 861 (1953).

(5) E. A. Popenoe and V. du Vigneaud, *ibid.*, **205**, 133 (1953).

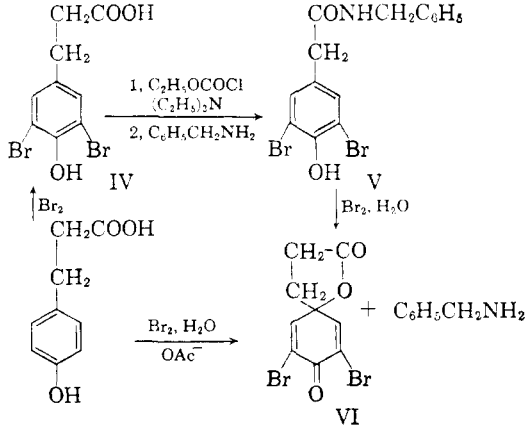
(6) C. Ressler and V. du Vigneaud, *ibid.*, **211**, 809 (1954).

(7) See, for example, F. L. Scott, R. E. Glick and S. Winstein, *Experientia*, **13**, 183 (1957).

anism *via* II is operative, this reaction should permit a selective chemical degradation of polypeptides affecting only those linkages involving amino acids which can form lactone intermediates of the type exemplified by III; histidine and tryptophan come to mind immediately. Consequently, a study of these systems seemed desirable and was undertaken.

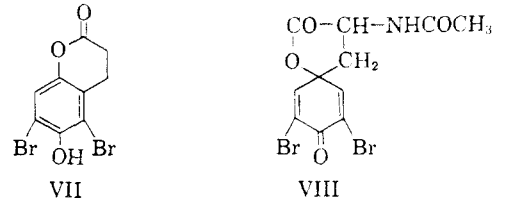
A recent report of work in this area carried on at the National Institutes of Health[8] prompts us to describe the results which have been obtained in our laboratories.

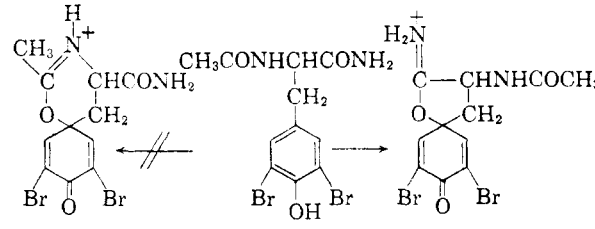
We chose to study first the reaction of amide derivatives of 3,5-dibromophloretic acid (IV) with bromine since this is the simplest model for the tyrosine amide systems. Reaction of N-benzyl-3,5-dibromophloretamide (V) with bromine under essentially the same conditions employed by du Vigneaud, *et al.*, resulted in liberation of benzylamine (isolated as the N-benzoyl derivative) with quantitative formation of the spiro-dienone-lactone VI ($C_9H_6O_3Br_2$). Under the same conditions in the absence of bromine N-benzyl-3,5-dibromophloretamide was completely unaffected, proving that benzylamine is not liberated from the amide to an appreciable extent by simple acid-catalyzed hydrolysis. The lactone VI also was formed directly by reaction of phloretic acid or its 3,5-dibromo derivative with aqueous bromine. The correctness of structure VI is indicated by elemental analysis, infrared data (no hydroxyl absorption, strong carbonyl absorption at 1785 cm.$^{-1}$ ($\gamma$-lactone) and 1690 cm.$^{-1}$ ($\alpha,\beta$-unsaturated ketone)) and ultraviolet data ($\lambda_{max}$ 258 m$\mu$ ($\epsilon$ 10,270)) in accord with expectations.[9] Reduction of the spiro-dienone-lactone with zinc–acetic acid under mild conditions yielded 3,5-dibromophloretic acid. It is apparent from these results that the cleavage reaction first observed with oxytocin occurs also in the simplest system and is, therefore, a general characteristic of such structures. The isolation of the spiro-dienone-lactone VI leaves no doubt as to the role of the aryl group and the general nature of the reaction.




Attempts to effect reduction of the spiro lactone VI to 3,5-dibromophloretic acid by aqueous hydrobromic acid or sodium bisulfite were unsuccessful under mild conditions. In the case of hydrobromic acid a slow reaction did take place, but the product was a neutral material with carbonyl absorption at 1720 cm.$^{-1}$, possibly a substance such as VII formed by acid-catalyzed rearrangement. In view of these observations the reported formation of $\beta$-sulfoalanyl dibromotyrosine[3,4] from oxytocin instead of the corresponding spiro-lactone-dienone is noteworthy.

Reaction of N-acetyltyrosine amide with bromine in aqueous methanol resulted in liberation of ammonia (isolated as benzamide) and formation of the spiro-dienone-lactone VIII, which also was obtained directly from N-acetyl 3,5-dibromotyrosine and bromine and which was readily reduced by zinc–acetic acid, but not aqueous hydrobromic acid, to N-acetyl 3,5-dibromotyrosine. Thus, attachment of an $\alpha$-acylamino substituent to the simple phloretic acid system, which simulates the situation in a peptide, does not alter the course of the cleavage reaction. It is especially interesting that the cleavage involves the tyrosyl–amino linkage and not the acetyl–amino linkage, a result in accord with previous findings that aryl participation to form a five-membered ring is more favorable than that leading to a six-membered spiro ring.[10,11]




We conclude from these studies with the phloretic acid and tyrosine systems that the oxidative cleavage of amides represents a potentially useful method for the specific chemical degradation of polypeptides containing tyrosine units. The same conclusion has been reached for the related case of tryptophan derivatives[8] and it is likely that the case of histidine will also be favorable. In addition there are opportunities for further selectivity in the attack by bromine on tyrosine, tryptophan and histidine units since the maximum rates of reaction of these species with bromine doubtless will occur at different *p*H values.

## Experimental[12]

**3,5-Dibromophloretic Acid (IV).**—A solution of 56 g. of bromine in 150 ml. of acetic acid was added dropwise with

(8) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4747, 4748 (1958).

(9) A. L. Nussbaum, O. Mancera, R. Daniels, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3263 (1951).

(10) E. J. Corey and C. K. Sauers, *ibid.*, **79**, 248 (1957).

(11) R. Heck and S. Winstein, *ibid.*, **79**, 3105, 3114 (1957).

(12) We are indebted to Mr. J. Nemeth and associates for the microanalyses and to Mr. P. McMahon for the infrared spectra.

stirring to a solution of 24.9 g. of phloretic acid[13] in 150 ml. of acetic acid. The addition took one hour, and stirring was continued for an additional half-hour. The acetic acid and excess bromine were evaporated under reduced pressure and the residue was treated with a dilute solution of sodium bisulfite. The product was dissolved in dilute sodium hydroxide and precipitated by the careful addition of concentrated hydrochloric acid. The yield of pale yellow solid, m.p. 99–105°, was 35.4 g. (74%). Recrystallization from benzene–hexane or carbon tetrachloride gave colorless crystals, m.p. 109–110° (lit.[14] m.p. 106–108°).

*N*-Benzyl-3,5-dibromophloretamide (V).—3,5-Dibromophloretic acid (12.0 g., 0.0375 mole) was dissolved in 100 ml. of benzene. Triethylamine (4.04 g., 0.04 mole) was added, followed by ethyl chloroformate (4.34 g., 0.04 mole). The mixture was allowed to stand at room temperature for 90 minutes. The triethylamine hydrochloride was removed by filtration, and benzylamine (5.35 g., 0.05 mole) was added. The solution was heated at reflux for 12 hours, cooled and diluted with hexane. The oil which precipitated was allowed to settle and the supernatant layer was decanted and stored at −10° overnight. The white crystals which separated weighed 2.20 g. and melted at 124–128°. A second crop of material was obtained by dissolving the oil in benzene, cooling, decanting and adding hexane to the solution. This gave an additional 0.38 g. of the amide. The product was recrystallized from benzene–hexane to a constant melting point of 132–133.5°.

*Anal.* Calcd. for $C_{16}H_{15}O_2NBr_2$: C, 46.51; H, 3.66; N, 3.39. Found: C, 46.54; H, 3.77; N, 3.42.

The oily material was taken up in methanol containing 1.0 g. of sodium methoxide. The mixture was warmed on the steam-bath for one hour, the solvent removed and the residue heated at reflux with an excess of benzylamine for two hours. The reaction mixture was dissolved in ether–methylene chloride, washed with dilute acid, sodium bicarbonate and water, dried over magnesium sulfate and the solvent evaporated. The residue was recrystallized from benzene–hexane to give an additional 7.45 g. of material, m.p. 125–127°. The total yield was 13.03 g. (84%).

**Cleavage of N-Benzyl-3,5-dibromophloretamide with Bromine Water.**—N-Benzyl-3,5-dibromophloretamide (0.2065 g., 0.5 mmole) was dissolved in 400 ml. of 3:1 water–methanol. The solution was cooled to 10° and a similarly cooled solution of bromine (1.75 g., 11.0 mmoles) and 72% perchloric acid (0.12 ml.) in 25 ml. of methanol was added all at once. The mixture became turbid immediately. It was allowed to stand at 5–10° for three hours, after which the solvent and excess bromine were removed under reduced pressure. The residue was taken up in ether and extracted with dilute hydrochloric acid. The extract was made strongly basic with sodium hydroxide and shaken with 1 ml. of benzoyl chloride. On standing overnight, 0.0678 g. (65%) of benzylbenzamide, m.p. 98–100°, precipitated. The melting point was undepressed on mixture with an authentic sample prepared from benzylamine and benzoyl chloride.

The ether solution from above was extracted with dilute sodium bicarbonate, washed with saturated salt, dried over magnesium sulfate and evaporated to give 0.1615 g. (100%) of white solid. After recrystallization from methanol–water it melted at 155–168°. Two more recrystallizations raised the melting point to 170–172°, undepressed on mixture with the dienone-lactone VI prepared from 3,5-dibromophloretic acid. The infrared spectrum was also superimposable with that of the dienone-lactone described below. Acidification of the bicarbonate extract gave no precipitate.

A control experiment under exactly the same conditions as above omitting the bromine gave a quantitative return of starting material.

**Preparation of 7,9-Dibromospiro[5.4]1-oxadeca-6,9-dien-2,8-dione (VI).**—Bromine water was added dropwise to a solution of 1.0 mmole of 3,5-dibromophloretic acid in 100 ml. of 1:1 methanol–water containing 2.0 mmoles of sodium acetate. The bromine was decolorized rapidly until an estimated one equivalent had been taken up. The methanol and excess bromine were removed under reduced pressure. The precipitated solid was filtered, washed well with water and air-dried to give 0.3352 g. of white crystals, m.p. 167–172°. The compound was taken up in ether, washed with dilute sodium bicarbonate and saturated salt solution and dried over magnesium sulfate. The solvent was removed and the residue was recrystallized from methanol–water and then twice from benzene–hexane, m.p. 173–174.5°. The infrared spectrum in chloroform had strong bands at 1785, 1690 and 1600 cm.$^{-1}$. The ultraviolet spectrum in methanol had $\lambda_{max}$ at 258 m$\mu$, $\epsilon_{max}$ 10,270.

*Anal.* Calcd. for $C_9H_6O_3Br_2$: C, 33.57; H, 1.88. Found: C, 33.79; H, 1.93.

The compound could be prepared in a similar manner by the action of bromine on phloretic acid.

**Reduction of VI with Zinc and Acetic Acid.**—The dienone-lactone (80.3 mg.) was dissolved in 25 ml. of dry ether and 5 ml. of glacial acetic acid was added followed by 650 mg. of zinc dust. The mixture was stirred at room temperature for 15 minutes. The zinc was removed by filtration and the filtrate washed with 200 ml. of water in three portions. The ether solution was washed with dilute sodium bicarbonate, the aqueous solution acidified and extracted with ether. The organic solution was washed with saturated salt, dried over magnesium sulfate and evaporated to dryness. This gave 46.8 mg. of a yellow gum which, after two recrystallizations from benzene–hexane yielded a solid melting at 108–109°. The melting point was not depressed on mixture with an authentic sample of 3,5-dibromophloretic acid.

**Attempted Reduction of VI with Hydrobromic Acid.**—The dieneone lactone (0.1605 g., 0.5 mmoles) was dissolved in 50 ml. of methanol and 50 ml. of 1 *N* hydrobromic acid was added. The solution turned yellow immediately. It was allowed to stand at room temperature for 30 minutes. The solvent was removed under reduced pressure and the residue taken up in ether. The ether solution was washed with saturated salt solution and dried over magnesium sulfate. The solvent was evaporated to give 0.1650 g. of a yellow gum which could not be induced to crystallize. The gum was dissolved in ether and washed with sodium bicarbonate. No precipitation occurred on acidification of the aqueous solution. The ether solution was dried over magnesium sulfate and the solvent evaporated. The infrared spectrum of the still gummy product had bands at 1785 and 1690 cm.$^{-1}$, characteristic of the starting material, and a third band at 1720 cm.$^{-1}$.

**Attempted Reduction of VI with Sodium Bisulfite.**—To a solution of 100 mg. of the dienonelactone in 25 ml. of methanol was added a solution of 150 mg. of sodium bisulfite in 10 ml. of water followed by 2 ml. of 1 *N* hydrochloric acid. The mixture was stoppered and allowed to stand for one hour. The solvent was removed and the residue suspended in water, filtered and washed with water. This gave 75.4 mg. of white powder, m.p. 170–173°, undepressed after admixture with starting material.

**Reaction of N-Acetyl-3,5-dibromotyrosine with Bromine.**—N-Acetyl-3,5-dibromotyrosine (381 mg., 1.0 mmole) was dissolved in 60 ml. of 1:1 methanol–water containing 200 mg. of sodium acetate and the solution was treated dropwise with bromine until a yellow color persisted. The excess bromine and part of the methanol were removed by concentration at reduced pressure and the solid which precipitated was collected, washed and dried, 252 mg., m.p. 212.5–215° dec. Two recrystallizations from ethanol–water afforded pure spiro-lactone VIII, m.p. 219.5–220.5° dec. The infrared spectrum (Nujol) showed absorption at 1775 ($\gamma$-lactone), 1690 ($\alpha,\beta$-unsaturated ketone), 1667 (amide carbonyl), 1600 (C=C) and 1515 cm.$^{-1}$ (amide N–H bending).

*Anal.* Calcd. for $C_{11}H_9Br_2NO_4$: C, 34.85; H, 2.39; N, 3.70. Found: C, 35.74; H, 2.75; N, 3.67.

Reduction with zinc–acetic acid as described above for VI yielded N-acetyl-3,5-dibromotyrosine, mixture m.p. undepressed with an authentic sample.

**Reaction of N-Acetyltyrosine Amide with Bromine.**—A solution of 444.5 mg. (2 mmoles) of N-acetyltyrosine amide[15] in 50 ml. of aqueous methanol (2:1) containing 0.1 ml. of 70% perchloric acid was treated with 2 g. of bromine as described above for N-benzyl-3,5-dibromophloretamide. Concentration of the reaction mixture and filtration yielded 587

(13) E. Bowden and H. Adkins, THIS JOURNAL, **62**, 2422 (1940).

(14) G. Habild, *Z. physiol. Chem.*, **285**, 127 (1950).

(15) D. W. Thomas, R. V. MacAllister and C. Niemann, THIS JOURNAL, **73**, 1548 (1951).

mg. of the spiro-lactone VIII, m.p. 213–217°, which was recrystallized to give 470 mg. of pure material, m.p. 219–220°, undepressed upon admixture with that described above and having an identical infrared spectrum.

Basification of the aqueous filtrate described above and treatment with benzoyl chloride afforded benzamide, m.p. 127.5–128°.

URBANA, ILL.

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

# The Oxidative Cleavage of Tyrosyl–Peptide Bonds. I. Cleavage of Dipeptides and Some Properties of the Resulting Spirodienone-lactones

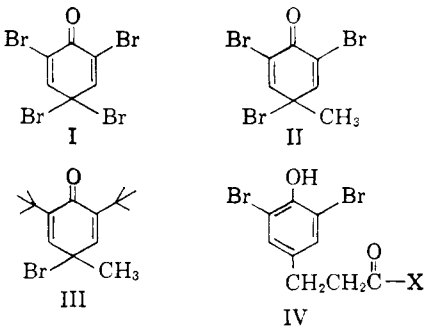
BY GASTON L. SCHMIR, LOUIS A. COHEN AND BERNHARD WITKOP

RECEIVED NOVEMBER 29, 1958

The reaction of phloretic acid with bromine or N-bromosuccinimide at *p*H 4.6 involves an oxidative participation between the phenolic ring and the carboxylate anion and leads to a spirodienone-lactone VII. A similar participation occurs with phloretylglycine (a tyrosyl–peptide model) leading to cleavage and release of glycine in 80% yield. The oxidative bromination of N-carbobenzyloxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine results in the formation of a spirolactone and the release of isoleucine in 40% yield. Aqueous hydrobromic or hydrochloric acid effects a reduction of the spirolactone to dibromophloretic acid while dilute sulfuric acid causes a rearrangement involving oxygen migration.

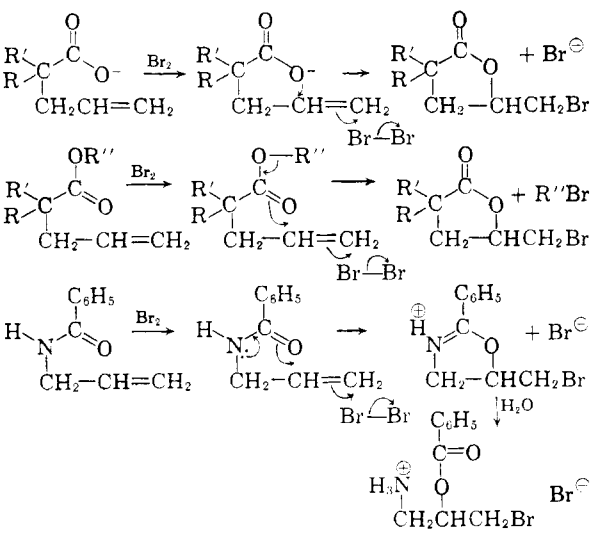
Recent studies in this Laboratory[1] have demonstrated the facile cleavage of tryptophyl–peptide bonds by the action of N-bromosuccinimide (NBS) in aqueous systems. We now wish to report some of our results from a study of the action of bromine and of NBS on derivatives of tyrosine and simpler analogs.

Under certain conditions the bromination of 2,6-disubstituted phenols leads to the formation of 4-bromo-2,6-disubstituted cyclohexadienones. For example, 2,6-dibromophenol forms I,[2] 2,6-dibromo-*p*-cresol forms II[3] and 2,6-di-*t*-butyl-*p*-cresol yields III.[4] Similar bromination of a phenol such as IV might lead to an interaction of the carbonyl function (CX) with the bromodienone *via* an internal displacement reaction. The facility of such displacements on double bonds has been amply demonstrated with carboxylic acids,[5] esters[5,6] and amides[7] as participating groups (Chart I).




The bromination of *p*-hydroxyphenylpropionic acid (phloretic acid) (V) in aqueous acetic acid led to the formation of the expected 3,5-dibromophloretic acid (VI) in high yield. However, quite different results were obtained in the reaction of V with bromine or NBS in acetate buffer at *p*H 4.6. The course of the reaction could be followed readily by measuring the rapid and extensive changes in the ultraviolet spectrum. The addition of three equivalents of brominating agent led to the appearance of a very intense peak at 260 mμ, the optical density attaining its maximum in less than three minutes. When the reaction was conducted on a preparative scale in acetonitrile–acetate buffer, a crystalline product began to separate shortly after the addition of reagent was begun. The same substance was obtained by the action of one equivalent of NBS on VI. The product, m.p. 174–176°, was a neutral compound whose infrared spectrum no longer indicated a phenolic hydroxyl group. The dienone-lactone structure VII (Chart II) was assigned on the basis of elemental analysis, infrared and ultraviolet spectra and subsequent transformations.

CHART I



The course of the reaction may be considered to follow either path a or b. The occurrence of a concerted displacement reaction (path a) is postu-

(1) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4747, 4748 (1958).
(2) J. H. Kastle and A. S. Loevenhart, *Am. Chem. J.*, **27**, 32 (1902).
(3) K. Fries and G. Oehmke, *Ann.*, **462**, 1 (1928).
(4) C. D. Cook, N. G. Nash and H. R. Flanagan, THIS JOURNAL, **77**, 1783 (1955).
(5) R. T. Arnold, M. deMoura Campos and K. L. Lindsay, *ibid.*, **75**, 1044 (1953).
(6) W. P. Miller, Ph.D. Thesis, University of Minnesota, 1957.
(7) L. Goodman and S. Winstein, THIS JOURNAL, **79**, 4788 (1957).