[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

Carbamic Acid Peptides. A New Type of Peptide and a New Possible Source of Ammonia from Proteins[1]

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In spite of the fact that carbamic acid is, formally, the simplest amino acid and is, therefore, a potential structural unit of protein molecules, information concerning its peptides is lacking in the chemical literature. We report herewith the preparation and some of the properties of three of the simplest tripeptides of this new class, $NH_3^+CHRCONHCONHCHR'COO^-$ (ϵ δ γ β α). Hypothetically, these substances might be regarded as formed by the condensation of one molecule of carbamic acid with two amino acids: $NH_2CHRCOOH + HNHCOOH + HNHCHR'COOH$. Experimentally, they are prepared from derivatives of hydantoic acid and may also be named δ-(α-aminoacyl) hydantoic acids.

Carbamic acid peptides may obviously be regarded as derivatives of urea. The idea that urea groups might be present in protein molecules was advanced as early as 1875 by Schutzenberger[3] and has been presented by several workers since that time. The chief experimental evidence which has been given in support of this structure is that some proteins give a larger percentage of carbon dioxide on alkaline hydrolysis than on acid hydrolysis. Hydantoic acids,[4] polypeptide hydantoins,[5] and carbonyl bis-peptides,[6] are among the compounds which have been suggested as the source of this ureide linkage. More recently the isolation of citrulline,[7] a carbamido amino acid, suggests that the urea group may exist in proteins.

The three carbamic acid peptides which we report are: glycylcarbamylglycine (δ-glycylhydantoic acid of glycine) $NH_3^+CH_2CONHCONHCH_2COO^-$ (I); glycylcarbamyl-*d,l*-alanine (δ-glycylhydantoic acid of *d,l*-alanine) $NH_3^+CH_2CONHCONHCH(CH_3)COO^-$ (II); and *d,l*-alanylcarbamylglycine, (δ-(*d,l*-alanyl) hydantoic acid of glycine) $NH_3^+CH(CH_3)CONHCONHCH_2COO^-$ (III). The general method of preparation was similar to Fischer's method of preparing peptides by amination of the corresponding halogenated derivatives.

$$XCHRCOX + NH_2CONHCNR'COOH \xrightarrow[\text{dioxane}]{\text{boiling}} XCHRCONHCONHCHR'COOH + NH_3\ (\text{liq}) \longrightarrow NH_2CHRCONHCONHCHR'COO^-.$$

(X = Cl or Br)

An alternative method for the preparation of the halogenated acyl hydantoic acids is catalytic hydrogenation of their benzyl esters[8]

$$XCHRCONHCONHCHR'COOCH_2C_6H_5 + H_2 \xrightarrow[\text{palladium}]{\text{colloidal}} XCHRCONHCONHCHR'COOH + C_6H_5CH_3$$

The δ-(chloroacetyl)-hydantoic acid of glycine (IV), $ClCH_2CONHCONHCH_2COOH$, was first prepared by this method. Preparation of this acid by hydrolysis of its ethyl ester was impossible because both aqueous and non-aqueous alkali cleaved the δ-N-acyl linkage, a reaction which has often been observed with N-acyl ureas.[9] Johnson's method of preparing δ-acetylthiohydantoic acids[10] does not lead to the corresponding chloroacetylhydantoic acids. Six δ-(haloacyl)-hydantoic acids and δ-acetylhydantoic acid of glycine, $CH_3CONHCONHCH_2COOH$ (V), were prepared. The titration curves in Fig. 1 show that acids IV and V are slightly stronger than the hydantoic acid of glycine.

One would expect the pK of these acids to be about the same as the pK of the corresponding acylated peptides. The pK of acid IV is approximately 3.4 (± 0.1) and the pK reported for chloroacetylglycine is 3.37.[11]

Liquid ammonia was used in aminating the halogenated acylhydantoic acids because aqueous ammonia caused hydrolysis of the δ-N-acyl link-

(1) This paper is from the doctoral dissertation of Charlotte I. Damerel, The Johns Hopkins University, 1939. Original manuscript received March 17, 1942.

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(3) Schutzenberger, *Bull. soc. chim.*, **24**, 4 (1875).

(4) Lippich, *Ber.*, **41**, 2953 (1908); *Z. physiol. Chem.*, **90**, 441 (1914).

(5) Johnson, *Chem. News*, **113**, 127 (1916); Johnson and Bates, THIS JOURNAL, **38**, 1087 (1916); Johnson and Hahn, *ibid.*, **39**, 1255 (1917); Renfrew and Johnson, *ibid.*, **51**, 254, 1784 (1929); Hahn and Gilman, *ibid.*, **47**, 2941 (1925).

(6) Brigl and Held, *Z. physiol. Chem.*, **152**, 230 (1926); Klarmann, *Chem. Rev.*, **4**, 101 (1927).

(7) Wada, *Biochem. Z.*, **257**, 1 (1933).

(8) Bergmann, Zervas, Fruton, Schneider and Schleich, *J. Biol. Chem.*, **109**, 340 (1935).

(9) Stoughton, *J. Org. Chem.*, **2**, 514 (1938); Baum, *Ber.*, **41**, 536 (1908); Titherley and Stubbs, *J. Chem. Soc.*, 299 (1914); Abderhalden and Riez, *Fermentforsch.*, **12**, 216 (1931).

(10) Wheeler, Nicolet and Johnson, *Am. Chem. J.*, **46**, 456 (1911).

(11) Zief and Edsall, THIS JOURNAL, **59**, 2245 (1937).

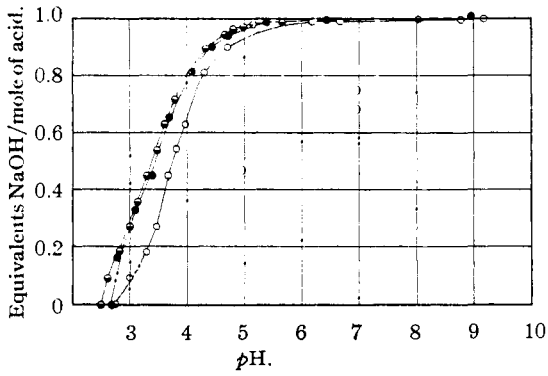


Fig. 1.—Titration curves of hydantoic acid of glycine —○—; δ-chloroacetylhydantoic acid of glycine (IV), —◑—; δ-acetylhydantoic acid of glycine (V) —●—.

age as mentioned previously. Compounds I, II and III were crystalline substances having amphoteric properties similar to those of the usual peptides. Aqueous solutions of these acids were readily attacked by sodium hydroxide, heat and hydrochloric acid. Compound III, unlike I and II, when exposed to the air immediately after drying, had the interesting property of taking up one molecule of water in five to ten minutes of exposure to the air. Although several hydrates of peptides have been reported,[12] as far as we know, those previously prepared have been more stable in the anhydrous state than compound III. This suggests further studies of the structural units responsible for the hydration of proteins.

The titration curves in Fig. 2 for aqueous solutions of compounds I, II and III indicate that these substances are amphoteric and have dissociation constants of about the same value. The approximate values, accurate to within 0.2 unit, are 3.3 for pK_1 and 7.6 for pK_2. These values are not significantly different from some of those reported for the polypeptides of glycine and of alanine.[13] For glycylglycine, pK_1 = 3.12, pK_2 = 8.07; for glycylalanine, pK_1 = 3.15, pK_2 = 8.25; for diglycylglycine, pK_1 = 3.26, pK_2 = 7.91. The formol titration curves for these aminoacylhydantoic acids, also found in Fig. 2, indicate that formaldehyde shifts the titration curves of these compounds in the same manner as it does those of amino acids and polypeptides.[14]

It is possible that the detection of linkages such

(12) Greenstein, *J. Biol. Chem.*, **124**, 255–262 (1938); Dyer, THIS JOURNAL, **63**, 266 (1941); Smith and Brown, *ibid.*, 2606.

(13) C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins." Charles C. Thomas, Springfield, Ill., 1938, p. 613.

(14) *Ibid.*, p. 192.

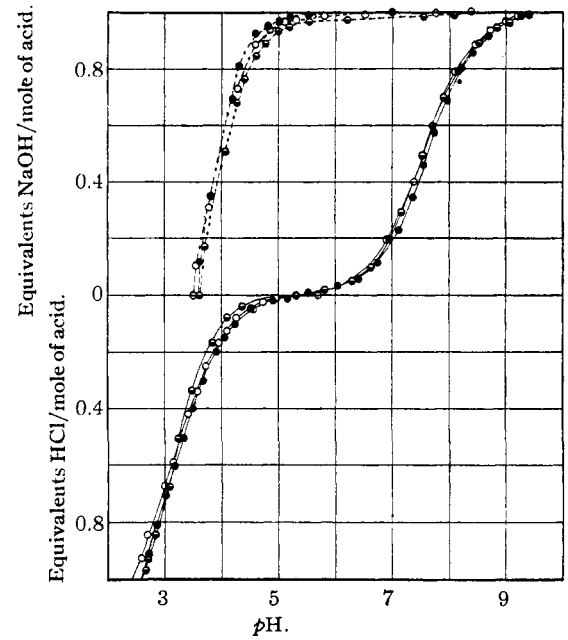


Fig. 2.—Titration curves of δ-(α-amino)-acylhydantoic acids: δ-glycylhydantoic acid of glycine (I) —○—; δ-glycylhydantoic acid of *d,l*-alanine (II) —◑—; δ-(*d,l*-alanyl)-hydantoic acid of glycine (III) —●—; aqueous solution ——; formaldehyde solution — — —.

as these, if they do exist in proteins, may be accomplished by a study of hydrolytic peculiarities. For this reason we are reporting herewith an orienting study of the behavior of these subtances under the influence of alkaline, "neutral" and acidic agents to serve as guides to kinetic studies on these substances in the future. We have used titration curves as our chief reliance in analysis. The differentiation by this method between the various substances which can be present is good and the small number of possible cleavage products from these synthetic materials permits a considerable amount of information to be obtained with a small sample. The presence of a few per cent. of a by-reaction product can usually be detected readily by inspection. The differences in rate of ammonia evolution which were observed were so great that only a qualitative test was necessary to detect them.

Predictions as to the hydrolytic behavior of the substances might be made on the basis of analogy with known compounds containing similar linkages. Thus the γ–δ–ε-linkage might be regarded as similar to that of a secondary amide such as diacetamide, while the β–γ–δ-linkage might be likened to that in urea. The analogy with urea itself is not sound, however, because of the possi-

bility of the formation of cyanates from it and its mono-substituted derivatives[15] which may be blocked by N—N′ disubstitution. Better analogies are to be found in N-acetyl-N′-methylurea, $CH_3CONHCONHCH_3$, and in acetylhydantoic acid, $CH_3CONHCONHCH_2COOH$, both of which have the same atomic groupings in the β-γ-δ-ϵ-chain.

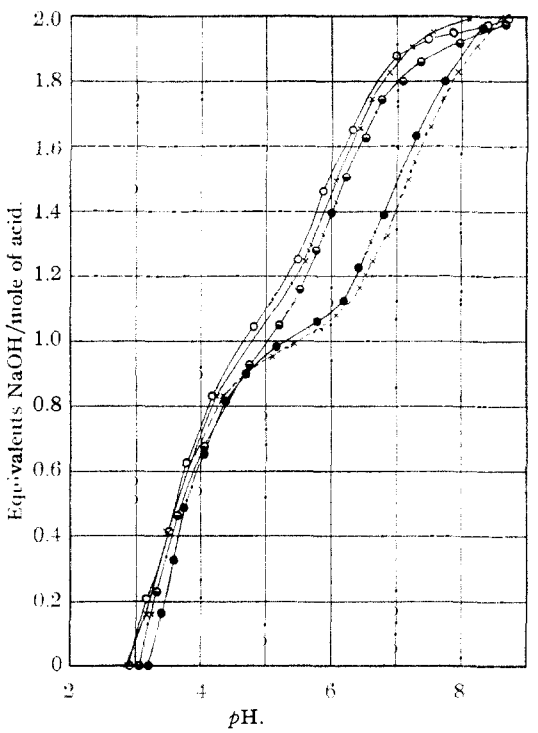


Fig. 3.—Titration curves in formaldehyde solution of δ-(α-amino)-acylhydantoic acids after twenty-four-hour treatment with 0.3 *N* sodium hydroxide at room temperature. The sodium hydroxide was neutralized with hydrochloric acid before each titration: acid I —○—; acid II —◒—; acid III —●—. Solutions of equivalent amounts of glycine and hydantoic acid —×— and of alanine and hydantoic acid - - -×- - - similarly treated.

Alkaline Hydrolysis

Secondary amides are hydrolyzed most readily by alkalies even when cold.[16,17] Acetylmethylurea appears to hydrolyze with more difficulty, although strictly comparable data are not available.[18] Acetylhydantoic acid cleaves at the δ-ϵ-linkage to give acetate and hydantoate ions.[9] By analogy one would predict that the new peptides would cleave at the δ-ϵ-linkage to give the appropriate amino-acid anion and the appropriate hydantoate ion.

The titration curves in Fig. 3 summarize the data on the alkaline hydrolysis of peptides I, II and III.

Glycylcarbamylglycine (I) and glycylcarbamylalanine (II) after treatment with sodium hydroxide gave formol titration curves similar to the curve for a solution containing equivalent amounts of glycine and hydantoic acid. Alanylcarbamylglycine (III) gave a curve similar to that of a solution of equivalent amounts of alanine and hydantoic acid. Acetylhydantoic acid was treated with sodium hydroxide in the same manner as these aminoacyl hydantoic acids and the titration curve indicated complete decomposition into acetate and hydantoate ions. The alkaline hydrolysis of the carbamyl peptides may thus be represented by the equation

$$\underset{\text{R}}{\text{NH}_2\text{CHCONHCONH}\underset{\text{R}'}{\text{CHCOO}^-} + \text{OH}^-} \longrightarrow \underset{\text{R}}{\text{NH}_2\text{CHCOO}^-} + \underset{\text{VI}}{\underset{\text{R}'}{\text{NH}_2\text{CONHCHCOO}^-}}$$

The titration curves in Fig. 3 show that the major portion of the attack upon peptides II and III with cold dilute alkali follows this course. The amount of ammonia formed, however, and the speed of its formation are both greater than are compatible with the assumption that it is formed only by a secondary cleavage of the hydantoate of alanine (VI). It does not seem unreasonable to suppose that a small amount of the peptide is attacked at the γ-δ-linkage, thus

$$\text{NH}_2\text{CH}_2\text{CONHCONHCH(CH}_3\text{)COO}^- + \text{OH}^- \longrightarrow \text{NH}_2\text{CH}_2\text{CONH}_2 + {}^-\text{OOCNHCH(CH}_3\text{)COO}^-$$

The rate of formation of ammonia from glycine amide is sufficiently greater than that from the hydantoate of alanine to account for the speed of the evolution of ammonia. Comparison of the titration curves of the hydrolyzed material with the controls in Fig. 3 shows that the total amount of this side reaction is small, although the qualitative test shows that it is greater for compound III than for compound II.

It is obvious that these compounds will give the same behavior as hydantoic acids on hydrolysis with hot, strong alkali, freeing stoichiometric quantities of carbonate ion and ammonia.

Acid Hydrolysis

Diacetamide[19] is hydrolyzed with fair speed

(15) Fawsitt, *Z. physik. Chem.*, **41**, 601 (1902).
(16) Ulffers and Janson, *Ber.*, **27**, 93 (1894).
(17) Titherley and Stubbs, *J. Chem. Soc.*, **105**, 306 (1914).
(18) Hofmann, *Ber.*, **14**, 2727 (1881).
(19) Hentschel, *ibid.*, **23**, 2395 (1890).

by dilute mineral acids and is cleaved much more rapidly than acetamide. Likewise acetylmethylurea[18] and benzoylhydantoic acid[10] decompose at the δ-ε-bond. We should predict from these analogies that our new peptides would cleave at the δ-ε-bond in dilute mineral acid.

The titration curves in Fig. 4 summarize the data on the dilute acid hydrolysis of peptides I, II and III.

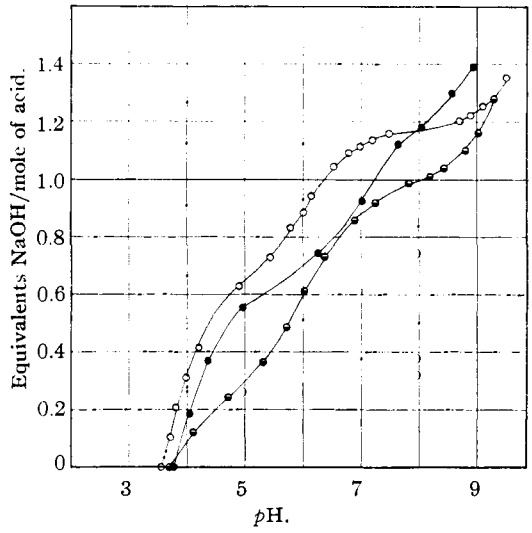


Fig. 4.—Titration curves in formaldehyde solution of δ-(α-amino)-acetylhydantoic acids, after heating with 0.3 *N* hydrochloric acid. The hydrochloric acid was neutralized with sodium hydroxide before the titration: acid I —O—; acid II —◒—; acid III —●—.

The interpretation of the titration curves in Fig. 4 is much more complex than that of the curves for alkaline hydrolysis. Since the number of products which can result from the hydrolysis of acid I is smaller than that from either II or III, we shall analyze it first.

It will be noted that the equivalence point for the hydrolysis products of acid I is just short of 1.2 moles. If the acid had been converted to glycine and hydantoic acid, the equivalence point would have been at two moles. It might be assumed that these were the primary products but that the hydantoic acid had subsequently been 80% converted to hydantoin by the heating in acid. Experiment shows that hydantoic acid is about only 50% converted to hydantoin under comparable conditions of time, temperature and acidity. We must, therefore, account for the lower equivalence found. Inspection of the curve shows a discontinuity in the neighborhood of 0.7 mole showing that the more acidic constituent (hydantoic acid?) should have about this equivalence. It is obvious that a mixture of 0.2 mole of hydantoic acid with 1.0 mole of glycine cannot give a break at 0.7.

The only other possible constituent of the mixture which could shift the curve to the more acid range is unchanged starting acid I. If we assume that half of the material survives the hydrolysis, then only half would be changed into glycine and hydantoic acid. If 60% of the latter were converted into hydantoin, we should have a mixture of 0.5 mole of acid I, 0.5 mole of glycine and 0.2 mole of hydantoic acid. The titration of such a mixture made up synthetically is recorded in Fig. 5. It will be noted that, except for a small discrepancy in the equivalence, this mixture agrees well with that found hydrolytically. The loss of 0.05 equivalent of acid on mixing is not immediately explicable.

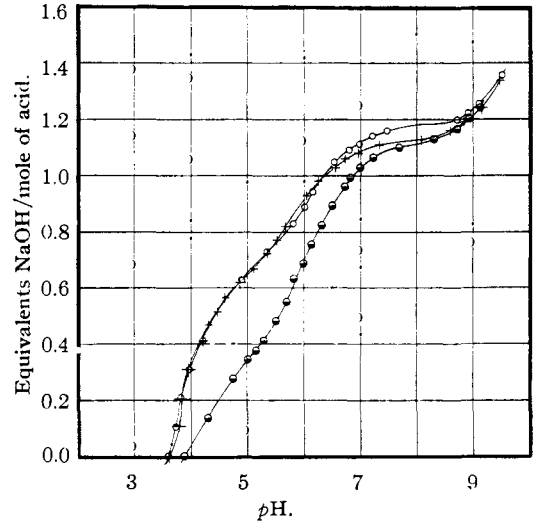


Fig. 5.— —O— Curve 1 of Fig. 4 repeated; —×— synthetic mixture of acid I 0.0005 mole, glycine 0.0005 mole, hydantoic acid 0.0002 mole, hydantoin 0.0003 mole, 15.00 ml. of 0.3 *N* HCl + equiv. vol. of 0.3 *N* NaOH + 12 ml. of neutral formaldehyde; titrated with NaOH. —◒— is acid I solution identical with that in curve 1 but heated for five to six hours in hydrochloric acid before titration.

If the explanation of the curve in the preceding paragraph is the correct one, a longer period of heating should lower the equivalence point and shift the curve to the alkaline region because of increasing destruction of the most acidic constituent, acid I. This is the effect actually found, as shown in Fig. 5.

We believe that these results justify the conclu-

sion that dilute mineral acid attacks acid I slowly and at the δ–ε-bond and that the hydantoic acid formed by this cleavage is partially and progressively converted to hydantoin.

In contrast to the hydrolysis of acid I, the hydrolysis of acid II, glycylcarbamylalanine, gives nearly the correct equivalence. We find also that the hydantoic acid of alanine, which would be formed by cleavage of acid II, condenses much more rapidly than hydantoic acid itself, to form methyl hydantoin. In the period corresponding to that of the hydrolysis it is completely condensed. The equivalence shown by the titration curve would be given by any mixture of unchanged starting acid and glycine which could be obtained by the hydrolysis. On the other hand, the presence of unchanged starting acid would shift the curve noticeably toward the acid region. The curve for glycine and hydantoin given in Fig. 6 is almost exactly superimposable on this curve, indicating that the major products of the hydrolysis are glycine and methyl hydantoin. It is thus evident that the ease of condensation of the hydantoic acid of alanine displaces the reaction to the right and causes acid II to be more completely attacked by dilute mineral acid than acid I.

The curve for the hydrolysis products of acid III, alanylcarbamylglycine, resembles that of acid I with the characteristic difference between the glycine in the former and the alanine in the latter showing up in the last half of the titration curve. This characteristic difference is shown again in Fig. 6 in another connection.

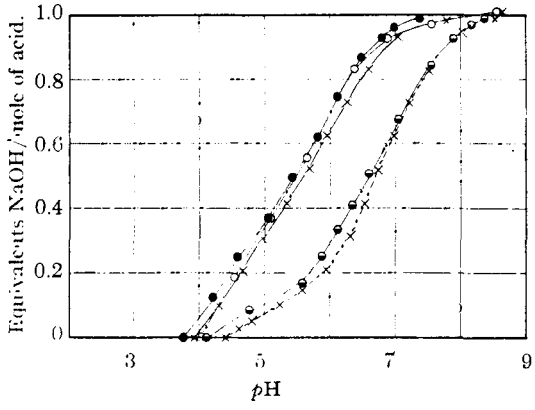
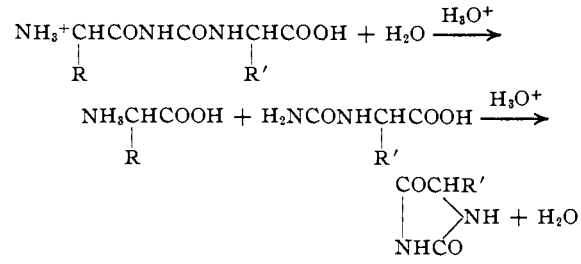


Fig. 6.—Titration curves, in formaldehyde solution, of heated aqueous solutions of δ-(α-amino)-acylhydantoic acids: acid (I), --○--; acid (II), —◒—; acid (III), --●—; solutions of equivalent amounts of glycine and hydantoin, -×-; and of alanine and hydantoin - - ×- - -, similarly treated.

We interpret these results as indicating that all three acids cleave in dilute mineral acid at the δ–ε-bond according to the equation

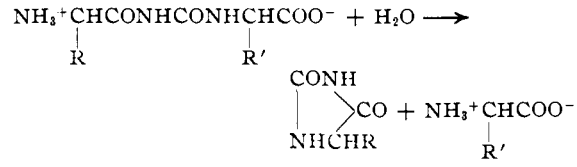


"Neutral Hydrolysis"

Diacetamide[19] and other secondary amides[16] are attacked by boiling water. Methylacetylurea[18] is stable under these conditions although it will decompose at 150°. From these observations we might predict again that cleavage of the new peptides would take place at the δ-ε-bond.

Acetylhydantoic acid was heated with distilled water for comparison. After prolonged heating the original acid could still be recrystallized from the solution although the titration curve indicated slight decomposition into acetic and hydantoic acids.

Figure 6 summarizes the results of formol titrations on heated aqueous solutions of the three new peptides. It will be noted that the decompositions are all very close to quantitative and, by comparison with synthetic mixtures, that the cleavage has taken place at the β–γ-bond instead of the δ–ε-bond as predicted and as found in acidic and alkaline media. This is immediately established in the cases of acids II and III by the positions of the curves. In the case of acid I a mixture of glycine and hydantoin could conceivably be formed by cleavage either at the β–γ-bond or at the δ–ε-bond. The lack of hydantoic acid as a byproduct in the hydrolysis of acid I, however, is evidence that the hydrolysis took place at the β–γ-bond since under the conditions of the reaction hydantoic acid does not condense rapidly to hydantoin. We can thus formulate all three reactions as



This conclusion was confirmed by the isolation of hydantoin from compound I, alanine from com-

pound II and glycine and methyl hydantoin from compound III.

The striking deviation from the behavior of analogous compounds which is found here must be ascribed to the structural differences between the compounds and the substances chosen as analogs. The most immediately apparent difference lies in the charges present on the peptides. We might conclude, therefore, that the peculiar course of the reaction is due to the directive influence of the charges. A final decision upon this point would require a more detailed kinetic analysis to make certain as to the exact species involved in the reaction since either peptide positive ion or peptide negative ion is present in sufficient amounts to account for the reaction and the attack may be by water, hydroxyl ion or hydrogen ion. It should be noted that tetra- or pentapeptides of this type could be prepared with charges removed from the carbamate linkage at both ends or at either end to find which charge is decisive.

To duplicate more closely the drastic conditions used in protein hydrolysis, peptide I was hydrolyzed by boiling with 5 normal hydrochloric acid solution. To measure the extent of the side-reaction caused by the powerful reagent employed, the hydrolyzing mixture was swept with an inert gas and the percentage of carbon dioxide formed was measured. It was found that 16.3% of the acid decomposed in this manner. Addition of sodium hydroxide to a portion of the hydrolyzate led to the immediate formation of ammonia. These products are those which would be expected from the secondary decomposition of the products formed by cleavages at either β–γ, γ–δ, or δ–ϵ-bonds. A preparation which behaved like a mixture of hydantoin and glycine was isolated from the residue, accounting for the product of δ–ϵ-cleavage, the major reaction in more dilute acid. This reaction is similar to that of the hydantoic acids of glycine and alanine in drastic acid hydrolysis, as reported by Lippich.[4]

If a carbamyl-peptide linkage were actually present in a protein, it might thus give rise on drastic acid hydrolysis only to a fraction of the amount of carbon dioxide or ammonia calculated stoichiometrically. The drastic hydrolysis performed may be illuminating with respect to the interpretation of results from protein hydrolysis. One must conclude that carbon dioxide and ammonia arising from protein hydrolysis with acid cannot be ascribed with certainty to a reaction proceeding according to simple stoichiometry.

The results of this research also emphasize the importance of studying the effect of less drastic hydrolyzing agents than constant boiling hydrochloric acid upon proteins.

One of the authors (C. I. D.) wishes to express her appreciation of a grant-in-aid from the Hynson, Westcott and Dunning Fund in the pursuance of this research.

Experimental Part

Benzyl Ester of the Hydantoic Acid of Glycine.—To a concentrated solution of 6 g. (0.030 mole) of crude glycine benzyl ester hydrochloride[20] made slightly basic with normal sodium hydroxide was added a concentrated solution of 2.5 g. (0.030 mole) of potassium cyanate. The mixture was warmed for two to three minutes on the steam-bath and then cooled with stirring in an ice-bath. A slight excess of normal hydrochloric acid was added and the mixture was allowed to stand in the ice-bath for half an hour before filtering out the white solid which had separated. Yield of the crude product was 50%. If the solid appeared crystalline it was recrystallized from hot water; if it was hard and lumpy it contained benzyl alcohol which was best removed by recrystallizing from chloroform. The sample used for analysis was recrystallized several times from chloroform and dried in a Fischer pistol at 100° for two hours; m. p. 124.5-126°.

Anal. Calcd. for $C_{10}H_{12}N_2O_3$: C, 57.66; H, 5.81. Found: C, 57.74; H, 5.79.

The hydrochloride of the benzyl ester of glycine was prepared from glycyl chloride hydrochloride and benzyl alcohol,[20] the method used by Bergmann in obtaining his benzyl esters. Our experience indicates that this method is more reliable than the standard method of saturation of a mixture of glycine and benzyl alcohol with hydrogen chloride gas. The hydrochloride was prepared according to the method of Fischer[21] except that commercial methanol instead of absolute ethyl alcohol was used to precipitate the glycine. It was found that crude cloudy acetyl chloride caused the reaction to be complete in one hour and gave a better yield than pure acetyl chloride. This was probably because the particles of glycine were more completely dispersed in the crude chloride.

Ethyl Ester of δ-Chloroacetylhydantoic Acid of Glycine.—Three grams (0.026 mole) of dry crude hydantoic acid ethyl ester[22] was placed in a round-bottom flask which could be connected by a ground glass joint to a reflux condenser carrying a calcium chloride tube at the top. The solid was covered with a mixture of 50-75 cc. of anhydrous benzene and 2.5 cc. (0.033 mole) of pure chloroacetyl chloride and the suspension refluxed on the hot-plate for thirty minutes. During this time the solid slowly dissolved and the solution became pale yellow. The solution was cooled in an ice-bath for thirty minutes. A yellow oil first settled to the bottom and then fine white

(20) Ruggli, Ratti and Henzi, *Helv. Chim. Acta.*, **12**, 361 (1929).
(21) Fischer, *Ber.*, **38**, 2915 (1905).
(22) Harries and Weiss, *ibid.*, **33**, 3418 (1900).

crystals appeared. The yellow oil solidified and was collected with the crystals by suction filtration. The solid was recrystallized from boiling water. The yield of the recrystallized product was 53%; m. p. 145-146°.

Anal. Calcd. for $C_7H_{11}ClN_2O_4$: C, 37.75; H, 4.94; Cl, 15.94; mol. wt., 222.5. Found: C, 37.57; H, 4.80; Cl, 16.06; mol. wt.,[23] 226, 227.

Longer heating of the reaction mixture caused more of the yellow oil to be formed which reduced the yield. A vacuum evaporation of the benzene filtrate likewise increased the amount of oil. This preparation may also be carried out in molten chloroacetic acid, which was used by Jacobs and Heidelberger[24] for chloroacetylation of substituted urea derivatives, but the yield is less than with benzene. All attempts to hydrolyze this ester resulted in the formation of hydantoic acid and chloroacetic acid.

Benzyl Ester of δ-Chloroacetylhydantoic Acid of Glycine.—A mixture of 6.8 g. (0.033 mole) of the dry finely powdered crude benzyl ester of the hydantoic acid of glycine, 3 cc. (0.039 mole) of pure chloroacetyl chloride, and 100 cc. of anhydrous benzene was refluxed for an hour in the apparatus described for the ethyl ester. The mixture slowly became yellow and at the end of about thirty minutes crystals of the chloroacetyl ester started to come out of the boiling solution. After an hour the mixture was so full of solid that considerable bumping occurred. The hot mixture was cooled, finally in ice, and filtered. The solid was recrystallized from 95% ethyl alcohol using Norite; yield of the recrystallized product was 70%; m. p. 179.5-180°; slight decomposition on a re-melt.

Anal. Calcd. for $C_{12}H_{13}ClN_2O_4$: C, 50.60; H, 4.61. Found: C, 50.42; H, 4.52.

δ-Chloroacetylhydantoic Acid of Glycine (IV). A. By Hydrogenation of the Benzyl Ester.—Into a 2-liter, three-necked, round-bottomed flask was placed a mixture of 10 g. (0.035 mole) of recrystallized chloroacetylhydantoic acid benzyl ester, 300 cc. of water, 1200 cc. of methanol, 20 drops of palladium chloride solution, 10 drops of glacial acetic acid, and about 5 cc. of Norite. The middle neck of the flask was fitted with a condenser. One of the side necks contained a glass tube reaching to the bottom of the flask through which hydrogen was to be passed directly from the tank and the other side neck contained an outlet tube to carry off the exit gas. The whole apparatus was placed on the steam-bath under the hood and hydrogen was passed in at a moderate rate for four hours with constant heating. During this time the crystals of the ester slowly dissolved and the odor of toluene could be detected. The mixture was cooled and filtered by suction and the clear filtrate was evaporated under vacuum to a volume of 5-10 cc. and white crystals settled out. The mixture was washed from the flask with as little water as possible and filtered. The residue was recrystallized from absolute alcohol; yield of recrystallized product, 65%; m. p. 198-200° with decomposition. The crystals were insoluble in both cold and boiling benzene; they were much more soluble at room temperature in dioxane than in water and alcohol. The sample used for analysis was recrystallized three times from absolute alcohol and dried in a Fischer pistol at 100°.

Anal. Calcd. for $C_5H_7ClN_2O_4$: C, 30.84; H, 3.63; Cl, 18.23; N, 14.40; neutralization equivalent, 194.5. Found: C, 30.92; H, 3.58; Cl, 18.12[25]; N, 14.51; neutralization equivalent, 195.

B. By Direct Chloroacetylation of the Hydantoic Acid of Glycine.—Two hundred milliliters of dioxane distilled from sodium, and 10 cc. (0.13 mole) of pure chloroacetyl chloride were boiled gently in an open 500-cc. Erlenmeyer flask on a hot-plate covered with heavy asbestos paper. Ten grams (0.085 mole) of finely powdered dry crude hydantoic acid prepared from glycine and potassium cyanate[26] was added gradually in small amounts over a period of fifteen to thirty minutes. Most of the solid went into solution about one minute after it was added but the crystalline residue left at the bottom of the flask soon became an oil which would have colored the solution red on prolonged heating. As soon as the oil started to become discolored the clear liquid was quickly poured into another flask, the oil remaining at the bottom of the reaction flask. A large amount of a fine white solid separated as the decanted solution cooled. After standing for about thirty minutes in cold water at a temperature not lower than 11°, the mixture was filtered and more of the product, slightly yellow, was obtained from the filtrate by precipitation with petroleum ether. A mass of fine glittering needles separated when the combined solids were recrystallized from boiling water; yield of the recrystallized product was 55%. A mixed melt with the crystals from A showed no depression.

When the oil which formed was allowed to become red the whole solution became colored and the yield was reduced because of the difficulty of purifying the colored crystals. The use of the apparatus described for the esters instead of the open Erlenmeyer, the addition of all of the hydantoic acid at the beginning of the reaction, or the use of crude dioxane, all increased the amount of the red oil. The oil itself did not solidify and no product could be obtained from it. In many of the runs the oil appeared to form a coating over the unreacted hydantoic acid.

Attempts were made, without success, to chloroacetylate hydantoic acid in molten chloroacetic acid, in boiling benzene, in boiling toluene, and in boiling chloroacetyl chloride. In all cases, if any product was obtained, it was hydantoin. Dioxane was used for the chloroacetylation after the product desired was prepared by the method of A and found to dissolve readily in this solvent. It was also found later that the reaction would occur to a slight extent in ethyl acetate.

δ-Chloroacetylhydantoic Acid of *d,l*-Alanine.—Ten grams (0.076 mole) of the hydantoic acid of alanine prepared from potassium cyanate and alanine,[27] and 10 cc. (0.13 mole) of pure chloroacetyl chloride were treated in dioxane in the same manner as the corresponding acid of glycine. All of the solid dissolved, however, and there was no discoloration; yield of the recrystallized product, 51%; m. p. 181-181.5°, with decomposition.

Anal. Calcd. for $C_6H_9ClN_2O_4$: Cl, 17.00; neutraliza-

(23) The method used was that of Menzies and Wright, THIS JOURNAL, **43**, 2314 (1921). Acetone was used as the solvent.

(24) Jacobs and Heidelberger, *ibid.*, **39**, 1439 (1917).

(25) Analysis by Elizabeth Packard.

(26) McMeekin, Colin and Weare, THIS JOURNAL, **57**, 626 (1935).

(27) Boyd, *Biochem. J.*, **27**, 1838 (1933).

tion equivalent, 208.5. Found: Cl, 16.98[28]; neutralization equivalent, 208.

δ-(α-Chloropropionyl)-hydantoic Acid of Glycine.—This acid was prepared as above from the hydantoic acid of glycine and α-chloropropionyl chloride, the latter prepared by the method of Brown.[29] No oil formed during the first fifteen minutes of the reaction. The yield of the recrystallized product was 51%; m. p. 208.5-211°, with decomposition.

Anal. Calcd. for $C_6H_9ClN_2O_4$: C, 34.53; H, 4.35; neutralization equivalent, 208.5. Found: C, 34.36; H, 4.38; neutralization equivalent, 211.

δ-(α-Chloropropionyl)-hydantoic Acid of *d,l*-Alanine.—This acid was prepared as above from the hydantoic acid of *d,l*-alanine and α-chloropropionyl chloride. No oil formed. The yield of the recrystallized product was 56%; m. p. 191-192.5°, with decomposition.

Anal. Calcd. for $C_7H_{11}ClN_2O_4$: neutralization equivalent, 222.6. Found: neut. eq., 225.

δ-(α-Chloropropionyl)-hydantoic Acid of *l*-Leucine.—This acid was prepared as above from the hydantoic acid of *l*-leucine[27] and α-chloropropionyl chloride. No oil formed and no solid separated on cooling the dioxane solution but petroleum ether precipitated a very fine white solid. Yield of recrystallized product was 46%; m. p. 147-148°; re-melt, 148-148.5°.

Anal. Calcd. for $C_{10}H_{17}ClN_2O_4$: neutralization equivalent, 264.6. Found: neut. eq., 269.

δ-(α-Bromopropionyl)-hydantoic Acid of Glycine.—Seventy-five milliliters of dioxane and 10 ml. (0.093 mole) of α-bromopropionyl bromide were boiled gently in an open Erlenmeyer on a hot-plate and ten grams (0.085 mole) of the hydantoic acid of glycine gradually added over a period of fifteen minutes, at the end of which time the reddish oil, which had formed as soon as the reaction started, appeared to go into solution. On cooling, the red solution gave no solid but with petroleum ether a thick reddish yellow oil settled out and some yellow solid formed. The oil solidified on standing in ice. However, if the petroleum ether was decanted from the oil and the oil washed with a small portion of the ether, the residual oily mass could be recrystallized from water using Norite as efficiently as the solidified oil. The yield of the product twice recrystallized (glittering white needles) was about 10%; m. p. 201-204°, with decomposition.

Anal. Calcd. for $C_5H_9O_4N_2Br$: C, 28.46; H, 3.59; Br, 31.59. Found: C, 28.74; H, 3.62; Br, 31.58.[30]

δ-Acetylhydantoic Acid of Glycine (V).—This acid was prepared from acetyl chloride and hydantoic acid in the same manner as the halogenated derivative. The solution became yellow quickly and an oil separated. A few crystals separated on cooling the solution and more crystals were obtained by precipitation with petroleum ether. The yield was about 0.3 g. of the recrystallized product from 10 g. of the hydantoic acid; m. p. 234-235°, with decomposition.

Anal. Calcd. for $C_7H_8O_4N_2$: C, 37.48; H, 5.04; neutralization equivalent, 160.1. Found: C, 37.59; H, 5.12; neutralization equivalent, 160.

This acid could not be obtained by the use of acetic anhydride or of acetyl chloride in boiling benzene. It is quite possible that Johnson's suggested method for preparing this acid[10] might give better yields.

δ-Glycylcarbamylglycine (I).—Ten grams (0.057 mole) of the dry finely powdered recrystallized δ-chloroacetylhydantoic acid of glycine was placed in a dry 500-cc. Erlenmeyer flask fitted with a stopper containing two glass tubes, one reaching just to the lower end of the stopper and the other extending one-third of the distance into the flask. The flask was allowed to stand stoppered for ten to fifteen minutes in a dry ice-acetone mixture in a Dewar flask. The shorter tube was connected by rubber tubing to the vent of the hood. About 200 cc. of liquid ammonia was added through the longer tube and the tube was then closed by rubber tubing and screw clamp. The mixture was left in the Dewar for six to eight hours until all of the solid had dissolved in the liquid ammonia. The ammonia was then allowed to evaporate and the white solid left in the flask was transferred by washing with as little methanol as possible to a 200-cc. round-bottomed flask. The methanol mixture was evaporated to dryness, using suction and a trap containing concentrated sulfuric acid cooled in ice. During the evaporation care was taken not to heat the solution to a temperature higher than 40°. After the evaporation the white solid, which should have no odor of ammonia, was transferred with as little water as possible to a small beaker. If the mixture was alkaline it was acidified with the smallest possible excess of dilute hydrochloric acid. The aqueous mixture was filtered and more solid was precipitated from the filtrate with methanol. The combined solids from water and methanol were recrystallized from hot water as quickly as possible. The yield of the recrystallized solid (very fine white crystals) was 70%. The sample used for analysis was recrystallized three times and dried in a Fischer pistol at 100°; m. p. 192.5-194°, with decomposition.

Anal. Calcd. for $C_5H_9N_3O_4$: C, 34.27; H, 5.18; N, 24.00; neutralization equivalent, 175.1. Found: C, 34.21; H, 5.22; N, 23.62; neutralization equivalent, 176.

δ-Glycylcarbamyl-*d,l*-alanine (II).—Five grams (0.027 mole) of the dry, finely powdered, recrystallized δ-chloroacetylhydantoic acid of *d,l*-alanine was placed in a 50-cc. round-bottomed long-necked flask and cooled in a dry ice-acetone mixture in a Dewar. The solid was covered with 50 cc. of liquid ammonia and it immediately dissolved. The ammonia was allowed to evaporate slowly over a period of eight to twelve hours and the residue extracted with methanol and evaporated to dryness by suction as above. The dry residue was extracted with as little water as possible, the mixture made very slightly acidic and then filtered. When treated with acetone, the filtrate gave a white oil which solidified on standing but the solid contained so much chloride ion that it was discarded. The residue from the water mixture could not be recrystallized from either water or a water-alcohol mixture although it dissolved on heating in these solvents. It was finally washed several times with small portions of water and then dissolved in water at room temperature in the proportion of half a gram to 100 cc. Acetone was added to this aqueous

(28) Analysis by Blanche Skidmore.
(29) Brown, THIS JOURNAL, **60**, 1325 (1938).
(30) Analyzed by Shirley Meserve.

solution and gave a precipitate of very fine white crystals which usually gave no test for chloride ion. If chloride was present the solid was dissolved in water and reprecipitated with acetone until the chloride ion test was negative. The yield of the crude solid filtered from the first aqueous mixture was 77%. For analysis, crystals free from chloride ion were dried at 100° in a Fischer pistol using phosphorus pentoxide.

Anal. Calcd. for $C_6H_{11}O_4N_3$: N, 22.22; neutralization equivalent, 189.1. Found: N, 22.10; neutralization equivalent, 192.

δ-*d,l*-Alanylcarbamylglycine (III).—Two and three-tenths grams (0.0091 mole) of the dry finely powdered recrystallized δ-(α-bromopropionyl)hydantoic acid of glycine was treated with liquid ammonia in the manner described for acid II. The same results occurred with the following exceptions: after evaporation of the ammonia the solid was much more soluble in methanol than the solids in previous preparations; the crude aqueous mixture was slightly acidic, indicating that all of the ammonia had evaporated; the filtrate from the crude aqueous mixture gave with acetone a precipitate containing only a trace of bromide ion so that some of the product could be extracted from this filtrate. As with acid II the product could not be recrystallized from water or a water-alcohol mixture. The total yield of the crude solid filtered from the aqueous mixture and from the first acetone precipitation was 55%. Different samples of the acetone-precipitated solid, free from bromide ion, were dried in screwcap vials from one to two hours in a Fischer pistol. When cool, the vials were quickly stoppered and weighed, samples removed to tared watch glasses, and the vials quickly stoppered and weighed again. Table I shows the results obtained when the samples, spread out in thin layers on tared watch glasses, were weighed at various time intervals. Samples washed with petroleum ether before drying behaved in the same manner as those not washed with the ether. Before analysis the sample was allowed to come to constant weight as indicated in the table. The hydrate was in the form of very fine crystals; m. p. of hydrate: softens at 180°; melts 190-195°, with decomposition.

TABLE I

ABSORPTION OF WATER BY δ-*d,l*-ALANYLCARBAMYLGLYCINE (III)

Sample no.[a]	Weight removed from vial, g.	Weight after ( ) minutes	Weight if 1 mole of water absorbed by 1 mole of III
1	0.1192	0.1281(15)	
		.1290(90)	0.1306
		.1292(900)	
2	.1190	.1290(30)	
		.1289(240)	.1303
		.1289(900)	
3	.0967	.1049(15)	
		.1053(30-45)	.1059
		.1053(60)	
4	.1040	.1116(5)	
		.1137(60)	.1139
		.1136(1140)	
5	.0523	.0572(5)	.0573
		.0572(20)	

[a] Samples 1 and 2 came from one preparation, sample 3 from a second preparation, and samples 4 and 5 from a third preparation. The dried sample from which 1 and 2 were taken was larger than the other dried samples. Sample 5 is from sample 4 re-dried.

Anal. Calcd. for $C_6H_{13}O_5N_3(C_6H_{11}O_4N_3 + H_2O)$: N, 20.29; neutralization equivalent, 207.1. Found: N, 20.43; neutralization equivalent, 207.

General Technique of Titrations.—All of the titrations were performed electrometrically with a *p*H meter accurate to within 0.1 *p*H unit. Calomel and glass electrodes were used. Boiled distilled water was used for dissolving the samples. The 0.1 *N* carbonate-free sodium hydroxide was measured from a 10-ml. buret graduated in 0.05 ml. As the volume of standard base required for the 0.1-0.2 g. samples used for the titrations varied from 5 to 10 milliliters the accuracy of the titrations, assuming readings to 0.01 ml., was 2-4 parts per thousand. In titrations involving the same type of determination, an attempt was made to keep the concentrations the same in all of the titrations.

The neutralization equivalents were calculated from the end-points taken as the mid-point of most rapid *p*H change in the curves obtained by plotting milliliters of standard base against *p*H. For purposes of uniformity the mole ratio of acid to base is plotted against *p*H in this paper.

Amphoteric Properties of Acids I, II and III.—One-tenth to fifteen-hundredths gram samples of acids I, II and III were dissolved as completely as possible at room temperature in 20 ml. of water and the mixtures titrated with 0.1 normal sodium hydroxide or hydrochloric acid by the procedure described above. The results were plotted as in Fig. 2 and the *p*H at the mid-point of these curves was assumed in the discussion to be approximately equal to the pK values. This assumption was justified on the basis of the following determinations. The dissociation curve for acid I at constant 0.05 molar ion concentration was determined[31] at room temperature using the *p*H meter with a glass electrode for the measurements in the acid solution and the same meter with a hydrogen electrode for the measurements in alkaline solution. Values of 3.2 for pK_1 and 7.8 for pK_2 were obtained. In order to evaluate the accuracy of the method the dissociation curve for glycine at 0.05 molar ion concentration was determined by exactly the same procedure. The values of pK_1 and pK_2 for glycine were within 0.1 unit of the accurate values reported.[13] The simple titration curve for acid I resembled so closely its dissociation curve at constant ion concentration that it was felt, considering the approximate method of measuring the *p*H, that titration curves would serve as well as dissociation curves to give approximate values of the dissociation constants of these acids.

The procedure used for the formol titrations followed somewhat the procedure of Dunn and Loshakoff[32]; 0.1-0.15 g. samples were dissolved as completely as possible in 15-20 cc. of water and 12 cc. of neutral formaldehyde added. The mixture was titrated as described with 0.1 normal sodium hydroxide. Dunn and Loshakoff recommended the use of larger samples and of 0.3 normal sodium

(31) C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1938, p. 152.

(32) Dunn and Loshakoff, *J. Biol. Chem.*, 113, 359 (1936).

hydroxide but it was found in determinations with acid I that the end-points in the dilute solutions were the same as those in the more concentrated solutions.

Action of Sodium Hydroxide on Acids I, II and III.—One-tenth to fifteen-hundredths gram samples in 50-100-cc. beakers were dissolved in 15.00 ml. of 0.3 normal sodium hydroxide and covered with watch glasses with strips of wet red litmus adhering to their convex sides. These solutions were allowed to stand at room temperature for twenty-four hours. Solutions containing equivalent amounts of glycine and the hydantoic acid of glycine, and of alanine and the hydantoic acid of glycine, were similarly treated, and a blank containing only the 15.00 ml. of sodium hydroxide was allowed to stand in the same manner. After ten to fifteen minutes the litmus above the solution of acid III became decidedly blue; after thirty to sixty minutes the litmus above the solutions of acid II and of the mixture of alanine and hydantoic acid became faintly blue. Litmus suspended above glycine amide similarly treated became blue in five to ten minutes. No change was observed for the other solutions or for the blank. At the end of twenty-four hours the blank was titrated with 0.3 normal hydrochloric acid and the results showed that the concentration of the base was unchanged by standing. To the other solutions was added a volume of 0.3 normal hydrochloric acid equivalent to the 15.00 ml. of sodium hydroxide, and 12 ml. of neutral formaldehyde. The solutions were then titrated with 0.1 normal sodium hydroxide as described above. The results are plotted in Fig. 3.

Action of Heat on Solutions of Acids I, II and III.—Solutions of 0.1 to 0.15 g. samples in 15–20 ml. of water in small covered beakers were heated from one to five hours at 90-100°. To the cooled solution 12 ml. of neutral formaldehyde was added and the solutions titrated with 0.1 normal sodium hydroxide. The results are plotted in Fig. 6. Solutions of equivalent amounts of glycine and hydantoin, and of alanine and hydantoin, were also titrated similarly in formaldehyde and the curve was like that of the heated solutions of acids I and III.

Solutions of about 0.3 g. each of acids II and III in 20 cc. of water were heated for at least five hours and attempts were made to identify the products of the reaction. The solutions were evaporated to 5-10 cc. before analysis. From II, alanine was precipitated with acetone and from III, glycine was precipitated with methanol. Methanol does not readily precipitate alanine from an aqueous solution. Alanine and glycine were identified by their great solubility in water as contrasted with the original acids, and by their decomposition points. As the decomposition point of alanine is about 40° higher than that of glycine this method of differentiation is adequate. From the acetone filtrate of II a substance was isolated which had a melting point similar to that of a mixture of hydantoin and alanine; from the methanol filtrate of III the hydantoin of alanine was isolated and identified by a mixed melt. Heating gives a turbid liquid at 145-146° and a clear liquid at 149-150°.

A solution of two grams of acid I in 20 cc. of water was heated and on cooling crystals of hydantoin, identified by a mixed melt, separated. The filtrate from these crystals yielded only a substance which behaved like a mixture of hydantoin and glycine.

Action of Hydrochloric Acid on Heated Solutions of Acids I, II and III.—One-tenth to fifteen hundredths gram samples, each dissolved in 15.00 ml. of 0.3 normal hydrochloric acid in 50-100-ml. beakers covered with watch glasses, were heated from one to five hours at a temperature of 90-100°. To the cooled solutions were added a volume of 0.3 normal sodium hydroxide equivalent to the hydrochloric acid and 12 ml. of neutral formaldehyde. The solutions were titrated with 0.1 *N* sodium hydroxide. A blank determination showed the concentration of the hydrochloric acid to be unchanged by heating. The results are plotted in Fig. 4.

No attempts have yet been made to isolate any products from these hydrochloric acid reactions. The test for ammonia described in the preceding section is given in a shorter time than with the same materials untreated with hydrochloric acid. The presence of the acid, and the fact that heat must be avoided in concentrating any of the neutralized solutions, make the analysis of these solutions difficult.

One hundred eighteen and one-tenth milligrams of hydantoic acid (0.001 mole) was heated for two and a half hours on a steam-bath with 15 ml. of 0.3 *N* hydrochloric acid. The acid was then neutralized with an equivalent amount of sodium hydroxide and the solution titrated with 0.0999 *N* sodium hydroxide. In two determinations the equivalence was found at 0.49 and 0.54 mole of base per mole of hydantoic acid used. This shows that hydantoic acid is about half condensed to hydantoin by this treatment.

In a similar experiment with the hydantoic acid of alanine, equivalence was reached after heating when less than 0.01 mole of base had been added per mole of the hydantoic acid derivative originally used. This shows that the process of heating in acid condenses the hydantoic acid of alanine to methyl hydantoin nearly quantitatively in the time required for fifty per cent. conversion of the unmethylated compound.

For the drastic hydrolysis of acid I with hydrochloric acid the following procedure was used. 0.6219 g. of acid I dissolved in 50 ml. of five molar hydrochloric acid was boiled for three and one-quarter hours in a carbon dioxide-free apparatus constructed in such a manner that all of the gases and vapors from the reaction were carried over by a stream of nitrogen through a reflux condenser into a measured volume of standard barium hydroxide solution contained in a bulbed flask. At the end of the time, titration of the barium hydroxide with standard oxalic acid indicated that 0.0254 g. of carbon dioxide had been evolved. Assuming one mole of acid to give one mole of carbon dioxide this corresponded to 16.3% decomposition. The residue gave an immediate positive test for ammonia when treated with cold sodium hydroxide as described above. A substance which behaved somewhat like a mixture of hydantoin and glycine was isolated from the hydrochloric acid solution.

Summary

1. The first representatives of the class of carbamic acid peptides have been prepared.

2. A method for preparing substituted acyl hydantoic acids has been elaborated.

3. It has been found that liquid ammonia will aminate haloacyl hydantoic acids which are cleaved by ordinary amination processes.

4. By the similarity of their titration curves with those of ordinary peptides it is inferred that isoelectric carbamic acid peptides are dipolar ions.

5. Cleavages of these peptides with hydrolytic agents have been studied.

6. A new source of ammonia and of carbon dioxide from the hydrolysis of proteins is suggested.

7. It is demonstrated that the isolation of a given quantity of ammonia or carbon dioxide cannot be ascribed to the presence of an equivalent amount of a certain linkage since these may be produced in non-stoichiometric proportions.

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Catalytic Debenzylation. The Effect of Substitution on the Strength of the O-Benzyl and N-Benzyl Linkages[1]

BY RICHARD BALTZLY AND JOHANNES S. BUCK

The growing synthetic importance of debenzylation procedures[2] in which the protective benzyl group is removed catalytically from ethers, esters and amines makes it desirable to see whether the lability of the benzyl group can be increased by convenient substitution. This would be especially useful in amine syntheses in which the N-debenzylations sometimes call for rather vigorous treatment.

Furthermore, the effect of constitutive factors on the stability of the benzyl linkages must be fundamentally related to the general scheme by which substituents on the benzene ring influence the activity of the system at various locations in the ring itself, and such influences must ultimately be accounted for in a general explanation of the behavior of aromatic systems.

The results presented here seem to give a definite answer to the practical part of the problem only. Most substitutions studied increase the stability of the benzyl linkage and the few instances in which the stability is decreased correspond to intermediates through which preparation of benzyl tertiary amines is considerably less convenient than with the unsubstituted benzyl compounds. Use of such hyperactive benzyl derivatives (involving especially the α-menaphthyl group) would be advisable only in special cases.

All the reductions here reported were performed with palladized charcoal.[3] In our experience, catalyst prepared from the same batch of charcoal with constant proportions of metal used has a highly reproducible activity, as measured by the rate of reduction of benzyl alcohol. However, rates of reduction of different substances are probably only roughly comparable particularly if possible effects of hindrance be considered. This rough comparison of rates (involving in some cases a zero rate) is the only measure available to determine stability of O-benzyl linkages. It had been intended to study the competitive reduction of dibenzyl ethers until it was discovered that the substituted benzyl alcohol produced by the cleavage of the first O-benzyl bond was reduced more rapidly than the original ether, so that it was useless to seek an answer in that way.

The equivalent method of competitive cleavage of dibenzylamines and dibenzylmethylamines has no such disadvantage and was used to determine the effect of substitution in the ring. It is a reasonable assumption that substitution in the ring would affect the stability of O-benzyl and N-benzyl linkages in the same fashion and a few equivalent cases in the two series (compare no. 19, Table I, with nos. 1–6, Table II) are in agree-

(1) Presented at the Detroit meeting of the American Chemical Society, April, 1943.

(2) *Cf.* among others Bergmann and Zervas, *Ber.*, **65**, 1192 (1932); British Patent 318,488; Baltzly and Buck, THIS JOURNAL, **62**, 164 (1940); Buck and Baltzly, *ibid.*, **63**, 1964 (1941); King and Work, *J. Chem. Soc.*, 1307 (1940). For a fundamentally related method see Papa, Schwenk and Whitman, *J. Org. Chem.*, **7**, 587 (1942).

(3) Ott and Schröter, *Ber.*, **60**, 633 (1927); Hartung, THIS JOURNAL, **50**, 3370 (1928); Hartung and Crossley, *ibid.*, **56**, 158 (1934). These last authors consider palladium to have a greater debenzylating action than platinum in an absolute sense. Their experiments were with both metals supported on charcoal. Judged by the reduction of benzyl alcohol, palladized charcoal debenzylates more rapidly than platinum-oxide platinum-black. Use of the latter would have interfered greatly with the present experiments, in some cases preventing the debenzylation by reducing the ring and in all competitive debenzylations greatly complicating the identification of products.