havior at the melting point, which is characteristic for all the N-bromoimino compounds reported here, is a sudden change to a black char.

Hydrolysis of XII.—Aliquots of a 2×10^{-4} *M* solution of XII in acetonitrile–water (1:9) released 100% of the imino-nitrogen as ammonia under the standard conditions of ninhydrin assay.

Compound XII (100 mg.) was refluxed (80°) for 2 hours in 10 ml. of acetonitrile–water (1:1). Liberated bromine gradually evaporated. The solution was concentrated *in vacuo* and as the acetonitrile evaporated, white crystals formed. They were collected (75 mg., 95%) and their infrared spectrum (Nujol) was indistinguishable from that of authentic lactone X (R = Bz), m.p. 161–162°. Preliminary experiments showed that a reaction time of 2 hours was necessary for complete hydrolysis.

2-Bromoimino-3-benzamido-5-methyl-5-bromomethyltetrahydrofuran (XIII, R = Bz).—DL-2-Benzamido-4-methyl-4-pentenoic acid amide (0.116 g., 0.5 mmole) was treated with NBS (0.095 g., 0.525 mmole) as described above for the preparation of XII. The yield of crystalline XIII (R = Bz) collected after concentration of the reaction mixture was 0.031 g. (15%), m.p. 141–144° dec.; λ_{max}^{KBr} 6.01 μ (C=N), 6.06 μ (amide C=O). The analytical sample was dried *in vacuo* at 65° for 3 hours.

Anal. Calcd. for $C_{13}H_{14}N_2O_2Br_2$: C, 40.02; H, 3.62; Br, 40.97. Found: C, 40.59; H, 3.71; Br, 40.91.

The compound gave a positive reaction when rubbed on moist starch–iodide paper. Higher yields could undoubtedly be obtained by doubling the relative concentration of NBS.

2-Bromoimino-3-*p*-toluenesulfonamido-5-methyl-5-bromomethyltetrahydrofuran (XIII, R = Ts).—DL-2-*p*-Toluenesulfonamido-4-methyl-4-pentenoic acid amide (0.085 g., 0.3 mmole) was treated with NBS (0.057 g., 0.32 mmole) as described above; yield of XIII (R = Ts), 0.016 g. (12%), m.p. 124–126° dec., λ_{max}^{KBr} 6.05 μ (C=N). The compound gave a positive starch–iodide paper test.

Anal. Calcd. for $C_{13}H_{16}N_2O_3SBr_2$: Br, 36.47. Found: Br, 36.30.

2-Oxa-3-bromoimino-5-(tosyl)-aza-7-bromobicyclo[3,2,1]-octane (XVI). The Reaction of N-Tosylbaikiain Amide with NBS.—N-Tosyl-DL-baikiain amide (280 mg., 1 mmole) dissolved in 25 ml. of warm acetonitrile was added rapidly with shaking to a solution of NBS (400 mg., 2.2 mmoles) in 100 ml. of water. An immediate turbidity occurred and crystallization commenced within 5 minutes. The reaction mixture was kept at 0° for 2 hours, and the product was filtered off; yield of XVI, 350 mg. (80%), m.p. 142–143° dec., λ_{max}^{KBr} 6.00 μ (C=N). The analytical sample was dried *in vacuo* for 4 hours at 65°.

Anal. Calcd. for $C_{13}H_{14}N_2O_3SBr_2$: C, 35.63; H, 3.22; N, 6.39; Br (total), 36.48; Br^+, 18.24. Found: C, 35.67; H, 3.69; N, 6.52; Br (total), 35.89; Br^+, 18.7.

Area measurements of the n.m.r. spectrum (60 Mc/sec., $CDCl_3$ solvent), using the four aromatic protons as a reference area, confirm the presence of 14 hydrogen atoms. The compound is soluble in warm alcohols, ethyl acetate, chloroform and benzene. Loss of bromine was observed upon attempted recrystallization. No precipitation occurred when aqueous silver nitrate was added to a warm ethanol solution of the compound.

Hydrolysis of the Bicyclic Lactone XVI.—Aliquots of a 4×10^{-4} *M* solution of XVI in acetonitrile–water (1:4) gave a ninhydrin color yield of 15% after 15 min. at 100° and 20% after 1 hour. A 4×10^{-4} *M* solution of XVI in acetonitrile–1 *M* formate buffer, *p*H 5 (1:4), gave a color yield of 22% in 15 min. and 75% in 1 hour. Ammonium chloride solutions were treated in a similar way to obtain the reference 100% color yields.

N-Tosyl-4-hydroxy-5-bromopipecolic Acid Lactone.—When XVI (300 mg.) was refluxed for 1 hour in 10 ml. of 90% acetic acid, the liberated bromine gradually evaporated. The solvent was removed *in vacuo* and the residue was distributed between ethyl acetate and aqueous sodium bicarbonate. Evaporation of the ethyl acetate layer gave the lactone of N-tosyl-4-hydroxy-5-bromopipecolic acid (95 mg.). The analytical specimen was recrystallized twice from methanol; m.p. 179–181°, λ_{max}^{KBr} 5.54 μ (γ-lactone).

Anal. Calcd. for $C_{13}H_{14}NO_4SBr$: C, 43.34; H, 3.92; N, 3.89. Found: C, 43.37; H, 3.96; N, 3.91.

N-Tosyl-4-hydroxy-5-bromopipecolic Acid.—The bicarbonate layer was acidified with concentrated hydrochloric acid and the precipitate was extracted into ethyl acetate. Removal of the organic solvent left N-tosyl-4-hydroxy-5-bromopipecolic acid (100 mg.). The analytical specimen was recrystallized once from aqueous ethanol and once from ethyl acetate–chloroform; m.p. 193–195°, λ_{max}^{KBr} 5.75 μ (carboxyl C=O).

Anal. Calcd. for $C_{13}H_{16}NO_5SBr$: C, 41.28; H, 4.26; N, 3.70. Found: C, 41.49; H, 4.44; N, 3.63.

Preliminary experiments showed that the above reaction conditions were necessary for complete hydrolysis and also gave the maximum yield of lactone. Over 60% of XVI remained when it was treated under the conditions which effected complete hydrolysis of XII.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Rearrangements of Dehydroproline Derivatives

BY ALEXANDER V. ROBERTSON, JOHN E. FRANCIS AND BERNHARD WITKOP

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The action of N-bromosuccinimide on N-carbobenzyloxy-3,4-dehydro-DL-proline amide at *p*H 7–9 in a *remarkably easy Hofmann rearrangement* leads with loss of ammonia to a compound $C_{12}H_{13}NO_4$ which on the basis of its oxidation and reduction products, its O,O-diacetate and extensive n.m.r. data has been assigned the *unusual dicarbinolamide* structure II, formally the condensation product of maleic dialdehyde with benzyl carbamate. Whereas the action of base on esters and amides of N-benzoyl-3,4-dehydroproline fails to move the double bond into the 2,3-position, it produces the *optically active hydantoin* V from N-carbobenzyloxy-3,4-dehydro-L-proline amide and aromatizes N-tosyl-3,4-dehydro-DL-proline methyl ester to methyl pyrrole-2-carboxylate with *elimination of p-toluenesulfinate.* The betaine *3,4-dehydro*-DL-*stachydrine* was obtained from 3,4-dehydro-DL-proline by methylation under neutral conditions. This betaine, unlike all other dehydroproline derivatives, was unstable and rearranged easily to the isomeric *2,3-dehydrostachydrine* which was identical with the dehydration product from the two diastereoisomeric 3-hydroxystachydrines from *Courbonia virgata.*

A. The Dicarbinolamide II.—Certain aspects of the action of N-bromosuccinimide (NBS) on N-acyl-3,4-dehydroprolinamides have been discussed in the preceding paper.[1] The N-tosyl-, N-benzoyl- and N-carbobenzyloxy- derivatives all give high yields of ammonia at alkaline *p*H, but no cleavage at *p*H 4 or lower. This reaction has now been studied on a preparative scale with N-carbobenzyloxy-3,4-dehydroprolinamide (I). At *p*H 7–9 two equivalents of NBS are consumed and one equivalent of ammonia is released. A crystalline product to which the interesting structure of N-carbobenzyloxy-2,5-dihydroxy-Δ^3-pyrroline (II) has been assigned is readily isolated in 60% yield.

(1) N. Izumiya, J. E. Francis, A. V. Robertson and B. Witkop, *J. Am. Chem. Soc.*, **84**, 1702 (1962).

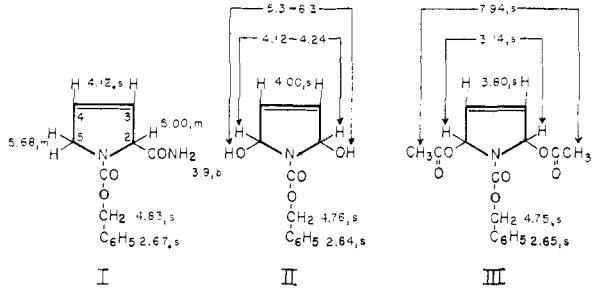


Chart I.—N.m.r. data (expressed in τ-values; s = singlet, m = multiplet, b = broad) for N-carbobenzyloxy-3,4-dehydro-DL-prolinamide (I), N-carbobenzyloxy-2,5-dihydroxy-Δ^3-pyrroline (II) and N-carbobenzyloxy-2,5-diacetoxy-Δ^3-pyrroline (III).

The compound is stable at room temperature but not at elevated temperatures, and exists in two forms which melt at 82° and 124°. These could be polymorphs or the pair of *cis/trans* isomers. The present evidence does not allow a clear decision between these alternatives. In the first experiments only the lower melting form was obtained. Three months later, when more material was required, the higher melting form crystallized during the isolation procedure, and it was discovered that all of the samples originally melting at 82° now had m.p. 124°. Repeated attempts to reisolate the lower melting form have failed. The infrared spectra of the two forms in chloroform are identical and have one carbonyl peak for the urethane group. There is a minor difference in the n.m.r. spectra (see below), and the infrared spectra in Nujol are markedly different: *inter alia* the form m.p. 82° has two carbonyl peaks for the urethane at 5.77 and 5.88 μ, whereas the form m.p. 124° has only one carbonyl peak at 5.97. These differences may reflect hydrogen-bonding of the carbonyl in the crystals.

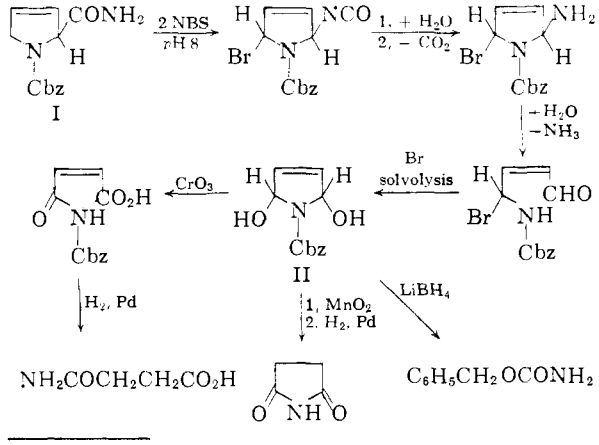
The composition $C_{12}H_{13}NO_4$ of the compound indicates the loss of a carbon and a nitrogen atom from the starting material and a gain of one oxygen atom. The ammonia released in the NBS-reaction must come from the amide nitrogen of I. The ultraviolet spectra of both forms are superposable on that of the starting material proving that the N-carbobenzyloxy unit is intact. In addition to the carbonyl absorption (above), the infrared spectra show strong bands between 2.8–3.1 μ for hydrogen-bonded OH (NH not excluded). The n.m.r. spectra are very informative, and τ-values for protons are given in formulas I-III (Chart I). The two singlets for the carbobenzyloxy group in II are almost identical with their positions in the starting material I. A singlet for two protons at 4.00, typical of the olefinic protons in dehydroproline and its derivatives[2] clearly proves that the double bond of I is still present in II. The broad peak centered at 5.8 disappears after deuterium exchange, indicating the presence of two active hydrogens. The shift in the bands for the protons on C-2 and C-5 in I implies a change of substituents at these carbons. The isomer of II melting at 82° shows two singlets, each of one proton area at 4.12 and 4.24, which are attributed to the protons on C-2 and C-5. The isomer melting at 124° over the same τ range shows only one band corresponding in area to two protons.

A diacetate III was prepared in high yield in pyridine and acetic anhydride. The ultraviolet and infrared spectra of this diacetate show the expected absorption. The n.m.r. spectrum consists of five very sharp singlets, with areas and chemical shifts corresponding to the assignments in formula III. The downfield shift of the band for the protons on C-2 and C-5 in III compared with their positions in II is typical.[3] The chemical equivalence of the two olefinic protons, of the two protons on C-2 and C-5 and of the two methyls of the acetoxy groups is convincing evidence for structure III.

In the O-acetyl determination,[4] III gives a value above the theoretical for a diacetate. This is due to a release of volatile acid in the hydrolysis of II. A satisfactory correction factor was obtained by an identical acetyl determination on pure II. The dicarbinolamide apparently undergoes cleavage in the alcoholic alkali to maleic dialdehyde followed by disproportionation to volatile acids. Catalytic reduction of both II and III caused general decomposition.

Oxidation of the dicarbinolamide II with manganese dioxide followed by catalytic reduction gave succinimide (>40% yield). Lithium borohydride reduction of II caused cleavage of the pyrroline ring on each side of the nitrogen, and benzyl carbamate was isolated in 61% yield. Chromic acid oxidation of II gave N-carbobenzyloxymaleamic acid (5% yield) as the only crystalline product, and the structure of this new compound was established by its analysis and by catalytic reduction to succinamic acid. All of these chemical transformations are consistent with the proposed structure II.

A mechanism for the formation of II[1] and its oxidation products may be



(2) The splitting patterns of protons in dehydroproline and its derivatives, including the dicarbinolamide II and its acetate III, merit a discussion which would be too extensive for the present paper. A detailed treatment will be published separately (A. V. Robertson and B. Witkop, *Austral. J. Chem.*, in press).

(3) L. M. Jackman, "Applications of NMR Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 55.

(4) Method of E. P. Clark, "Semimicro Quantitative Organic Analysis," Academic Press, Inc., New York, N. Y., 1943, p. 73.

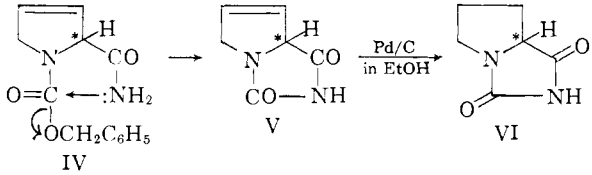
The scheme is a general outline of the types of reactions thought to be occurring. It is not intended to imply, for example, that the allylic bromination and solvolysis necessarily take place at the stages shown.

The possibility of using the dicarbinolamide as a Mannich intermediate for the synthesis of Δ^6-tropenones in condensations of the Robinson–Schöpf type is being examined.

The dicarbinolamide II offers a welcome model for the study of the complex epimerization and tautomerization reactions as they have been observed with cyclic peptides such as ergotamine. Recent extension of this work by the Sandoz group (Dr. A. Hofmann, personal communication) has led to the recognition of at least three tautomers of ergotamine one of which is postulated to have a dicarbinolamide structure comparable to II.

Attempts to prepare the N-benzoyl and N-tosyl analogs of II from the corresponding dehydroproline amides have not been successful, despite the fact that the yields of ammonia at spectroscopic concentrations are as great as for the carbobenzyloxy compound.[1] However, the lower solubilities of the benzoyl and tosyl compounds in the acetonitrile–water reaction mixture caused complications. A preparative experiment with N-carbobenzyloxyproline (which also cleaves to ammonia at spectroscopic concentrations[1]) gave no solid product and the rate of consumption of NBS was extremely slow compared with that for the dehydroproline compounds.

B. Dehydroproline Hydantoins.—N-Carbobenzyloxy-3,4-dehydro-DL-prolinamide (IV) rapidly rearranged in hot aqueous alkali to the hydantoin of 3,4-dehydro-DL-proline (V). The intramolecular

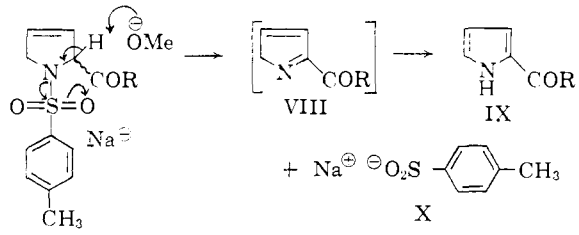


IV V VI

displacement of benzyl alcohol from N-carbobenzyloxyamino acid amides by base,[5] with formation of hydantoins, has many precedents. The present reaction is noteworthy because the double bond stays in the unconjugated 3,4-position.

The hydantoin V was also prepared from 3,4-dehydro-DL-proline and potassium cyanate. The Δ^3- rather than Δ^2-double bond position was proved by the presence of two olefinic protons in the n.m.r. spectrum, and by the optical activity of the hydantoin prepared from 3,4-dehydro-L-proline (optical purity 95%) and potassium cyanate. The levorotatory hydantoin was 78% optically pure as determined by reduction to partially racemic L-proline hydantoin (VI) of $[\alpha]^{20}D$ −100°. The pure hydantoin (VI) of L-proline has $[\alpha]^{20}D$ −128°. The easy racemization of optically active hydantoin by base is well known.[6]

C. Aromatization of N-Tosyldehydroproline Derivatives under the Influence of Base.—Whereas N-tosyldehydroproline is not affected by 1.0 *N* sodium hydroxide at room temperature, the solution of its amide, ester or glycine peptide acquires a strong absorption peak at 265 mμ on standing for a few minutes at 20°. The nature of this transformation became apparent in an experiment on a preparative scale. When N-tosyldehydroproline methyl ester (VII, R = OCH_3) was treated with 1.0 *N* sodium methoxide in methanol, methyl pyrrole-2-carboxylate (IX, R = OCH_3) was isolated in a yield exceeding 90%. The mechanism of this aromatization is pictured as:[7]



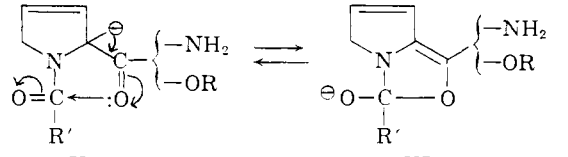
VII VIII IX X

R = -NH_2, -OCH_3, -$NHCH_2COOH$; R' = -H, -CH_3

This kind of elimination may be of interest also in the proline series where the preparation of Δ^1-pyrroline-2-carboxylic acid still poses difficulties. The action of *hot* alkali will probably be required in this case as well as in the conversion of N-tosyldehydrobaikiain amide to α-picolinic acid which has been observed spectroscopically by J. E. Francis.

The presence of sodium *p*-toluenesulfinate (X) in the aqueous solution was shown by the ready consumption of permanganate.

Neither aromatization nor migration of the $\Delta^{3,4}$-unsaturation was observed in the reaction of N-benzoyl- or N-(*p*-phenylazobenzoyl)-3,4-dehydroproline ester even with sodium hydride in refluxing toluene. The anion XI may be considerably stabilized by the contribution of a bicyclic anion XIa.



XI XIa

D. Labile and Stable Dehydroproline Betaines.—Methylation of 3,4-dehydro-DL-proline with methyl iodide and methanolic sodium hydroxide[8] yields a crystalline hygroscopic betaine (XII) chloride which was characterized as the picrate, m.p. 176°, undepressed on admixture with a sample of the picrate (m.p. 174–175°) of anhydro-3-hydroxystachydrine[9] prepared by dehydration

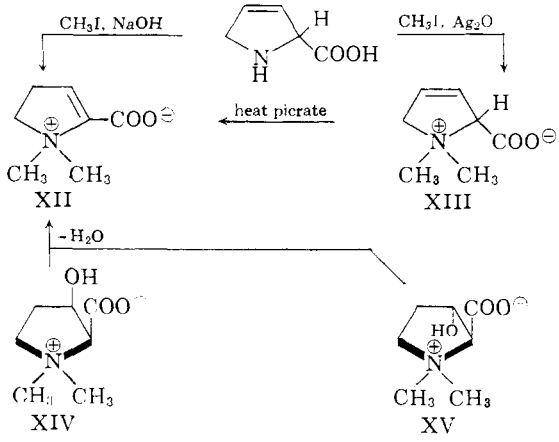
(5) *Cf.* L. A. Cohen and E. M. Fry, *J. Am. Chem. Soc.*, **78**, 5863 (1956).

(6) *Cf.* M. Bovarnick and H. T. Clarke, *ibid.*, **60**, 2426 (1938).

(7) *Cf.* W. Paterson and G. R. Proctor, *Proc. Chem. Soc.*, 248 (1961).

(8) *Cf.* H. King, *J. Chem. Soc.*, 337 (1941).

(9) J. W. Cornforth and A. J. Henry, *ibid.*, 597 (1952). We are greatly obligated to Dr. Cornforth, The National Institute for Medical Research, Mill Hill, England, for placing this sample at our disposal. The parent 3-hydroxyproline (presumably having the *trans*-arrangement of carboxyl and hydroxyl groups as in XV), a new natural amino acid and building block of collagen [*Cf.* J. D. Ogle, M. A. Logan and R. B. Arlinghaus, *Federation Proc.*, **20**, 1 (1961); *Arch. Biochem. Biophys.*, **94**, 85 (1961)] is accessible *via* the (largely stereospecific) hydroboration of N-carbobenzyloxy-3,4-dehydroproline ester [F. Irreverre, A. V. Robertson and B. Witkop, unpublished].



of *cis*- (XIV) or *trans*-3-hydroxystachydrine (XV) from *Courbonia virgata*.[9] The infrared spectra of the two picrates are also identical. Conversion of the synthetic picrate to the hydrochloride, m.p. 183°, and catalytic reduction with platinum oxide in dilute hydrochloric acid yields DL-stachydrine, characterized as the picrate, m.p. and m.m.p. 192°. The 2,3-position of the double bond in XII has been postulated on the basis of the formation of N,N-dimethyl-β-alanine by permanganate oxidation[9]; it is confirmed by the n.m.r. spectrum of the hydrochloride in D_2O[10] which shows the presence of only one olefinic proton. A singlet is observed for the two methyl peaks which are equivalent because of the plane of symmetry through the ring and carboxyl due to the trigonal C-2.

The migration of the double bond from the 3,4- to the 2,3-position in the course of methylation under alkaline conditions is avoided when the silver salt of 3,4-dehydroproline (which forms rapidly in *anhydrous* methanol) is treated with methanolic methyl iodide. The resulting betaine XIII forms a picrate melting at 141°. Above this temperature thermal migration of the double bond occurs and the melt solidifies to the picrate of XII which then melts at 175° as above. The n.m.r. spectrum of the hygroscopic hydrochloride XIII in D_2O clearly shows all the proton peaks expected from a Δ^3-pyrroline. The most significant signals are a peak for two olefinic protons and two singlets for the methyl groups which are not equivalent because C-2 is tetrahedral.

Experimental[11]

N-Carbobenzyloxy-2,5-dihydroxy-Δ^3-pyrroline (II). A. Lower Melting Form.—N-Carbobenzyloxy-3,4-dehydroprolinamide[1] (4.0 g., 16.2 mmoles) and disodium hydrogen phosphate (15 g.) were dissolved in a mixture of acetonitrile (120 ml.) and water (400 ml.). The solution was stirred magnetically while N-bromosuccinimide (6.0 g., 2 equivalents) was added in small portions at such a rate that one portion was consumed (no color with starch–iodide paper) before the next was added. Consumption of reagent was: *ca.* 1 equiv. in 5 min., 1.5 equiv. in 15 min., 1.8 equiv. in 30 min., 1.9 equiv. in 60 min. The remaining portion was then added and the starch–iodide test was still positive after another hour. The initial *p*H of 10 dropped to 8.5 upon the first addition of NBS and then gradually changed to 7.0 when all of the reagent had been added. The slight excess of reagent was decomposed with a few drops of aqueous sodium thiosulfate, and the acetonitrile was removed by evaporation *in vacuo* (bath 40°). The resulting aqueous emulsion was extracted with chloroform (4 × 50 ml.). Ninhydrin analysis of the aqueous layer showed the presence of 15.0 mmoles of ammonia. The chloroform extracts were dried over magnesium sulfate and evaporated *in vacuo* to yield a slightly red gum (3.9 g.). The gum crystallized on being warmed with 10 ml. of ethyl acetate–cyclohexane (1:1) and the white product was collected and washed with 5 ml. of cold solvent mixture (2.4 g., 62%). The analytical sample was rapidly recrystallized twice from ethyl acetate–cyclohexane (1:1) and dried at room temperature and pressure over silica gel; m.p. 81–82°; $\lambda_{max}^{meOH}(\epsilon_{max})$, 251 mμ (200), 257 (250), 263 (205), 267 (140); $\lambda_{max}^{CHCl_3}$, 5.84 μ; λ_{max}^{Nujol} 5.77, 5.82 μ; $\nu_{max}^{CCl_4}$ (1 mg./ml., 1-cm. cell, Beckman IR7), 3585(s), 3600(sh), 3415(broad), 3340(broad) cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{13}NO_5$: C, 61.27; H, 5.57; N, 5.96; Br, 0.0. Found: C, 61.60; H, 5.76; N, 5.74; Br, 0.05.

B. Higher Melting Form.—Three months later the above sample had m.p. 122–124° and, when the preparation was repeated, the higher melting form was obtained directly. A sample was recrystallized for analysis as above, m.p. 122–124°. The spectral data were the same as given above for the form m.p. 81–82°, except for the infrared carbonyl region in Nujol, λ_{max} 5.97 μ. The n.m.r. spectra showed bands of area and chemical shift corresponding to the assignments on formula II, and the slight difference between the two forms was noted in the Discussion.

Anal. Calcd. for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.96; acetyl, 0.0. Found: C, 61.54; H, 5.85; N, 5.77; "acetyl," 6.8 (see Discussion).

N-Carbobenzyloxy-2,5-diacetoxy-Δ^3-pyrroline (III).—One gram of the dicarbinolamide II (m.p. 124°) was dissolved in pyridine (5 ml.) and acetic anhydride (2 ml.) at room temperature. Two days later the excess of anhydride was destroyed with 5 ml. of methanol and the solution was evaporated *in vacuo*. The residue in ether was washed in turn with dilute hydrochloric acid, 5% sodium bicarbonate and water. The ether layer was evaporated and the residue was recrystallized from ether–cyclohexane, yielding 0.95 g., m.p. 94–95°; ultraviolet absorption as for II; λ_{max}^{Nujol} 5.70 μ (acetate C=O), 5.78 μ (urethane C=O), no N—H or O—H stretching absorption; n.m.r. assignments as on formula III and in the Discussion.

Anal. Calcd. for $C_{16}H_{17}NO_6$: C, 60.18; H, 5.37; N, 4.39; acetyl, 27.0. Found: C, 60.45; H, 5.32; N, 4.67; obsd. acetyl, 32.8; cor. acetyl, 27.8.

The correction factor for the acetyl value was obtained by adjusting the observed value for II to the molecular weight for III.

Manganese Dioxide Oxidation of II.—One gram of the dicarbinolamide II (m.p. 124°) was dissolved in 100 ml. of chloroform and magnetically stirred in a stoppered flask for 3 days with 10 g. of manganese dioxide.[12] The reaction mixture was filtered and the manganese dioxide was washed with 50 ml. of chloroform. The filtrate and washings were evaporated and the residue was treated with 5 ml. of hot ethyl acetate–cyclohexane (1:1) and left at 0°. Next day starting material (0.35 g., identified by infrared spectrum in chloroform) was filtered off. The residue (0.50 g.) in the filtrate could not be crystallized. It was hydrogenated in 5 ml. of ethanol with 50 mg. of 10% palladium-on-carbon. Thirty ml. of hydrogen was absorbed in 20 min. and thereafter a much slower uptake was observed. The rate was almost negligible after 17 hours by which time the total absorption was 108 ml. The catalyst was filtered off and the filtrate was evaporated leaving a gum (210 mg.) which was distributed between water (20 ml.) and chloroform (10 ml.). The aqueous layer was evaporated to give a crystalline residue (108 mg.) which was recrystallized from ethyl acetate–hexane; m.p. 122–125°. The infrared spectrum in chloroform was indistinguishable from that of authentic succinimide.

Benzyl Carbamate by the Lithium Borohydride Reduction of II.—Two grams of the dicarbinolamide II (m.p. 124°) dis-

(10) We are greatly obliged to Dr. H. Conroy for assistance with the n.m.r. spectra and their interpretation.

(11) All melting points are corrected; all boiling points are uncorrected. The analyses were made by Mr. H. G. McCann and associates of the Analytical Services Unit of this Laboratory.

(12) J. Attenburrow, *et al.*, *J. Chem. Soc.*, 1094 (1952).

solved in 10 ml. of tetrahydrofuran was added slowly to a slurry of 400 mg. of lithium borohydride in 10 ml. of tetrahydrofuran. Thirty minutes later the excess of borohydride was destroyed by the cautious addition of water and the reaction mixture was distributed between water (20 ml.) and ether (80 ml.). The ether layer was washed with water until neutral (3 × 10 ml.). Removal of the ether gave a residue which was crystallized (780 mg.) from ether–hexane; m.p. after three crystallizations, 82–84°. The infrared spectrum in chloroform and the n.m.r. spectrum in $CDCl_3$ were identical with those for authentic benzyl carbamate.[13]

N-Carbobenzyloxymaleamic Acid by the Chromic Oxide Oxidation of II.—Two grams of the dicarbinolamide (m.p. 81°) was dissolved in 10 ml. of warm glacial acetic acid, and 1.2 g. of chromic oxide in 1 ml. of water was added. An immediate vigorous reaction occurred and the flask was cooled in ice and left 1 hour. The green solution was acidified with 30 ml. of 2 *N* hydrochloric acid and extracted with chloroform (4 × 30 ml.). The extracts were dried over magnesium sulfate and evaporated. The gummy residue (2.05 g.) was dissolved in benzene (15 ml.) and extracted with saturated aqueous sodium bicarbonate (4 × 20 ml.). The gum (0.98 g.) remaining in the benzene layer could not be crystallized. The aqueous layer was acidified with concentrated hydrochloric acid (20 ml.) and extracted with chloroform (4 × 50 ml.). The chloroform layer was evaporated and part of the residue (0.36 g.) crystallized on being scratched after the addition of benzene. The crystals (90 mg.) were collected and recrystallized from benzene, giving **N-carbobenzyloxymaleamic acid**, m.p. 150–151°.

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45. Found: C, 57.78; H, 4.53.

Succinamic Acid.—The acid was hydrogenated in ethanol over 10% palladium-on-carbon. Two moles of hydrogen was rapidly absorbed and the catalyst was filtered off. Evaporation of the filtrate gave a crystalline residue which had m.p. 152–155° after recrystallization from ethanol. Its infrared spectrum (KBr) was identical with that of authentic succinamic acid.

Anal. Calcd. for $C_4H_7NO_3$: C, 41.02; H, 6.03; N, 11.96. Found: C, 41.01; H, 6.14; N, 11.89.

3,4-Dehydro-DL-proline Hydantoin (V). Method A.—N-Carbobenzyloxy-3,4-dehydroprolinamide (I, 500 mg.) was heated on a steam-bath with 10 ml. of 1 *N* sodium hydroxide. The starting material suddenly reacted as the temperature rose to 100° and a clear solution resulted. The solution was cooled and ether extracted. Evaporation of the ether extract left a liquid (163 mg.) which was identified as benzyl alcohol by its infrared spectrum in chloroform. The aqueous phase was acidified and extracted with ether. The ether extract was evaporated and the residue (117 mg.) was recrystallized from benzene, m.p. 153–154°. The ultraviolet spectrum in methanol showed only end absorption. The infrared spectrum in Nujol was identical with that for the sample prepared by method B. The n.m.r. spectrum showed a singlet at τ 3.97 typical of two olefinic protons in the dehydroproline series.

Anal. Calcd. for $C_6H_6O_2N_2$: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.95; H, 4.62; N, 20.21.

Method B.[14]—3,4-Dehydro-DL-proline (1 g.) in 2 ml. of water was heated at 100° for 10 minutes with 1 g. of potassium cyanate (fume hood). Concentrated hydrochloric acid (5 ml.) was cautiously added and heating was continued for 10 minutes. The solution was evaporated *in vacuo* and a dry residue was obtained by repeated evaporation with small volumes of methanol. The powder was extracted with 50 ml. of boiling benzene. Removal of solvent left crystalline hydantoin which was recrystallized from benzene; m.p. 153–155°.

Anal. Calcd. for $C_6H_6N_2O_2$: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.04; H, 4.46; N, 20.36.

3,4-Dehydro-L-proline hydantoin was prepared by method B above from 3,4-dehydroproline, $[\alpha]^{20}D$ −380° (*c* 1, H_2O). The product had $[\alpha]^{20}D$ −191° (*c* 2, EtOH) and its infrared spectrum was indistinguishable from that of the racemic hydantoin.

Anal. Calcd. for $C_6H_6N_2O_2$: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.47; H, 4.62; N, 20.30.

L-Proline Hydantoin (VI).—3,4-Dehydro-L-proline hydantoin (138 mg., 1 mmole) was hydrogenated in 5 ml. of ethanol over 50 mg. of 10% palladium-on-carbon. Absorption of hydrogen ceased in 10 minutes at 1-mole uptake. The catalyst was filtered off and the filtrate was evaporated, leaving L-proline hydantoin, $[\alpha]^{20}D$ −100° (*c* 2, EtOH). An authentic sample prepared by method B above from proline, $[\alpha]^{20}D$ −85° (*c* 1, H_2O), had $[\alpha]^{20}D$ −128° (*c* 2, EtOH). The infrared spectra of the two specimens were superimposable.

Action of Alkali on N-Tosyldehydroproline Amide (VII).—N-Tosyl-DL-dehydroproline amide (26.6 mg., 0.1 mmole) was dissolved in 95% ethanol (50 ml.). Two quartz cuvettes (10-mm. light path) were filled with 3.8 ml. of 1.0 *N* sodium hydroxide. To the blank was added 0.2 ml. of 95% ethanol, and to the sample cell was added 0.2 ml. of the prepared amide solution with stirring. The ultraviolet spectrum was quickly run, and then rerun at intervals. The spectrum changed rapidly, as the peak at 230 mμ shifted toward 220 mμ, and a new peak appeared at 265 mμ. The extinction values are shown at various intervals.

Time, min.	λ_{max} 220, ϵ =	λ_{max} 265, ϵ =
0	11,120	1600
10	12,000	5100
20	12,400	6850
32	12,500	7600
135	17,000	10,000
	15,200[a]	14,100[a]

[a] The final readings were taken when the solution was heated for 35 minutes at 40–50° with 0.5 *N* sodium hydroxide.

Under comparable conditions the action of alkali failed to change the spectrum of an aqueous solution of N-tosyl-DL-dehydroproline.

Action of Sodium Methoxide on N-Tosyldehydroproline Methyl Ester: Methyl Pyrrole-2-carboxylate.—N-Tosyl-DL-dehydroproline methyl ester (281 mg., 1 mmole) was dissolved with heating in 1 *N* sodium methoxide in dry methanol (5 ml.). The solution was refluxed for 1 hour, cooled, and evaporated *in vacuo* to about 0.5 ml. It was taken up in chloroform and washed with ice-water. The chloroform extract was dried over sodium sulfate and evaporated to dryness *in vacuo*. There remained a white crystalline solid (115 mg.), m.p. 70–73°. The compound was recrystallized from cyclohexane to give colorless needles, m.p. 72–73°. The mixed melting point with an authentic sample of methyl pyrrole-2-carboxylate, m.p. 71.5–73°, was 72–73°. The infrared spectra of the two compounds were identical in every detail. A small portion of the aqueous wash, when acidified with acetic acid, decolorized permanganate.

Treatment of N-Benzoyl-DL-dehydroproline Methyl Ester with Sodium Methoxide.—A 10^{-3} *M* solution of N-benzoyl-DL-dehydroproline ester in methanol was prepared. In two quartz cuvettes (10-mm. light path) was placed 0.1 *N* sodium methoxide (2.7 ml.). To the blank cell was added 0.3 ml. of methanol. To the sample cell was added 0.3 ml. of the ester solution with mixing. The ultraviolet spectrum was taken immediately and at intervals thereafter. There was no change in the spectrum after 1.5 hours.

On a larger scale, 1 *M* sodium methoxide failed to rearrange the ester after 60 hours at room temperature and 3.5 hours at reflux. After 16 hours the saponified material was N-benzoyldehydroproline, as shown by infrared spectrum and the infrared spectrum of the ester prepared from it.

Treatment of N-Tosyldehydroprolylglycine with Sodium Hydroxide.—N-Tosyl-DL-dehydroprolylglycine (16.2 mg., 0.05 mmole) was dissolved with difficulty in 250 ml. of 95% ethanol.

In 10-mm. quartz cuvettes were placed 3.8 ml. of 1 *N* sodium hydroxide. To the blank was added 0.2 ml. of 95% ethanol with mixing. To the sample cell was added 0.2 ml. of the dipeptide solution with rapid mixing (10 seconds). The ultraviolet spectrum was taken immediately and at intervals after that. As in the case of the amide, the peak at 230 mμ shifted toward 220, and a new peak appeared at 265 mμ. The ε values at intervals are:

(13) "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1947, p. 168.

(14) *Cf.* W. J. Boyd, *Biochem. J.*, **27**, 1838 (1933).

Time, min.	λ_{max} 265, ϵ =	λ_{max} 220, ϵ =
0	1200	9000
10	2100	10,200
20	3000	10,800
30	3900	11,700
50	5450	12,000
60	6150	12,500
90	7950	12,500
120	9400	13,000
235	12,450	13,600
340	14,100	14,000

DL-Dehydroproline Methyl Ester Hydrochloride.—DL-Dehydroproline (5.0 g.) in methanol (100 ml.) was cooled to 4° in ice, as dry HCl gas was bubbled into the solution. The input of gas was halted after 30 minutes, and the solution was allowed to stand overnight at 0°. The yellow solution was evaporated to dryness *in vacuo* at 60°, and finally at the oil-pump. The hydrochloride was obtained as a brownish crystalline mass. It was dissolved in hot methanol, treated with charcoal, filtered, and crystallized by the addition of ether. When dried *in vacuo* over calcium chloride, the tan crystals weighed 5.67 g., m.p. 129–131°. From the mother liquors, a second crop (746 mg.), m.p. 115–123°, was obtained. The second crop was crystallized twice from methanol–acetone; m.p. 125–127°. It was finally purified by sublimation to a white crystalline solid, m.p. 134–136°. The infrared spectra of the sublimed and unsublimed materials were identical.

Anal. Calcd. for $C_6H_{10}NO_2Cl$: C, 44.07; H, 6.16; Cl, 21.71. Found: C, 44.15; H, 6.48, Cl, 21.60.

***p*-Phenylazobenzoyl-DL-dehydroproline Methyl Ester.**—Dry pyridine (10 ml.) was heated to about 60° and to it was added quickly dehydroproline methyl ester hydrochloride (682 mg.). The mixture was stirred vigorously for 20 seconds, and *p*-phenylazobenzoyl chloride (m.p. 92–94°, 1.7 g.) was added and washed into the flask with 5 ml. of pyridine. The mixture was stirred at 60°, and in 15 minutes all went into solution. The dark red solution was maintained at about 60° for 2 hours. The solution was cooled and poured into ice and 6 *N* hydrochloric acid (40 ml.). A flocculent orange precipitate formed, which was collected by filtration. The substance dissolved in hot methanol, but could not be crystallized from aqueous methanol or methanol–ether. Evaporation to near dryness yielded 345 mg. of pale orange powder, m.p. above 260°. The infrared (Nujol) showed a very small peak at 5.8 μ, but larger peaks at 6.25, 6.48, 6.83, 7.06, 7.27, 12.6 and 14.3 μ.

The aqueous filtrate was extracted with ethyl acetate several times, and the acetate layer washed with 5% bicarbonate and saturated salt solution. The acetate solution was then dried over sodium sulfate and evaporated to dryness *in vacuo*. The dried extract was taken up in acetone and refiltered to remove more orange material, and finally the dry neutral residue was dissolved in chloroform and filtered through a short column of neutral alumina (Woelm, activity grade 1). From the column, a reddish oil (1.39 g.) was obtained which crystallized partially on standing. The rest of the oil crystallized from acetone–ether with seeding, m.p. 111–114°. One recrystallization from acetone–ether yielded the pure material, m.p. 112–114°, as red crystals. For analysis, a sample was dried *in vacuo* for 24 hours over P_2O_5 at room temperature, then at 65° for 6 hours. In chloroform the infrared spectrum showed sharp peaks at 5.68, 6.06 and 6.14 μ.

Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 67.69; H, 5.26; N, 12.73.

***p*-Phenylazobenzoyl-DL-dehydroproline Amide.**—*p*-Phenylazobenzoyl-DL-dehydroproline methyl ester (208 mg.) was treated with methanolic ammonia for 16 hours in the usual manner. Evaporation of the solvent yielded 190 mg. of orange powder, m.p. 199–201°. The substance was recrystallized as orange-red plates from methanol–acetone; m.p. 202–203°. Addition of ether to the mother liquors produced a second crop, m.p. 202°. The yield was 161 mg. (81%). Recrystallization from methanol–acetone yielded the pure material, m.p. 204.5°. For analysis the sample was dried *in vacuo* at 100° over P_2O_5 for 20 hours. The infrared spectrum ($CHCl_3$) had peaks at 5.88, 6.06, 6.14 and 6.31 μ.

Anal. Calcd. for $C_{18}H_{16}N_4O_2$: C, 67.48; H, 5.03; N, 17.49. Found: C, 67.50; H, 5.13; N, 17.77.

For purposes of comparison and systematization the analogous proline derivatives were prepared.

DL-Proline Methyl Ester Hydrochloride.—DL-Proline (7 g.) was suspended in methanol (100 ml.) and treated with gaseous HCl at 0° for 40 minutes. The solution was allowed to stand for 20 hours at 0°. Evaporation of the solvent left a pale yellow oil which crystallized on standing. The hygroscopic solid was suspended in acetone, collected by filtration and dried *in vacuo*, m.p. 120–121.5°. The mother liquors were evaporated to dryness, taken up in a small volume of methanol, and crystallized by addition of acetone and ether; m.p. 120–122°. The substance was too hygroscopic to obtain an estimate of the yield. It was recrystallized once from methanol–benzene–cyclohexane and once from methanol–benzene; m.p. 121–122.5°. It was dried at 65° over P_2O_5 for 5 hours *in vacuo*.

Anal. Calcd. for $C_6H_{12}NO_2Cl$: C, 43.51; H, 7.30; N, 8.46; Cl, 21.41. Found: C, 43.53; H, 7.68; N, 8.54; Cl, 21.39.

A portion of the substance was sublimed *in vacuo* onto a cold finger with the aid of an oil-bath at 80–90°, m.p. 121–121.5°. This sample was used for determination of the above values for nitrogen and chlorine.

***p*-Phenylazobenzoyl-DL-proline Methyl Ester.**—The proline derivative was prepared exactly as described for the dehydroproline derivative. Again the same high melting (above 305°) impurity was found. The pure orange-red crystalline ester melted at 104–106°.

Anal. Calcd. for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 67.41; H, 5.75; N, 12.33.

2,3-Dehydro-DL-stachydrine (XII).—3,4-Dehydro-DL-proline (1.3 g.), sodium hydroxide (1.3 g.), methanol (20 ml.) and methyl iodide (3 ml.) were boiled under reflux. Additional methyl iodide (2-ml. portions) was added after 2 hours and 20 hours, and heating was continued for a total of 24 hours. The solution was evaporated and the residue was dissolved in water and heated with excess of freshly precipitated silver chloride and filtered. The filtrate was evaporated and the oily residue was treated with absolute ethanol and filtered from sodium chloride. Another treatment with ethanol gave a residue free of sodium chloride, and trituration with acetone afforded crystalline material (515 mg.) which was very hygroscopic. Recrystallization from methanol–acetone gave colorless needles of 2,3-dehydro-DL-stachydrine hydrochloride, m.p. 180–182° dec. after drying *in vacuo* at 80° for 16 hours. A satisfactory analysis could not be obtained. The n.m.r. spectrum in D_2O shows a peak for one olefinic proton, and a singlet for two equivalent N-methyl groups.

The hydrochloride (175 mg.) was treated with an equivalent of saturated aqueous sodium picrate and the solution was kept at 0° overnight. 2,3-Dehydro-DL-stachydrine picrate (163 mg.) was collected and recrystallized from ethanol, m.p. 175–176°. The infrared spectrum of the picrate was identical with that of anhydro-3-hydroxystachydrine picrate,[8] m.p. 173–175°, and a mixed melting point showed no depression.

Anal. Calcd. for $C_7H_{11}N_2O_2 \cdot C_6H_3N_3O_7$: C, 42.17; H, 3.81; N, 15.13. Found: C, 42.27; H, 3.81; N, 14.71.

DL-Stachydrine.—2,3-Dehydro-DL-stachydrine hydrochloride (100 mg.) was hydrogenated at room temperature and pressure with Adams catalyst in water (10 ml.) containing 0.1 ml. of concentrated hydrochloric acid. Additional catalyst (50 mg.) was added after 4 hours and shaking was continued for 5 hours. The catalyst was filtered and, after removal of solvent, the residue was treated with 3 ml. of saturated aqueous sodium picrate yielding DL-stachydrine picrate as yellow plates (27 mg.) which were recrystallized from ethanol; m.p. 190–192° dec. The infrared spectrum was identical with that of authentic material, m.p. 197–198° dec., prepared from DL-proline using the method of Cornforth and Henry for quaternization.[9]

3,4-Dehydro-DL-stachydrine (XIII).—3,4-Dehydro-DL-proline (1.0 g.) and silver oxide (2.00 g.) were agitated with 20 ml. of *anhydrous* methanol. Formation of the silver salt appeared complete after 45 minutes and the mixture was stirred with 6 ml. of methyl iodide. After 1 hour the precipitate was filtered off and the filtrate was evaporated. The oily residue (1.24 g.) was treated with picric acid (2.3 g.) in ethanol (30 ml.), giving 3,4-dehydro-DL-stachydrine

(XIII) picrate as yellow needles (1.68 g.) which were recrystallized from ethanol; m.p. 140–141°, solidifying at 142° and remelting at 173–175°.

Anal. Calcd. for $C_7H_{11}N_2O_2 \cdot C_6H_3N_3O_7$: C, 42.17; H, 3.81; N, 15.13. Found: C, 42.38; H, 3.86; N, 15.13.

The picrate (500 mg.) was dissolved in 3 *N* hydrochloric acid and extracted 5 times with benzene. The colorless aqueous layer was evaporated *in vacuo* and the residue (250 mg.) was thoroughly dried and recrystallized from methanol–acetone to give the hygroscopic hydrochloride of 3,4-dehydro-DL-stachydrine, m.p. 180–182° dec. Its n.m.r. spectrum in D_2O shows a peak for two olefinic protons and two singlets for the non-equivalent N-methyl groups.

Conversion of 3,4-Dehydrostachydrine (XIII) into 2,3-Dehydrostachydrine (XII).—A tube containing 3,4-dehydrostachydrine (XIII) picrate (20 mg.) was placed in an oil-bath at 150°. When the compound had melted and resolidified, it was cooled and crystallized from ethanol yielding yellow plates of 2,3-dehydrostachydrine (XII) picrate, m.p. 173–175°, having an infrared spectrum identical with that of the authentic sample. The infrared spectra of the isomeric picrates show noticeable differences, as do those of the two hydrochlorides in KBr.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Alkylation and Cleavage of Methionine Peptides

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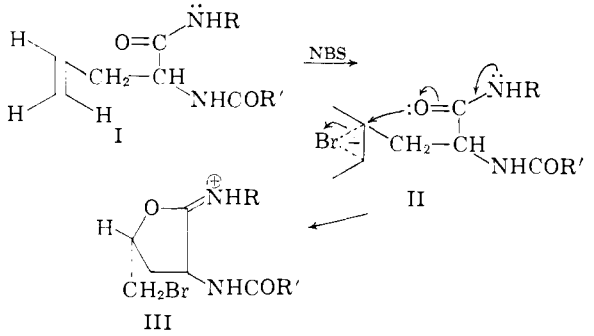
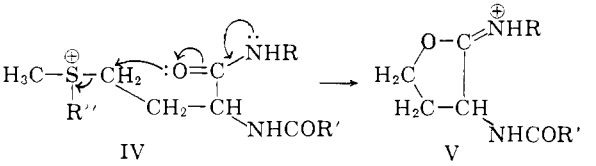
Intramolecular participation of the peptide carbonyl in the displacement of the sulfur function in sulfonium salts of methionine peptides has been utilized for peptide cleavage. Alkylation and decomposition of methionine peptides has been studied with regard to optimal conditions and the effect of the nature of the alkylating agent on the yields in the cleavage reaction.

The oxidative cleavage of peptides of tryptophan,[2] tyrosine,[3] histidine[4] and allylglycine[5] appears to proceed through participation of the C-peptide bond in the ring opening of a labile bromonium intermediate (I–III).[6]



In a peptide of a methioninesulfonium derivative IV, participation of the C–peptide group is easily possible by 1,5-interaction, concerted elimination of the sulfur function in the γ-position and formation of a homoserine (imino)lactone (V).[7]



The literature records numerous instances of such a breakdown of methioninesulfonium salts leading to homoserine or its lactone. However, the intramolecular assistance from the methionine carboxyl group in these elimination reactions has not always been properly recognized.

The decomposition of N-formylmethioninemethylsulfonium acetate to α-formamido-γ-butyrolactone on evaporation of an aqueous solution to dryness *in vacuo* implies an internal displacement of methyl sulfide by the carboxylate ion.[8] The sulfonium salt, derived from bis-(2-chloroethyl) sulfide and methionine, when heated in aqueous solution at 100° for several hours, yielded largely methionine and a small amount of homoserine.[9]

A heat-labile principle of cabbage juice was found to be a methioninemethylsulfonium salt giving rise to homoserine when autoclaved in water.[10] Homoserine was obtained from the methioninemethylsulfonium salt isolated from asparagus when boiled with alkali.[11] In the decomposition of methioninemethylsulfonium bromide in acid solutions methionine was regenerated, whereas in hot neutral and alkaline solutions homoserine and dimethyl sulfide were formed.[12]

The presence of homoserine, homoserine lactone and S-carboxymethylhomocysteine in addition to methionine, among the products of acid hydrolysis of ribonuclease, 50% inactivated by iodoacetate at *p*H 2.8, indicated elimination of the sulfur function of methionine.[13] The same prod-

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