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Selective Chemical Cleavage of Asparagine Peptides[1]

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Asparagine peptide was selectively cleaved by the Hofmann rearrangement followed by an alkaline treatment at the peptide linkage in which an amino group of the asparagine residue participated. Upon reaction of *N*-benzyloxycarbonyl-L-alanyl-L-asparagine with one equivalent of bromine and three equivalents of aqueous sodium hydroxide solution at 60°C, 1-(*N*-benzyloxycarbonyl-L-alanyl)-2-oxo-imidazolidine-5-carboxylic acid was obtained in quantitative yield. When this was treated with an aqueous methanolic sodium hydroxide solution at room temperature, a cleavage reaction occurred to give *N*-benzyloxycarbonyl-L-alanine and 2-oxo-imidazolidine-5-carboxylic acid. Under the same condition of the rearrangement, *N*-benzyloxycarbonyl-L-asparaginyl-L-phenylalanine was converted to 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carbonylphenylalanine. Since glutamine peptide could not give the corresponding ring compound, this cleavage reaction was found to be specific to asparagine peptide.

For the elucidation of the primary structure of protein, a number of chemical cleavage reactions specific to the particular amino acid residues have been presented as a supplementary method to the enzymic reactions which did not cover all usual amino acids in a sense of selective cleavage.[2] Nevertheless, neither chemical nor enzymic reaction of asparagine peptide has been known so far. In our previous papers,[3-5] chemical cleavage reactions of serine and threonine peptides were demonstrated. These reactions were based on formation of oxazolidone derivatives, by action of phosgene, which facilitated the removal of acyl group attached to the ring nitrogen atom by alkaline hydrolysis. It is expected that a similar cleavage reaction would occur readily too in the case of imidazolidone peptide where the ring O atom in oxazolidone peptide is replaced with NH group as shown in Fig. 1. Although α,β-diaminopropionic acid which will give the imidazolidone ring by reaction with phosgene, is not a constituent of natural protein, asparagine derivative can also be converted to the imidazolidone derivative *via* the Hofmann rearrangement reaction. In this respect, *N*-benzyloxycarbonyl-L-asparagine was actually converted to 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carboxylic acid by treatment with sodium hypochlorite at 60°C according to Schneider.[6] When *N*-acetylasparagine was treated with bromine and barium hydroxide at 90°C by Karrer and Schlosser,[7] 2-oxo-imidazolidine-5-carboxylic acid was obtained through the Hofmann rearrangement followed by cyclization to the imidazolidone ring and then elimination of acetyl group. Concerning oxidative reaction of peptide with sodium hypobromite, Goldschmidt *et al.*[8-12] and Carpenter *et al.*[13] had demonstrated chemical changes of *N*-terminal amino acid residue either to nitrile or to α-ketoacyl group for the purpose of the sequential analysis of peptide from *N*-terminal end. The other use of this rearrangement reaction had been investigated by Kovács *et al.*[14-16] in order to distinguish α- and ω-acid amide group in aspartic or glutamic acid residue in peptide chain.

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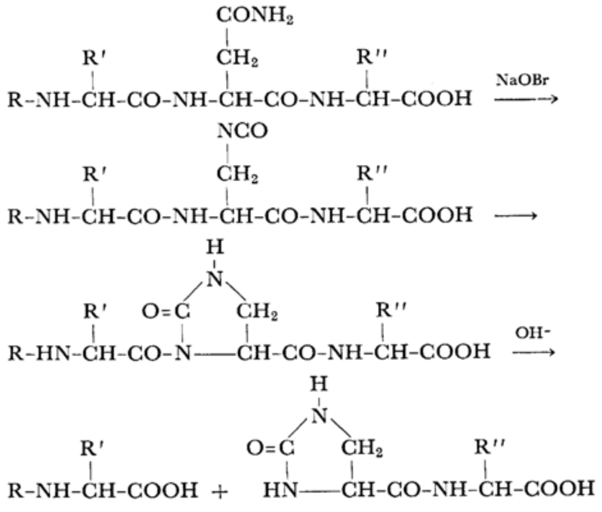

Fig. 1.

However, no attempts have been made to apply the Hofmann reaction to the selective cleavage of asparagine peptide according to a scheme outlined in Fig. 1. Asparagine peptides used in this reaction were prepared either by the mixed anhydride method or by the *p*-nitrophenyl ester method.

As a preliminary experiment *N*-benzyloxycarbonyl-L-alanyl-L-asparagine (I) was treated with one equivalent of bromine and five equivalents of aqueous sodium hydroxide at different temperatures as mentioned in Table 1. Theoretically, four equivalents of alkali are necessary to convert an acid amide to an amine in the usual Hofmann reaction, and one more equivalent should be added to neutralize the carboxyl group of I. From the reaction mixture, *N*-benzyloxycarbonylalanine (III) was obtained in crystalline state as one of the cleavage products. Although another product, *e. g.*, 2-oxo-imidazolidine-5-carboxylic acid (IV) could not be isolated, acid hydrolysis of a residue obtained by evaporation of the mother liquor gave α,β-diaminopropionic acid hydrochloride (V). Among a couple of the reaction conditions, the reaction at 60°C seemed to be most profitable in view of the yields of the products, III and V, as shown in Table 1. Although the results of the preliminary experiments suggest and strongly support the mechanism shown in Fig. 2, a route of the cleavage *via* imidazolidone peptide (II) could not be necessarily ascertained, since α,β-diaminopropionic acid would be possibly formed by the Hofmann reaction followed by the simple alkaline hydrolysis of asparagine residue without formation of imidazolidone ring.

TABLE 1. EFFECTS OF TEMPERATURE TO REACTION OF *N*-BENZYLOXYCARBONYL-L-ALANYL-L-ASPARAGINE WITH SODIUM HYPOBROMITE IN ALKALINE MEDIUM

Reaction Temp. (°C)	Yield of III (%)	Yield of V (%)
40	39	—
60	53	55
80	44	29

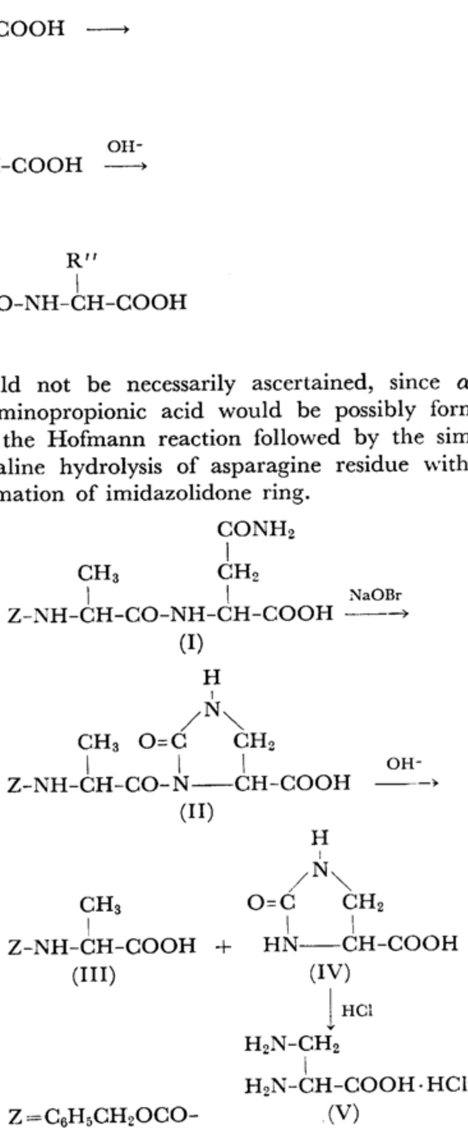

Fig. 2.

In order to prove the reaction mechanism, an isolation of the intermediate(II) was next attempted. A general mechanism of the Hofmann rearrangement by which a primary acid amide is converted to an amine is shown in Fig. 3. However, in the case of the reaction of *N*-benzyloxycarbonylalanylasparagine (I), the isocyanate, as soon as formed on step 4, will attack the nitrogen atom of the amino group of the original asparagine residue because of the electron-withdrawing effect of the carbon atom of the isocyanate group. As far as the formation of the isocyanate is concerned, only two equivalents of alkali are enough. Therefore, one equivalent of bromine and totally three equivalents of 1 N sodium hydroxide, that is, two for the formation of isocyanate and one for the neutralization of the carboxyl group, were used for the reaction of I. Thus, 1-(*N*-benzyloxycarbonyl-L-alanyl)-2-oxo-imidazolidine-5-carboxylic acid (II) was obtained in a quantitative yield as expected. This compound showed an absorption of the ring carbonyl group at 1760 cm^{-1} in its infrared spectrum.

$$Br_2 + 2NaOH \rightarrow NaOBr + NaBr \tag{1}$$
$$RCONH_2 + NaOBr \rightarrow RCONHBr + NaOH \tag{2}$$
$$RCONHBr + OH^- \rightarrow RCONBr^- + H_2O \tag{3}$$
$$RCONBr^- \rightarrow RNCO + Br^- \tag{4}$$
$$RNCO + OH^- \rightarrow RNHCOO^- \tag{5}$$
$$RNHCOO^- + OH^- \rightarrow RNH_2 + CO_3^{--} \tag{6}$$

Fig. 3.

The compound II was then treated with two equivalents of an aqueous methanolic sodium hydroxide solution at room temperature, giving III and IV in yields of 50 and 10% respectively. From the result of this stepwise reaction, it is now certified that the cleavage reaction takes place through the imidazolidone intermediate without splitting of the peptide linkages by simple alkaline hydrolysis.

When the asparagine residue is located at a position other than carboxyl terminal end, there is another possibility of ring formation; that is, the isocyanate group will link with the nitrogen atom of an amino acid residue adjacent to asparagine residue at its carboxyl side to form a hydrouracil ring instead of an imidazolidone ring. In order to check this possibility, *N*-benzyloxycarbonyl-L-asparaginyl-L-phenylalanine (VII) was treated under the same reaction condition, and 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carbonylphenylalanine (VIII) was obtained as a single product. This compound showed an infrared absorption at 1790 cm^{-1} which was characteristic of the ring carbonyl absorption of the imidazolidone as compared with authentic 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carboxylic acid. Furthermore, the removal of benzyloxycarbonyl group from VIII by catalytic hydrogenation gave a ninhydrin-negative product (IX). These facts indicated that the isocyanate group was not linked with the amino group of the phenylalanine residue, but certainly with that of the asparagine residue.

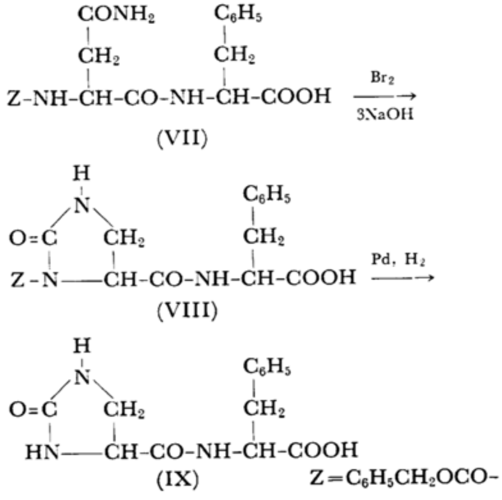

Fig. 4.

At the stage of the present investigation, a solubility of longer asparagine peptide in an aqueous alkaline solution gave some restriction for an applicability of this method. For instance, in the reaction of *N*-benzyloxycarbonyl-L-alanyl-L-asparaginyl-L-phenylalanine (XI) with one equivalent of bromine and three equivalents of aqueous 1N sodium hydroxide solution, a large amount of unreacted tripeptide was recovered and no 1-(*N*-benzyloxycarbonylalanyl)-2-oxo-imidazolidine-5-carbonylphenylalanine was obtained. The original tripeptide XI was almost insoluble in this reaction medium. When five equivalents of alkali were used, *N*-benzyloxycarbonylalanine was found in the reaction mixture by a thin-layer chromatography, but could not be isolated as crystals. However, a possibility of non-selective cleavage of the peptide bond by simple alkaline hydrolysis could not be excluded in this case, since ninhydrinpositive spots of amino acids were also found on a thin-layer chromatogram of the reaction mixture.

It must be investigated whether or not a glutamine residue will react in a similar way under the same condition as that of the cleavage reaction of asparagine peptide. In the Hofmann reaction of *N*-benzyloxycarbonyl-L-glutamine (XIIa), compound XIIIa was obtained as a reaction product. The removal of benzyloxycarbonyl group from XIIIa by catalytic hydrogenation yielded a free amino compound (XIV), which was then hydrolyzed by 6 N hydrochloric acid, giving three amino acids, *e. g.*, α,β-diaminobutyric acid, glutamic acid and glutamine. On the basis of this fact together with the results of the elementary analysis of XIIIa and of the observed molecular weight of dimethyl ester of XIIIa, the structure of this compound XIIIa was assigned as shown in Fig. 5. This may be formed by the intermolecular condensation of the isocyanate derivative with an intact glutamine molecule.

N-Benzyloxycarbonyl-L-alanyl-L-glutamine (XIIb) gave also a corresponding product (XIIIb) under the same condition of the Hofmann reaction as mentioned in Fig. 5. This product did not afford *N*-benzyloxycarbonylalanine by treatment

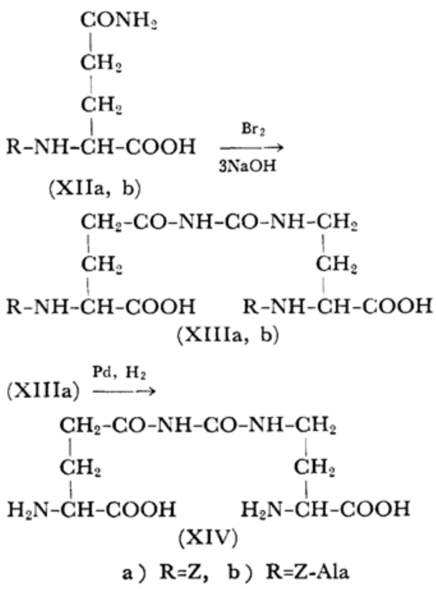

Fig. 5.

with aqueous sodium hydroxide in methanol at room temperature as expected from the structure.

From the results obtained above, it is now concluded that the isocyanate group formed as an intermediate in the Hofmann reaction could be very easily cyclized, giving a five-membered imidazolidone ring, but not a six-membered 2-hydroxytetrahydropyrimidine ring. Therefore, this selective cleavage reaction proceeded specifically only for asparagine peptide whereas glutamine peptide did not react similarly. For application of this reaction to the selective cleavage of asparagine residue in natural peptide or protein, however, the reaction condition should be somehow improved for solubility of the molecule and for prevention of the non-selective alkaline hydrolysis.

Experimental

All melting points are uncorrected. The infrared absorption spectra were obtained in Nujol mull with Nihon Bunko IR-S spectrophotometer. Thin-layer chromatography was carried out on silica gel G.

***N*-Benzyloxycarbonyl-L-alanyl-L-asparagine (I).** To a mixed solution of 13.4 g (0.060 mol) of *N*-benzyloxycarbonyl-L-alanine and 6.0 g (0.060 mol) of triethylamine in 150 m*l* of tetrahydrofuran, there was added 7.1 g (0.066 mol) of ethyl chloroformate at −2°C with stirring. After the reaction mixture had been stirred at −5°C for 20 min, a solution of 9.9 g (0.066 mol) of L-asparagine monohydrate in 66 m*l* of 1N sodium hydroxide solution was added. Stirring was continued at this temperature for 1 hr, and then at room temperature for additional 3 hr. Insoluble crystals of unreacted asparagine were filtered off; wt 2.9 g. The filtrate was acidified to pH 2 with 6 N hydrochloric acid, and an oily substance was obtained. It was extracted with totally 250 m*l* of ethyl acetate. After the extract had been allowed to stand in a refrigerator overnight, crystals formed were filtered; wt 5.5 g, mp 179—180°C (decomp.). Concentration of the mother liquor gave 3.2 g of the same crystals; total yield, 48%. Combined crystals were recrystallized from 94% ethanol to yield 8.3 g of pure crystals; mp 186—187°C (decomp.), $[\alpha]_D^{17}$ +18.7° (*c* 2.09, dimethylformamide).

Found: C, 53.20; H, 5.70; N, 12.29%. Calcd for $C_{15}H_{19}O_6N_3$: C, 53.40; H, 5.68; N, 12.46%.

***N*-Benzyloxycarbonyl-L-asparaginyl-L-phenylalanine *t*-Butyl Ester (VI).** To a solution of 5.16 g (0.020 mol) of *t*-butyl L-phenylalaninate hydrochloride[17,18] in 40 m*l* of dimethylformamide, there was added 2.02 g (0.022 mol) of triethylamine on ice-cooling. After stirring for 30 min, triethylamine hydrochloride formed was filtered off. To the filtrate, a solution of 7.74 g (0.020 mol) of *p*-nitrophenyl *N*-benzyloxycarbonyl-L-asparaginate in 40 m*l* of dimethylformamide was added and the reaction mixture was allowed to stand at room temperature. Crystals formed on addition of water were filtered off after allowing to stand overnight in a refrigerator; yield, 8.29 g (88%), mp 151—152°C. Recrystallization from ethyl acetate gave 7.60 g (81%) of pure crystals; mp 152.0—152.5°C.

Found: C, 64.16; H, 6.92; N, 8.99%. Calcd for $C_{25}H_{31}O_6N_3$: C, 63.94; H, 6.66; N, 8.95%.

***N*-Benzyloxycarbonyl-L-asparaginyl-L-phenylalanine (VII).** To 4.7 g of VI, 25 m*l* of trifluoroacetic acid was added, and the reaction mixture was stirred at room temperature for 1 hr. Crystals formed on addition of 70 m*l* of ether were filtered, washed with ether and then dried *in vacuo* over phosphrus pentoxide; yield, 3.9 g (94%), mp 202.5°C (decomp). Recrystallization from dimethylformamide - water gave needles; mp 210.5°C (decomp.).

Found: C, 60.98; H, 5.67; N, 10.28%. Calcd for $C_{21}H_{23}O_6N_3$: C, 61.01; H, 5.61; N, 10.16%.

***N*-Benzyloxycarbonyl-L-alanyl-L-asparaginyl-L-phenylalanine *t*-Butyl Ester (X).** Through a mixed solution of 14.0 g (0.031 mol) of VI and 3 m*l* of acetic acid in 400 m*l* of methanol, hydrogen gas was passed in the presence of 2 g of 10% palladium on charcoal with stirring for 6 hr. After removal of the catalyst by filtration, the filtrate was evaporated *in vacuo.* To a solution of the residue in 50 m*l* of dimethylformamide, there were added 3 g of triethylamine and a solution of 10.4 g (0.031 mol) of *p*-nitrophenyl *N*-benzyloxycarbonyl-L-alaninate[19] in 50 m*l* of dimethylformamide. The reaction mixture was stirred at room temperature for 24 hr. After addition of water, it was allowed to stand in a refrigerator overnight. Crystals formed were filtered and dried *in vacuo* at 70°C for 3 hr. Recrystallization from dimethylformamide - water gave 14.7 g (91%) of X; mp 164—165°C. Repeated recrystallization from the same solvent raised the melting point to 174—175°C (sintered at 164°C).

Found: C, 62.27; H, 6.80; N, 10.34%. Calcd for $C_{28}H_{36}O_7N_4$: C, 62.20; H, 6.71; N, 10.36%.

***N*-Benzyloxycarbonyl-L-alanyl-L-asparaginyl-L-phenylalanine (XI).** A mixture of 5.4 g (0.010 mol) of X and 30 m*l* of trifluoroacetic acid was stirred at room temperature for 1 hr, and then 100 m*l* of anhydrous

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ether was added. A gelatinous precipitate formed was filtered after cooling and washed with ether. It was reprecipitated from dimethylformamide - water, and then dried *in vacuo* over phosphorus pentoxide; yield, 4.7 g (94%), mp 196°C (decomp.). Elementary analysis was carried out after drying *in vacuo* at 90°C for 3 hr.

Found: C, 57.32; H, 5.95; N, 11.13%. Calcd for $C_{24}H_{28}O_7N_4 \cdot H_2O$: C, 57.36; H, 6.02; N, 11.15%.

***N*-Benzyloxycarbonyl-L-alanyl-L-glutamine (XIIb).** A mixed solution of 6.7 g (0.030 mol) of *N*-benzyloxycarbonyl-L-alanine and 3.0 g (0.030 mol) of triethylamine in 75 m*l* of dioxane, there was added 3.6 g (0.033 mol) of ethyl chloroformate at −5°C for 5 min with stirring. After the stirring had been continued at this temperature for 20 min, a solution of 4.8 g (0.033 mol) of L-glutamine in 33 m*l* of aqueous 1 N sodium hydroxide was added. The reaction mixture was stirred at −5°C for 40 min and then at room temperature for 2.5 hr. A small amount of undissolved glutamine was removed by filtration. The filtrate was acidified to pH 1 with 6 N hydrochloric acid, and extracted with ethyl acetate. Crystals formed in the extract were filtered and recrystallized from water to yield 7.1 g (67%) of needles; mp 167.5—168.5°C. Recrystallization from water raised the melting point to 169.0—171.5°C.

Found: C, 54.49; H, 6.18; N, 11.99%. Calcd for $C_{16}H_{21}O_6N_3$: C, 54.69; H, 6.02; N, 11.96%.

The Hofmann Reaction of *N*-Benzyloxycarbonyl-L-asparagine. To a solution of 3.3 g of sodium hydroxide in 70 m*l* of water, 4.4 g of bromine was added dropwise with stirring at 0—5°C. To the solution, 6.7 g (0.025 mol) of *N*-benzyloxycarbonyl-L-asparagine was added, and stirred at 50°C for 1 hr.[20)] After addition of sodium thiosulfate on cooling, the reaction mixture was washed with ether, and then acidified to pH 2 with 6 N hydrochloric acid to give an oily material which was crystallized after allowing to stand in a refrigerator overnight. It was filtered and recrystallized from water to yield 4.3 g (65%) of 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carboxylic acid; mp 164—168°C (decomp.). Recrystallization from methanol - ether raised the melting point to 190°C (decomp.), lit.,[6)] mp 194°C.

Found: C, 54.24; H, 4.58; N, 10.64%. Calcd for $C_{12}H_{12}O_5N_2$: C, 54.54; H, 4.58; N, 10.60%.

This compound gave crystalline *N,N'*-dicyclohexylammonium salt; mp 200°C (decomp.).

Found: C, 64.19; H, 7.92; N, 9.22%. Calcd for $C_{12}H_{12}O_5N_2 \cdot C_{12}H_{23}N$: C, 64.69; H, 7.92; N, 9.43%.

2-Oxo-imidazolidine-5-carboxylic Acid (IV). Through a mixed solution of 1.32 g (5.0 mmol) of 1-benzyloxycarbonyl-2-oxo-imidazolidine - 5 - carboxylic acid and a few drops of acetic acid in 50 m*l* of methanol, hydrogen gas was passed in the presence of 100 mg of 10% palladium on charcoal for 8 hr. After evaporation of the solvent, a residue obtained was crystallized from methanol - ether; mp 179.5°C (decomp.), lit.,[6)] 190—191°C (decomp.), lit.,[7)] 184°C (decomp.).

Found: C, 37.14; H, 5.00; N, 21.48%. Calcd for $C_4H_6O_3N_2$: C, 36.93; H, 4.65; N, 21.53%.

This carboxylic acid gave long needles of *N,N'*-dicyclohexylammonium salt; mp 214°C (decomp.) (sintered at 203°C).

Found: C, 61.81; H, 9.46; N, 13.48%. Calcd for $C_4H_6O_3N_2 \cdot C_{12}H_{23}N$: C, 61.71; H, 9.39; N, 13.49%.

Cleavage Reaction of *N*-Benzyloxycarbonyl-L-alanyl-L-asparagine (I). a) A mixture of 3.4 g (10 mmol) of I and a solution of 1.8 g (11 mmol) of bromine in 50 m*l* of aqueous 1 N sodium hydroxide was stirred at 78—80°C for 1 hr. After cooling, it was acidified to pH 2—3 with 6 N hydrochloric acid. Crystals of *N*-benzyloxycarbonyl-L-alanine (III) formed were filtered; wt 0.85 g, mp 77.5—81.0°C. The filtrate was extracted with 45 m*l* of chloroform. To a concentrated extract, petroleum ether was added to give needles of a second crop of III; wt 0.58 g, mp 81.5—84.0°C, total crude yield, 64%. Recrystallization of combined product from chloroform - petroleum ether gave 0.99 g (44%) of pure crystals; mp 84—85°C, $[\alpha]_D^{17}$ −14.6° (*c* 2.2, acetic acid). Lit.,[21)] mp 84°C, $[\alpha]_D^{17}$ −14.3° (acetic acid), lit.,[22)] mp 87°C (corrected), $[\alpha]_D^{27}$ −13.9° (*c* 2.0, acetic acid).

The aqueous layer from the chloroform extraction was evaporated *in vacuo* to dryness. A residue was triturated with 100 m*l* of anhydrous ethanol to remove insoluble inorganic salts. The solution was evaporated *in vacuo* to a residue which was then dissolved in 15 m*l* of water. The aqueous solution was passed through Dowex 50 (H form) column, and an effluent was evaporated *in vacuo* to give an oil. This oily material was heated with 6 N hydrochloric acid under reflux for 2 hr. Residue obtained by evaporation *in vacuo* was crystallized from 99% ethanol to yield 0.41 g (29%) of L-α,β-diaminopropionic acid monohydrochloride; mp 230—231°C (decomp.). It was recrystallized from water; mp 238—239°C (decomp.), $[\alpha]_D^{16}$ +21.2° (*c* 5.61, 1 N hydrochloric acid). Lit.,[23)] mp 245°C (decomp.) (corrected), $[\alpha]_D^{20}$ +25.09° (1 N hydrochloric acid), lit.,[7)] $[\alpha]_D^{20}$ +25.0° (1 N hydrochloric acid.).

Reactions at different temperatures shown in Table 1 were carried out by the similar procedure to that mentioned above.

b) To a solution of 2.4 g (0.060 mol) of sodium hydroxide in 60 m*l* of water, 3.2 g of bromine was added dropwise on ice-cooling. To the sodium hypobromite solution prepared above, 6.7 g (0.020 mol) of I was added. The reaction mixture was stirred at 60°C for 1 hr. After cooling and addition of 40 m*l* of water, the reaction mixture was acidified to pH 2 with 6 N hydrochloric acid. An oily material formed was extracted with totally 750 m*l* of ethyl acetate three times. The combined extracts were washed with water, and dried with sodium sulfate. Evaporation of the solvent *in vacuo* gave a solid of crude 1-(*N*-benzyloxycarbonylalanyl)-2-oxo-imidazolidine-5-carboxylic acid (II), which was then dried *in vacuo* over calcium chloride; yield 6.5 g (95%), mp 66°C (decomp.). This compound gave a crystalline dicyclohexylammonium salt

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20) In our experiment, crystalline 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carboxylic acid was obtained only when three equivalents of sodium hydroxide and one equivalent of bromine were used, although Schneider carried out the same reaction using much excess amount of sodium hydroxide and bromine.[6)]

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22) M. Hunt and V. du Vigneaud, *J. Biol. Chem.*, **124**, 669 (1938).

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from anhydrous ether solution; mp 212.5°C (decomp.), ν_{max} 1760 cm^{-1} (ring CO).

Found: C, 62.41; H, 7.94; N, 10.82%. Calcd for $C_{27}H_{40}O_6N_4$: C, 62.77; H, 7.80; N, 10.85%.

To a solution of 0.84 g (2.5 mmol) of II obtained above in 10 m*l* of methanol, there was added 5 m*l* of aqueous 1 N sodium hydroxide solution. The reaction mixture was stirred at room temperature for 2 hr. After acidification to pH 3 with 1 N hydrochloric acid, methanol was removed by evaporation *in vacuo*. Aqueous solution was extracted with chloroform three times. To the dried extract, petroleum ether was added to yield 0.28 g (50%) of needles of *N*-benzyloxycarbonyl-L-alanine (III). Its infrared spectrum and melting point were identical with those of the authentic sample respectively.

The aqueous layer from the chloroform extraction was evaporated *in vacuo*. The residue obtained was dried over phosphorus pentoxide, and dissolved in anhydrous methanol. To the solution, dicyclohexylamine and then anhydrous ether were added giving crystals of dicyclohexylammonium salt. They were filtered and dried. A solution of this salt in a mixture of methanol and water was treated with Dowex 50 (H form) by stirring for 30 min. After removal of the resin by filtration, the solvent was evaporated *in vacuo*. A residue obtained was crystallized from methanol - ether; yield, 30 mg (10%). Its infrared spectrum and melting point were identical with those of 2-oxo-imidazolidine-5-carboxylic acid (IV) respectively.

The Hofmann Reaction of *N*-Benzyloxycarbonyl-L-asparaginyl-L-phenylalanine (VII). To sodium hypobromite solution prepared from 23.3 m*l* of 1 N sodium hydroxide and 0.42 m*l* of bromine, there was added 3.1 g (7.5 mmol) of VII. The reaction mixture was stirred at 60°C for 1 hr. After cooling, crystals formed were filtered and dried *in vacuo* over phosphorus pentoxide; yield 3.1 g (100%), mp 179—181°C (decomp.). Recrystallization from methanol - ether gave pure crystals of 1-benzyloxycarbonyl-2-oxo-imidazolidine-5-carbonylphenylalanine (VIII) containing one mole of methanol as crystallization solvent; mp 191°C (decomp.), ν_{max} 1790 cm^{-1} (ring CO).

Found: C, 59.66; H, 5.79; N, 9.15%. Calcd for $C_{21}H_{21}O_6N_3 \cdot CH_3OH$: C, 59.58; H, 5.68; N, 9.48%.

When 23.645 mg of the crystals was heated at 80°C *in vacuo*, a constant weight of 22.067 mg was obtained after 15.5 hr. The loss in weight corresponds to 92.3% of the theoretical amount of one mole of methanol as crystallization solvent.

Through a solution of 0.75 g (1.8 mmol) of VIII in a mixture of 20 m*l* of methanol, 5 m*l* of acetic acid and 5 m*l* of water, hydrogen gas was passed for 6 hr in the presence of 100 mg of 10% palladium on charcoal. After removal of the catalyst by filtration, the solvent was evaporated *in vacuo*. A residue obtained was dried *in vacuo* over phosphorus pentoxide; yield, 0.40 g (78%). Recrystallization from water gave needles of 2-oxo-imidazolidine-5-carbonylphenylalanine (IX); mp 195°C (decomp.) (sintered at 100°C). This compound gave no ninhydrin reaction.

Found: C, 53.37; H, 5.81; N, 14.16%. Calcd for $C_{13}H_{15}O_4N_3 \cdot H_2O$: C, 52.87; H, 5.80; N, 14.23%.

The Hofmann Reaction of *N*-Benzyloxycarbonyl-L-glutamine (XIIa). To a solution of 4.0 g of bromine in 100 m*l* of 0.75 N sodium hydroxide, there was added 7.0 g (0.025 mol) of *N*-benzyloxycarbonyl-L-glutamine. The reaction mixture was stirred at 60°C for 1 hr. After cooling and addition of sodium thiosulfate, the reaction mixture was acidified to pH 2 with 6 N hydrochloric acid. Crystals formed were filtered off and dried *in vacuo* over phosphorus pentoxide; wt 5.6 g. It was recrystallized from methanol - water, and then from ethanol - water to yield needles of XIIIa; mp 181.5°C (decomp.).

Found: C, 55.96; H, 5.54; N, 9.96%. Calcd for $C_{26}H_{30}O_{10}N_4$: C, 55.91; H, 5.41; N, 10.03%.

This compound gave pure crystals of bis-dicyclohexylammonium salt, mp 192°C (decomp.).

Found: C, 65.11; H, 8.32; N, 9.09%. Calcd for $C_{26}H_{30}O_{10}N_4 \cdot 2C_{12}H_{23}N$: C, 65.19; H, 8.32; N, 9.12%.

The compound IIIa was converted to its dimethyl ester by diazomethane in the usual manner; mp 160—162°C (decomp.), mol wt: Found: 545 (by the Rast method using camphor). Calcd for $C_{28}H_{34}O_{10}N_4$: 586.

Hydrogenation of IIIa by the usual procedure using 10% palladium on charcoal gave compound XIV. It was recrystallized from water; mp 226—227°C (decomp.), R_f, 0.13 on a thin-layer chromatogram (*n*-butanol - acetic acid - water, 4 : 1 : 2).

Found: C, 41.25; H, 6.47; N, 18.87%. Calcd for $C_{10}H_{18}O_6N_4$: C, 41.37; H, 6.25; N, 19.30%.

Compound XIV was then hydrolyzed by refluxing with 6 N hydrochloric acid for 3 hr. The reaction product gave three spots of α,γ-diaminobutyric acid, glutamic acid and glutamine by ninhydrin coloration on a thin-layer chromatogram and a paper chromatogram. Thin-layer chramotography (*n*-butanol - acetic acid - water, 4 : 1 : 2): R_f, 0.16 (α,γ-diaminobutyric acid), 0.22 (glutamine), 0.30 (glutamic acid). Paper chromatography (phenol - water, 4 : 1): R_f, 0.18 (α,γ-diaminobutyric acid), 0.33 (glutamic acid), 0.59 (glutamine).

The Hofmann Reaction of *N*-Benzyloxycarbonyl-L-alanyl-L-glutamine (XIIb). To a mixture of 15 m*l* of 1N sodium hydroxide and 0.80 g of bromine, there was added 1.76 g of XIIb. The reaction mixture was stirred at 60°C for 1 hr. By a procedure similar to that in the reaction of XIIa, 1.49 g of the product XIIIb was obtained. It was recrystallized from methanol - ether - petroleum ether to yield needles; mp 192.5°C (decomp.).

Found: C, 54.29; H, 5.82; N, 11.78%. Calcd for $C_{32}H_{40}O_{12}N_6 \cdot CH_3OH$: C, 54.09; H, 6.05; N, 11.47%.

Even when this product was treated with 1 N sodium hydroxide in methanol at room temperature for 2 hr, the starting material was recovered without formation of *N*-benzyloxycarbonylalanine (III).

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