dried at 25° *in vacuo* for 24 hr. The compound foams at 160° with gas evolution and the residue is cysteic acid.

Anal. Calcd. for $C_7H_{15}O_5NS$: C, 37.33; H, 6.71; N, 6.22. Found: C, 37.52; H, 6.65; N, 6.42.

Solutions of the ester were subjected to a variety of conditions for 1 hr. followed by electrophoretic examination: (a) 98% formic acid; (b) 30% hydrogen peroxide; (c) performic acid, as prepared above; (d) 2 *N* hydrobromic acid; (e) water. In none of these mixtures was any significant amount of cysteic acid found. When solutions d and e were heated on steam for 0.5 hr. significant but approximately equal conversion to cysteic acid was observed. When a wet paper electropherogram containing the ester was dried at 80°, the material eluted and reanalyzed by electrophoresis, a significant conversion to cysteic acid was evident. Leucine *t*-butyl ester[8] showed no evidence of cleavage under any of these conditions.

Cleavage of Tyrosine Peptides with NBS. A. Isolation of the Dienone-Lactone.—To a solution of N-carbobenzyloxy-L-tyrosyl-S-benzyl-L-cysteine ethyl ester (0.268 g., 0.005 mole) in 25 ml. of acetonitrile and 25 ml. of 0.01 *N* sulfuric acid was added a solution of NBS (0.45 g., 5 equiv.) in acetonitrile (10 ml.) and acid (10 ml.) dropwise over 20 min. Crystals of the dienone-lactone began to separate in *ca.* 40 min. and after further dilution of the mixture with water and cooling, the product was collected (0.125 g., 55%). It melted at 217–219°. When mixed with an authentic sample,[1] the m.p. was 218–220°. Comparison was also made by ultraviolet and infrared spectra. No attempt was made to recover additional material from mother liquors, although its presence was evident. Other peptides were oxidized in a similar manner, using the modifications listed in Tables I–III.

B. **Ninhydrin Assay.**—The acylpeptide (10^{-4} mole) was dissolved in acetic acid (5 ml.) and the solution diluted with water (5 ml.); NBS (6 moles, 108 mg.) was added to the stirred solution and stirring continued for 2 hr. Solutions were centrifuged and 1 ml. of the supernatant diluted with 50% acetic acid to a concentration appropriate for colorimetry (25–50 ml.). To 1 ml. of the diluted mixture was added *ca.* 0.3 g. of sodium bicarbonate to raise the *p*H to 3–4 and ninhydrin assay performed by standard procedures.[21] Cleavage mixtures were examined by paper chromatography to verify the amino acid resulting from splitting of the peptide bond. The oxidation products of thioethers were invariably found to be sulfones, with negligible amounts of sulfoxides appearing on paper chromatograms. Ninhydrin yields were not increased by storage of reaction mixtures for 24 hr. or by heating on steam following the 2-hr. reaction time.

Standards for Chromatography and Ninhydrin Assay.—S-Benzyl-L-cysteine sulfoxide was prepared by oxidation of S-benzyl-L-cysteine with 30% hydrogen peroxide.[22] S-Methyl-L-cysteine sulfoxide,[23] S-carboxymethyl-L-cysteine sulfoxide,[18] S-methyl-L-cysteine sulfone[24] and S-carboxymethyl-L-cysteine sulfone[18,25] were prepared according to published procedures.

S-Benzyl-L-Cysteine Sulfone.—To a solution of N-carbobenzyloxy-S-benzyl-L-cysteine sulfone[1] (0.5 g.) in 3 ml. of glacial acetic acid in a dry 250-ml. flask was added 1 ml. of 32% hydrogen bromide in acetic acid. The mixture was left for 1 hr. with occasional shaking. Dry ether (100 ml.) was added and after several hours the precipitated solid (0.40 g.) was collected and washed with ether. The sulfone was recrystallized from hot water; m.p. 175° dec.

Anal. Calcd. for $C_{10}H_{13}O_4NS$: C, 49.38; H, 5.39; N, 5.76. Found: C, 49.60; H, 5.41; N, 5.76.

Direct oxidation of S-benzyl-L-cysteine, either with bromine or with hydrogen peroxide, invariably led to difficultly separable mixtures of sulfoxide and sulfone.

(21) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

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(23) S. Yurugi, *J. Pharm. Soc. Japan*, **74**, 519 (1954); *C. A.*, **49**, 8301 (1955); C. J. Morris and J. F. Thompson, *J. Am. Chem. Soc.*, **78**, 1605 (1956).

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

Oxidative Cleavage of Tyrosyl–Peptide Bonds. IV. The Oxidative Degradation of Ribonuclease and of S-Carboxymethylribonuclease[1]

BY JOHN G. WILSON[2] AND LOUIS A. COHEN

RECEIVED SEPTEMBER 21, 1962

Oxidative cleavage of ribonuclease with N-bromosuccinimide results in the splitting of five of the six tyrosyl–peptide bonds present, the tyrosyl–cysteic acid bond (from disulfide oxidation) failing to cleave. However, all six amide bonds are split in S-carboxymethylribonuclease, in which oxidation of sulfur proceeds only to the sulfone stage. It is suggested that the highly acidic sulfonic acid residue, when constrained in a complex matrix, may inhibit cleavage by protonation of an amide nitrogen. All amino terminals liberated agree with the published sequence of the enzyme. A limited correlation between tertiary structure and rate of cleavage or order of release of amino acids is indicated.

The application of oxidative cleavage with N-bromosuccinimide (NBS) to simple tyrosine peptides has been described in earlier communications in this series.[1,3] The ultimate utility of such a technique lies in its applicability to complex polypeptides and proteins. A number of factors exist, however, which may limit the ability of a protein to exhibit the straightforward behavior found among tyrosyl peptides: for example, limited penetration into a three-dimensional matrix; geometrical and conformational rigidity of peptide bonds, limiting free rotation; competition by suitably placed but extraneous nucleophiles; and side-reactions limiting the release of new amino terminals.[3a]

For an initial investigation, bovine pancreatic ribonuclease was chosen as the test protein, since it is available in a state of high purity, contains no tryptophan[4] and has a well-established sequence.[5] Although it was of interest to confirm the assignment of the six tyrosyl–amino acid bonds derived from enzymatic degradation studies,[5] our main concern was to examine the extent to which the factors enumerated above may complicate NBS degradation of a protein.[6]

Exploratory studies were performed by measuring the increase in absorption at 260 mμ following addition of successive amounts of NBS to aqueous solutions of ribonuclease. Figure 1, curve A, and Fig. 2 show the results of one such experiment. Curve C of Fig. 1 represents the expected optical density increases for the hypothetical case, in which all NBS added is directed toward tyrosine bromination and oxidation. The induction period appearing at the base of both curves represents a repression of dienone formation in 0.1 *N* sulfuric acid; in acidic media, bromination of the phenolic ring is essentially complete before dienone

(1) For paper III, *cf.* J. G. Wilson and L. A. Cohen, *J. Am. Chem. Soc.*, **85**, 560 (1963).

(2) Division of Plant Industry, C.S.I.R.O., Canberra, Australia.

(3) (a) G. L. Schmir and L. A. Cohen, *J. Am. Chem. Soc.*, **83**, 723 (1961); (b) G. L. Schmir, L. A. Cohen and B. Witkop, *ibid.*, **81**, 2228 (1959).

(4) A. Patchornik, W. B. Lawson, E. Gross and B. Witkop, *ibid.*, **82**, 5923 (1960).

(5) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **235**, 633 (1960); D. G. Smyth, W. H. Stein and S. Moore, *ibid.*, **237**, 1845 (1962); J. T. Potts, A. Berger, J. Cooke and C. B. Anfinsen, *ibid.*, **237**, 1851 (1962); E. Gross and B. Witkop, *ibid.*, **237**, 1856 (1962).

(6) No attempt was made, within the scope of this investigation, to follow loss of enzyme activity, or to isolate peptides as fragmentation products.

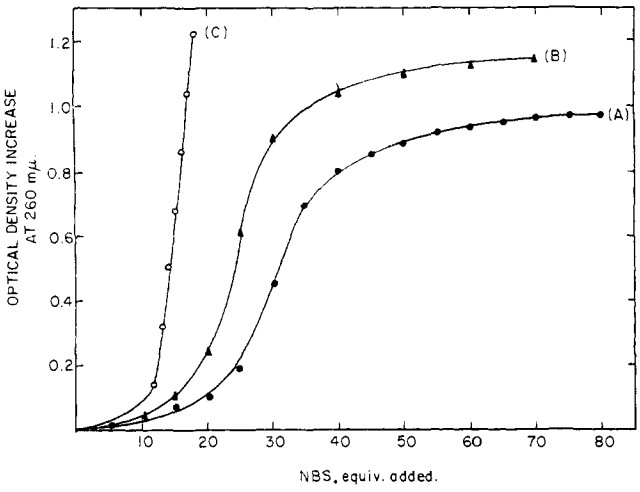
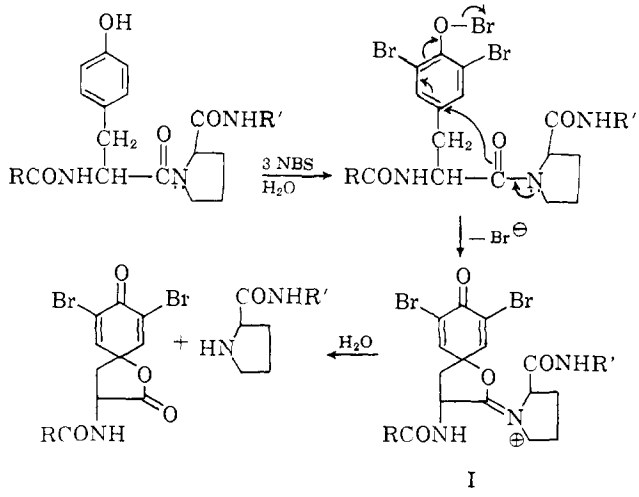


Fig. 1.—Action of NBS on RNase and CMRNase as shown by increase in optical density at 260 mμ; A, RNase; B, CRNase; C, theoretical curve (calcd. for dienone, ϵ = 10,200). Each point on C corresponds to the conversion of one tyrosine residue; concn. 2×10^{-5} *M* in 0.1 *N* H_2SO_4.

formation begins.[3] The induction period observed is greater than theoretical (Fig. 1, curve A, and Fig. 2) and suggests that, while there is no significant barrier to *ortho* bromination, dienone formation is hindered by conformational rigidity. The rapid increase in optical density after addition of thirty equivalents of NBS most likely represents a collapse of structure due to oxidative cleavage of one or more disulfide bonds. The maximum optical density attained corresponds to the oxidation of 4.6 tyrosine residues.

According to the sequence of ribonuclease,[5] cleavage of the six tyrosyl–peptide bonds should lead to the appearance, as new amino terminals, of glutamine, serine, valine, lysine, cysteic acid and proline. Resistance to cleavage of the tertiary amide bond of the tyrosyl-proline sequence was not anticipated, since N-carbobenzyloxytyrosylproline is cleaved by NBS to the extent of 85%. It is evident that nucleophilic participation which requires the formation of a quaternary immonium lactone (I) is not beyond the scope of the reaction.[6a]



I

NBS-oxidized ribonuclease was subjected to quantitative end-group assay *via* dinitrophenylation (proline was determined as the phenylthiohydantoin; Table I). Untreated ribonuclease revealed a small degree of contamination by a peptide with glutamic acid (or glutamine) as N-terminal, perhaps arising from

(6a) *Cf.* P. N. Craig, *J. Am. Chem. Soc.*, **74**, 129 (1952).

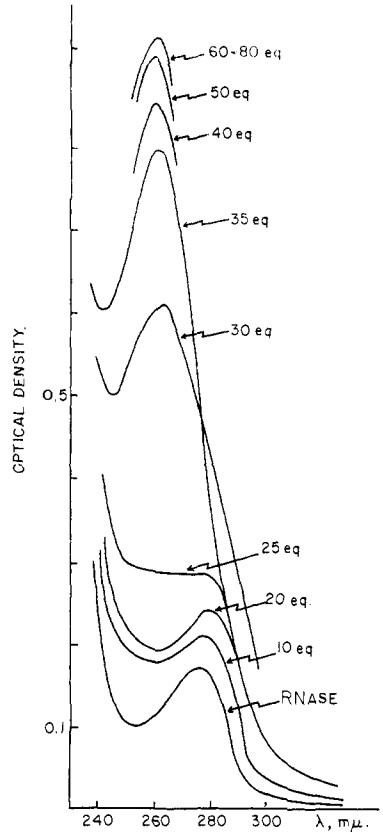


Fig. 2.—Ultraviolet spectra resulting from the addition of successive increments of NBS to ribonuclease in 0.1 *N* H_2SO_4.

the full sequence with loss of the original N-terminal lysine. In the presence of 0.01 *N* hydrobromic acid as a control, several other N-terminals are liberated in trace amount, possibly as the result of acid-catalyzed cleavage adjacent to aspartic acid residues.[7] The amount of NBS used (46 equiv.) is based on the consumption of three moles of oxidant per tyrosine residue, two per methionine and five per disulfide bond. Competitive consumption of oxidant by imidazole rings[3a] as well as by amino terminals[3a] must also be considered. It is evident from Table I that four of the six tyrosyl–peptide bonds are cleaved in fairly good yield. To what extent the lysine value includes a new amino terminal is indeterminate, since it had been shown previously that the amino groups of lysine are especially subject to destruction by NBS.[3a] Use of a considerable excess of NBS (80 equiv.) serves only to degrade amino terminals further, without significant increase in cleavage yield. The tyrosyl–cysteic acid bond was found to resist cleavage, accounting for the fact that optical density at 260 mμ approaches that corresponding to five dienone chromophores rather than six. The refractory behavior of the latter bond is in complete contrast to our results with model peptides[1] and to those of Thompson with oxidized insulin.[8] Since both native and performic acid-oxidized ribonuclease[9] fail to cleave at the tyrosyl–cysteic acid bond in acidic media, it is suggested that the inhibition process may be associated with interaction of the sulfonic acid residue with the preceding peptide bond. Thus, protonation would decrease considerably the ability of the amide to participate as a nucleophile, intramolecular protonation being far more effective than protonation by the me-

(7) *Cf.* B. Witkop, "Advances in Protein Chemistry," Vol. 16, Academic Press, Inc., New York, N. Y., 1961, p. 229.

(8) E. O. P. Thompson, *Austral. J. Biol. Sci.*, **13**, 106 (1960).

(9) W. Konigsberg, unpublished data. We are indebted to Dr. Konigsberg for making available to us the results of his studies.

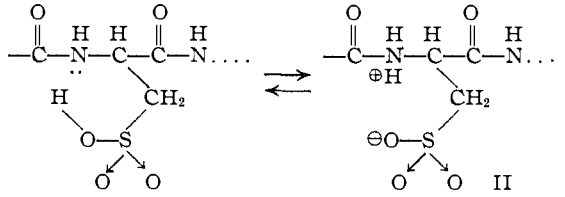
TABLE I

YIELDS OF NEW AMINO TERMINALS IN NBS OXIDATION OF RIBONUCLEASE[a]

Conditions	Yield by DNP method,[b] % Glu	Ser	Val	Lys	$CySO_3H$	Pro[c]	Ala	Thr
Control, H_2O	4			68				
50% AcOH, 4 hr.	4			63				
50% AcOH, 0.01 *N* HBr, 4 hr.	6		6	61			3	3
50% AcOH, 46 eq. NBS, 4 hr.	57	84	32	57	<1	55	3	4
50% AcOH, 80 eq. NBS, 4 hr.	29	68	35	26	<1	45		

[a] Results are average of several experiments. [b] Values adjusted for destruction during hydrolysis (*cf.* Experimental). [c] Determined in separate runs by phenylthiohydantoin method.

dium.[10] Evidently, such inhibition is not significant in smaller peptides where tertiary structure and rigidity are minor factors.



Further support for the inhibitory role of the sulfonic acid residue may be derived from studies with S-carboxymethylribonuclease (CMRNase). Spectral increase at 260 mμ following NBS oxidation (Fig. 1, curve B) approaches that expected for six dienone residues, oxidation now leading to a sulfone rather than to a sulfonic acid. It is also evident from the steeper slope of curve B that dienone formation occurs at

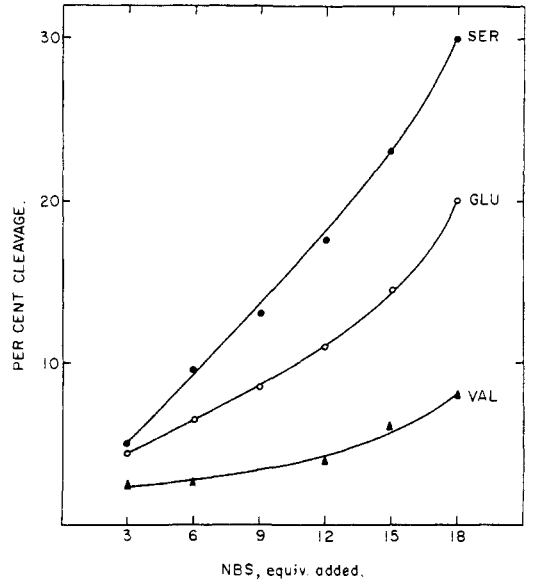


Fig. 3.—Action of limited amounts of NBS on ribonuclease in acetate buffer, *p*H 4.6 (DNP assay corrected for losses).

earlier stages in the oxidation process. Since tertiary structure has been modified and probably weakened in the course of formation of CMRNase, there is now an increase in flexibility of the polypeptide chain, permitting amide bonds to participate more readily in dienone-lactone formation. The results of quantitative assay for amino terminals in oxidized CMRNase are summarized in Table II. Cleavage of the tyrosyl–S-carboxymethylcysteine bond is now significant, actual yields estimated at greater than 50%.[11]

It was of interest to determine whether any selectivity in cleavage site could be observed with limited

(10) *Cf.* the effect of a sulfonic acid residue in catalyzing intramolecular ester cleavage, ref. 1; *cf.* also, M. Bender, *Chem. Rev.*, **60**, 53 (1960).

(11) Extensive instability of the DNP derivative to acid renders the use of a correction factor unreliable.

TABLE II

YIELDS OF NEW AMINO TERMINALS IN NBS OXIDATION OF CMRNase[a]

Conditions	Yield by DNP method,[b] % Glu	Ser	Val	Lys	CMCy-SO_3	Pro[c]
50% AcOH, 50 eq. NBS, 4 hr.	34	65	60	55	40	36
50% AcOH, 80 eq. NBS, 4 hr.	23	48	48	31	50	

[a] Results are average of several experiments. [b] Values adjusted for destruction during hydrolysis (*cf.* Experimental). [c] Determined in separate runs by phenylthiohydantoin method.

quantities of NBS and at a *p*H value within the range of enzymatic activity of ribonuclease. Accordingly, NBS was added to solutions of the enzyme in acetate buffer, *p*H 4.6,[12,13] and the liberation of three new amino terminals followed quantitatively. The results are summarized in Fig. 3. Although the total extent of cleavage in each case was low, it is of interest that valine appears to lag behind glutamic acid and serine, suggesting that the tyrosine residues preceding the latter two amino acids may be closer to the surface of the protein matrix.[14]

Experimental[15]

N-Carbobenzyloxy-L-tyrosyl-L-proline Methyl Ester.—N-Carbobenzyloxy-L-tyrosine (6.3 g.) was added to a solution of L-proline methyl ester hydrochloride[16] (3.3 g.) and triethylamine (2.8 ml.) in methylene chloride (40 ml.) followed by dicyclohexylcarbodiimide (4.3 g.) in 5 ml. of methylene chloride. The reaction mixture was stirred for 3 hours and left overnight. A few drops of glacial acetic acid was added to the mixture and the urea (3.9 g.) was filtered off and washed with methylene chloride. After evaporation of the combined filtrates, the residue was taken up in ethyl acetate and the solution washed with water, *N* hydrochloric acid, 5% sodium bicarbonate and water, any urea which separated being filtered off. The solution was dried ($MgSO_4$) and evaporated, leaving 7.0 g. of a glass which failed to crystallize.

N-Carbobenzyloxy-L-tyrosyl-L-proline.—The above ester (6.1 g.) was dissolved in ethanol (25 ml.) and the solution diluted with 10 ml. of water. A solution of 1.5 g. of sodium hydroxide in 20 ml. of water was added and the mixture stirred for 1 hr. Upon acidification of the mixture with *N* hydrochloric acid and dilution with water, an oil separated. It was separated from the solvent by decantation, taken into methylene chloride and the dried solution diluted to turbidity with ligroin (65°). Crystallization was slow and required several weeks in the cold for completion. The product, 4.3 g., m.p. 97–105°, was recrystallized from methylene chloride–ligroin; m.p. 95–102°.

Anal. Calcd. for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79. Found: C, 63.84; H, 5.94; N, 6.82.

The compound readily formed a crystalline dicyclohexylamine salt which separated from ethanol–ether as tiny needles, m.p. 203.5–205.5° with slight pre-sintering.

(12) At this *p*H value the activity of ribonuclease is low, but measurable; M. E. Maver and A. E. Greco, *J. Natl. Cancer Inst.*, **17**, 503 (1956).

(13) The NBS reaction is attended by a variety of side reactions in media more alkaline than *p*H 5 (ref. 3a).

(14) Studies on partial iodination of RNase suggest that the tyrosine residues preceding valine, lysine and proline are "buried" or less accessible to chemical modification; C. Y. Cha and H. A. Scheraga, *Biochem. and Biophys. Res. Commun.*, **6**, 369 (1961).

(15) Melting points are uncorrected. Ultraviolet spectra were run on a Cary recording spectrophotometer, model 14. The authors thank Mr. H. G. McCann and his associates of this Institute for performing the microanalyses.

(16) B. F. Erlanger, H. Sachs and E. Brand, *J. Am. Chem. Soc.*, **76**, 1806 (1954). In our hands the compound could not be crystallized and was used as a sirup.

Carbobenzyloxytyrosylproline was oxidized with NBS as previously described.[1] By ninhydrin assay, average cleavage yields were 85%.

N-2,4-Dinitrophenyl-S-carboxymethylcysteine Sulfone.—To a solution of S-carboxymethylcysteine sulfone[1] (0.63 g.) and sodium bicarbonate (1.1 g.) in water (25 ml.), fluorodinitrobenzene (1.1 g.) in ethanol (25 ml.) was added and the mixture stirred at room temperature for 4 hr. After dilution with water, the ethanol was removed *in vacuo* and the excess of reagent extracted with ether. Acidification with 6 *N* hydrochloric acid precipitated the crystalline derivative which was collected and washed well with water. The crude material (1.1 g.) was recrystallized twice from ethyl acetate–ligroin (65°) and once from aqueous ethanol to give yellow needles, sintering at 114–130°, resolidifying and melting again at 155–158°.

Anal. Calcd. for $C_{11}H_{11}O_{10}N_3S$: C, 35.02; H, 2.94; N, 11.14. Found: C, 35.11; H, 3.15; N, 11.21.

N-2,4-Dinitrophenyl-S-carboxymethylcysteine sulfoxide was prepared on the same scale and by the same method as above. It separated from water in prisms, m.p. 145–147°.

Anal. Calcd. for $C_{11}H_{11}O_9N_3S$: C, 36.57; H, 3.07; N, 11.63. Found: C, 36.49; H, 3.21; N, 11.35.

N-2,4-Dinitrophenyl-S-methylcysteine sulfone was prepared as above and was obtained as long needles from water; m.p. 176–178°.

Anal. Calcd. for $C_{10}H_{11}O_8N_3S$: C, 36.04; H, 3.33; N, 12.60. Found: C, 36.00; H, 3.62; N, 12.47.

Enzyme Oxidations.—Bovine pancreatic RNase (Sigma Chemical Co., chromatographic grade, Lot No. R60-B-069) was used without further purification. S-Carboxymethylribonuclease was prepared by mercaptoethanol reduction of the disulfide bonds in RNase, alkylation with iodoacetate and purification on Sephadex G-25 to a single, fairly symmetrical peak.[17]

To a solution of the protein (usually 0.5 μM) in 2 ml. of 50% acetic acid or *p*H 4.6 acetate buffer was added a solution of NBS in 2 ml. of acetic acid or acetate buffer–5% acetonitrile. The mixture was stirred for 4 hr. at 25° and the solvent removed by lyophilization.

Amino-Terminal Assay.—After lyophilization, the protein residue was taken up in 2 ml. of water to which was added 50 mg. of sodium bicarbonate followed by a solution of 50 mg. of fluorodinitrobenzene in 2 ml. of ethanol. After 4 hr. the mixture was carefully evaporated to dryness *in vacuo*. In early experiments, excess reagent was extracted with ether at this point; however, it was found that some peptide material was also extractable by ether since the carboxyl terminals were present as lactones and not as carboxylate anions. In most runs, therefore, excess reagent was carried through subsequent steps. The mixture of DNP-polypeptide fragments was hydrolyzed with 6 *N* hydrochloric acid under N_2 at 105–110° for 16–18 hr., using 3–4 ml. of acid. A few drops of glacial acetic acid was often used to facilitate transfer and to ensure complete solution. Dinitrophenol was removed by repeated sublimation and the residual ether-soluble DNP-amino acids were resolved by two-dimensional chromatography on Whatman No. 1 paper using the solvent systems toluene–pyridine–2-chloroethanol–0.8 *N* ammonium hydroxide (50:15:30:30) and 1.5 *M* phosphate buffer, *p*H 6.5.[18] The DNP-amino acids were eluted with 1% sodium bicarbonate and assayed spectrophotometrically at 360 mμ. Individual DNP-amino acids were identified by running them with an authentic specimen in the *tert*-amyl alcohol–*p*H 6.0 phthalate system. The identification and determination of the water-soluble DNP-cysteic acid were carried out by evaporation of the aqueous phase and (1) two-dimensional chromatography in the usual systems or (2) adsorption on a column of talc and elution with ethanol–*N* hydrochloric acid (4:1), other DNP derivatives remaining on the column.[19] Paper electrophoresis at *p*H 1.9 was also used to separate and identify DNP-cysteic acid.[20] Proline was determined by the Edman procedure from independent runs.[18]

Determination of Correction Factors.—Authentic DNP-amino acids, at appropriate concentrations, were subjected to the usual acid treatment in the presence of oxidized ribonuclease. Recovery factors were averaged from several runs for each compound: lysine, 81; valine, 73; glutamic acid, 70; serine, 31. The phenylthiohydantoin of proline was recovered in 55% yield. DNP-S-carboxymethylcysteine sulfone was destroyed to a considerable extent by acid (12% recovery). The major product showed a peak in the ultraviolet at 330 mμ rather than 360 mμ, but could not be identified. It was demonstrated by paper chromatography that reduction to the sulfoxide did not occur, nor did the material decarboxylate to DNP-S-methylcysteine sulfone to any significant extent.

(17) C. B. Anfinsen and E. Haber, *J. Biol. Chem.*, **236**, 1361 (1961).

(18) H. Fraenkel-Conrat, J. I. Harris and A. L. Levy, "Methods of Biochemical Analysis," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1955, p. 360.

(19) G. Biserte, J. W. Holleman, J. Holleman-Dehove and P. Sautière, "Chromatographic Reviews," Vol. 2, Elsevier Publishing Co., Amsterdam, 1960, p. 59.

(20) K. Wallenfels and A. Arens, *Biochem. Z.*, **332**, 217 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES 24, CALIF.]

Neighboring Carbon and Hydrogen. LI.[1] Dienones from $Ar_1\ominus$-3 Participation. Isolation and Behavior of Spiro(2,5)octa-1,4-diene-3-one

BY RICHARD BAIRD[2] AND S. WINSTEIN

RECEIVED OCTOBER 15, 1962

When the aryl group is *p*-hydroxyphenyl, Ar_1-3 participation in the phenoxide anion of 2-aryl-1-ethyl halides, designated by the symbol $Ar_1\ominus$-3, leads to spiro(2,5)octa-1,4-diene-3-one. Kinetic investigation of the behavior of 2-*p*-hydroxyphenylethyl bromide in basic methanolic solution shows that conditions are very favorable for $Ar_1\ominus$-3 participation; the intermediate spiro-dienone gives rise to monomeric methyl ether and higher molecular weight products in proportions dependent on the concentrations of methoxide and phenoxide ions. By spectroscopic methods it is possible to observe and study the kinetics of formation and decay of the intermediate spiro-dienone. Under specially designed conditions, it has been possible to isolate the unusually reactive spiro-dienone as a pure, crystalline solid and to study its behavior directly in solvolysis, hydrogenation, lithium aluminum hydride reduction and hydrogen bromide addition. The ultraviolet and n.m.r. spectra of the dienone are of some interest. Also, the acid-catalyzed rate of methanolysis of the spiro-dienone permits one to estimate the stationary state concentration of the ethylene phenonium ion intermediate in solvolysis of 2-*p*-anisylethyl toluenesulfonate. This is because the conjugate acid of the dienone may be taken as a model for the bridged-ion intermediate in anchimerically assisted ionization of a 2-*p*-anisylethyl derivative.

With suitable substrate structure and reaction conditions, it is possible to arrange for the formation of dienones through $Ar_1\ominus$-participation of a neighboring phenoxide-ion group.[1a,3] In the case of $Ar_1\ominus$-assisted formation of dienones, it has been indicated already in preliminary communications[3,4] that 2-*p*-hydroxyphenylethyl bromide (I-Br) gives rise under alkaline conditions to the intermediate dienone IV which can be detected spectroscopically[3] and even isolated in pure form.[4] The results of this study of the formation and behavior of this interesting substance are presented and discussed in the present manuscript.

(1) (a) Paper XLVI: R. Baird and S. Winstein, *J. Am. Chem. Soc.*, **84**, 788 (1962); (b) Paper XLVII: E. Friedrich and S. Winstein, *Tetrahedron Letters*, No. **11**, 475 (1962); (c) Paper XLVIII: E. Hedaya and S. Winstein, *ibid.*, No. **13**, 563 (1962); (c) Paper XLIX: S. Winstein, E. Vogelfanger, K. C. Pande and H. F. Ebel, *J. Am. Chem. Soc.*, in press; (e) Paper L: S. Winstein, E. Vogelfanger and K. C. Pande, *Chem. Ind.* (London), 2061 (1962).

(2) National Science Foundation Predoctoral Fellow, 1953–1955, 1956–1957. Present address: Dept. of Chem., Yale University, New Haven, Conn.

(3) S. Winstein and R. Baird, *J. Am. Chem. Soc.*, **79**, 756 (1957).

(4) R. Baird and S. Winstein, *ibid.*, **79**, 4238 (1957).