dried and evaporated to an oil which was dissolved in 10 ml. of hexane and applied to a column (15 × 1 cm.) of alumina (Merck). The octahydrodemethoxydesoxydesacetamidocolchicine was eluted with 20% benzene–hexane (200 ml.) after hexane (50 ml.) had removed only a trace of material. Slow sublimation at 40°/6 μ of the white solid, m.p. 49–50°, obtained by evaporation of the benzene–hexane eluant gave 250 mg. (60% yield) of material still melting at 49–50°; $[\alpha]^{25}D$ 0° (*c* 1.01, ethanol).

Anal. Calcd. for $C_{19}H_{25}O_3$: C, 75.5; H, 8.7; $-OCH_3$, 30.8; $C-CH_3$, 0. Found: C, 75.4; H, 8.7; $-OCH_3$, 30.9; $C-CH_3$, <0.5.

Octahydrodemethoxydesoxydesacetamidocolchicine Epoxide.—Perbenzoic acid oxidation of octahydrodemethoxydesoxydesacetamidocolchicine (VI) was carried out as described above for hexahydrodemethoxydesoxycolchicine and resulted in the consumption of one mole of perbenzoic acid per mole of compound. An oxidation reaction which consumed 82 mole % of perbenzoic acid and then became very much slower was treated as above in order to isolate epoxide. The chloroform residue was applied to the alumina column in hexane and this was followed by 10% benzene–hexane, 20% benzene–hexane and benzene. Evaporation of the benzene gave crystalline epoxide in 42% yield and this was recrystallized from hexane; m.p. 116–117°.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.7; H, 8.2. Found: C, 72.0; H, 8.2.

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[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

Syntheses of Aspartyl Amides and Peptides through N-Benzyl-*dl*-aspartic Acid

BY Y. LIWSCHITZ AND A. ZILKHA

RECEIVED JANUARY 18, 1954

The α-amides and α- and β-peptides of *dl*-aspartic acid have been synthesized, *via* N-benzyl-*dl*-aspartic acid, from a mixed anhydride prepared by the reaction of phosgene with this amino acid. A new method is given for the determination of the linkage in aspartyl amides and peptides by the biuret reaction.

Since N-benzyl-*dl*-aspartic acid (I) is readily synthesized[1] it was used for the preparation of aspartyl amides and peptides, with the expectation that the N-benzyl group would act as a reversible masking group; ring closure would give the corresponding anhydride with which amines or peptide esters would react. Acetic anhydride could not be used, since it removed water intramolecularly and, even at room temperature, acetylated the secondary α-amino group; these results will be published separately. It was found that phosgene reacted with I in dioxane, yielding compound II which, in accordance with analyses and general behavior, is probably a mixed anhydride of I and chloroformic acid (*cf.* Wieland and Bernhard[2]). Since the action of ammonia or benzylamine upon II yielded N-benzyl-*dl*-α-asparagine (III) and N^{α},-N'^{α}-dibenzyl-*dl*-α-asparagine (IV), respectively, with the evolution of carbon dioxide and hydrogen chloride, the α-carboxyl only was involved in the mixed anhydride formation. III and IV were easily converted to *dl*-α-asparagine (V) and N'^{α}-benzyl-*dl*-asparagine (VI) by catalytic hydrogenolysis (Chart I).

α-Amides were formed when II, either in the original dioxane solution or as the isolated compound, reacted with ammonia or benzylamine.

When the coupling reaction with glycine ethyl ester was carried out in dioxane, N-benzyl-α-*dl*-aspartylglycine ethyl ester (VII) was the only product; with isolated II suspended in dry toluene, mainly N-benzyl-β-*dl*-aspartylglycine ethyl ester (VIII) was obtained with a very small amount of VII, from which it could be separated by fractional crystallization. Catalytic hydrogenolysis of VII and VIII gave the free peptide esters IX and X; hydrolysis and subsequent hydrogenolysis the free dipeptides XII and XIII. N-Benzyl-α-*dl*-aspartylglycine (XI) was produced as an intermediary, but the corresponding β-compound could not be isolated. Hydrolysis of VIII and acidification with hydrochloric acid failed to precipitate the N-benzyl-β-aspartylglycine; this was also true for N-benzyl-β-aspartylalanine. This difference may serve as an additional means of distinguishing N-benzyl-α- and β-aspartylpeptides and as a method for their quantitative separation.

II reacted with *dl*-alanine ethyl ester, in dioxane or toluene, to give only N-benzyl-β-*dl*-alanine ethyl ester (XIV), which on direct hydrogenolysis yielded the dipeptide ester (XV), and on hydrolysis followed by reduction β-*dl*-aspartyl-*dl*-alanine (XVI).

Since the melting points of the peptides in the literature generally refer to optically active compounds, the nature of the linkage in each case had to be determined by other means.

N-Benzyl-α-*dl*-asparagine (III) melted at 180°, N-benzyl-β-*dl*-asparagine at 216°.[1] On hydrogenolysis the former yielded *dl*-α-asparagine which differs from *dl*-β-asparagine by (a) its greater water solubility, (b) its purple color with ninhydrin on paper chromatograms, contrasting with the yellowish-brown color given by the β-isomer, and (c) its red biuret reaction, differing from the bluish tinge shown by β-asparagine.[3]

N^{α},N'^{α}-Dibenzyl-α-*dl*-asparagine (IV) melted at 173°, its β-isomer at 215°.[4] On hydrogenolysis, compound VI was obtained (m.p. 235°) which, unlike the β-isomer,[5] did not form a N-carboxy anhydride with phosgene. This shows clearly that the α-carboxyl is not available.

α-*dl*-Aspartylglycine monohydrate (XII) (m.p. 155°), β-*dl*-aspartylglycine monohydrate (XIII) (m.p. 156°), their derivatives (VII–XI), and the peptide obtained with alanine (XVI) (m.p. 232°) were identified as follows: (a) In agreement with

(1) Max Frankel, Y. Liwschitz and Y. Amiel, THIS JOURNAL, **75**, 330 (1953).

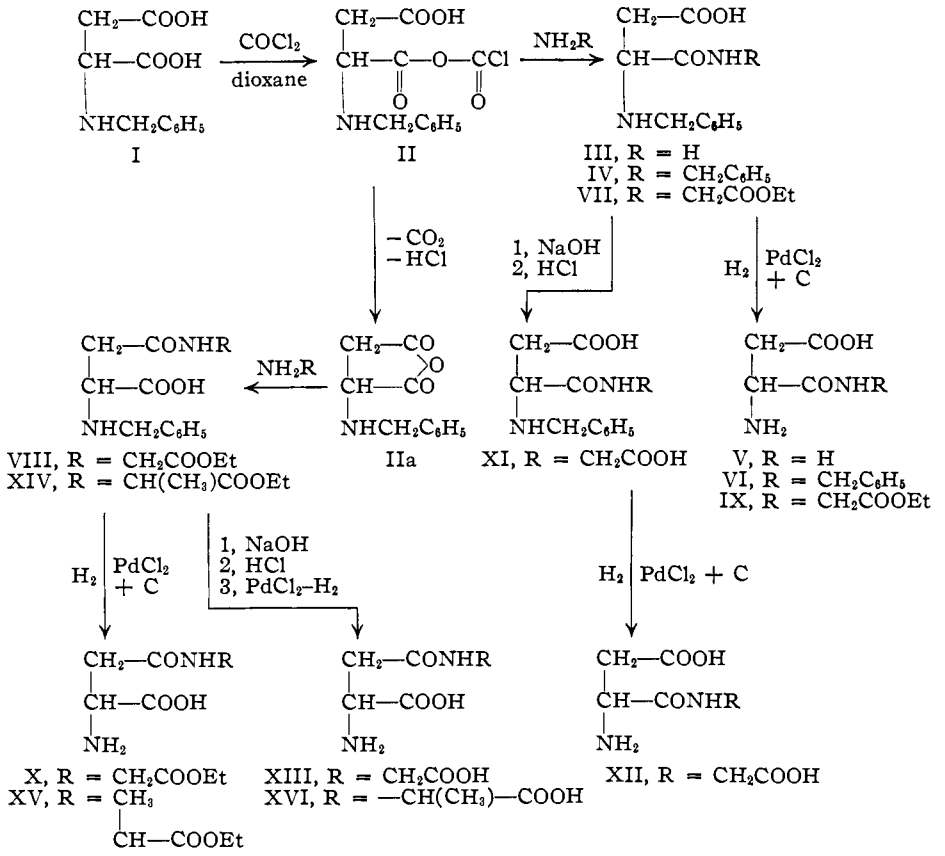
(2) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(3) E. Fischer, *Ber.*, **35**, 1095 (1902).

(4) F. H. McMillan and N. F. Albertson, THIS JOURNAL, **70**, 3778 (1948).

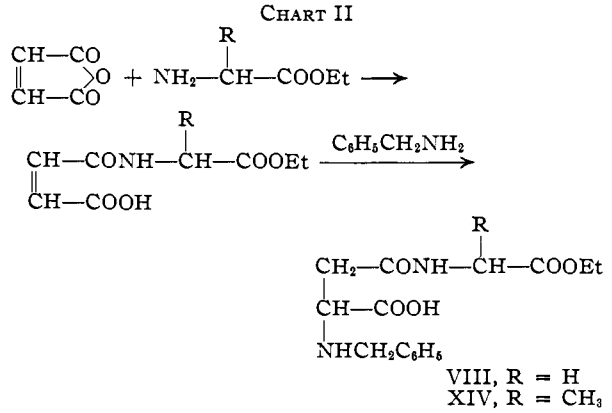
(5) Max Frankel, Y. Liwschitz and A. Zilkha, *ibid.*, **75**, 3270 (1953).

CHART I



the findings of LeQuesne and Young,[6] XII gave a purple spot with ninhydrin on chromatograms, XIII and XVI blue spots. (b) The β-peptides were identical with those prepared by a different method, summarized in Chart II, which will be published subsequently. (c) We were able to distinguish between α- and β-aspartyldipeptides by a new method based on the biuret reaction.

CHART II



As mentioned above, α-asparagine gives a red biuret color, β-asparagine an almost blue shade. The substitution of one amidohydrogen in β-asparagine by an alkyl group (*e.g.*, benzyl) results in suppression of the biuret reaction; this does not apply to analogous derivatives of α-asparagine. Thus in the case of α-amides of aspartic acid, it is quite irrelevant whether a benzyl radical is introduced into the α-amino or α-amido group, and both N-benzyl α-asparagine (III) and N′$^{\alpha}$-benzyl-α-asparagine (VI) give the normal red biuret color; even N$^{\alpha}$,N′$^{\alpha}$-dibenzyl-asparagine reacts positively. This behavior is attributable to the different strengths of the two carboxyl groups. The free α-carboxyl group which almost suppresses the biuret reaction in β-asparagine prevents it altogether if one hydrogen atom in the β-amido group becomes substituted. On the other hand, the weaker free β-carboxyl group does not interfere with the reaction. This rule also held for the α- and β-dipeptides of aspartic acid; thus a positive biuret reaction was given readily by IX and XII, but not by X, XIII, XV and XVI.

An explanation is required for the fact that although II is a mixed anhydride in which, of course, only one carboxyl group can be utilized, it yields both α- and β-peptides. The evidence for the structure of II consists in analytical values which are in accord with the suggested structure (see Experimental part), and the evolution of carbon dioxide in the coupling reaction. Given a 70% yield of the mixed anhydride (II) in the first phase of the reaction (the highest yield found for the isolated compound), the carbon dioxide evolution was practically quantitative. Production of this gas and of hydrogen chloride, which must be bound by a second mole of the reacting amine or amino acid ester, can only be explained on the basis of structure II. That this compound is not a mixture of two isomers, in which the α- and β-carboxyls of N-benzyl-*dl*-aspartic acid are linked to the chloroformyl residue, is proved by the fact that only the α-isomer is produced by coupling with ammonia or with a reactive amine, such as benzylamine. Therefore, if II is coupled with these or other reactive substances while still in dioxane solution, it reacts in the form described by the formula. If, however, it is first isolated and then coupled with less reactive substances (amino acid esters), it tends to lose carbon dioxide and hydrogen chloride; thus, it is transformed into the anhydride (IIa) which may couple in both ways.

Experimental

Micro-combustion analyses were made by Drs. Weiler and Strauss. Melting points were determined in a Fisher-

(6) W. J. LeQuesne and G. T. Young, *J. Chem. Soc.*, 24 (1952).

Johns apparatus and the ascending method of paper-partition chromatography was used.

Mixed Anhydride of N-Benzyl-*dl*-aspartic Acid[1] (I) and Chlorocarbonic Acid (II).—I (16 g.), which had been dried at 110°, was suspended in 350 ml. of dioxane (dried over sodium) in a three-necked flask equipped with a gas leading tube, a reflux condenser connected to a calcium chloride tube and a mechanical stirrer. Phosgene was bubbled in with stirring and the temperature maintained at 50–60°. After about 30 minutes, the solution cleared, but the introduction of phosgene was continued for another 15 minutes. Finally, the excess phosgene and the solvent were removed *in vacuo* at a temperature not exceeding 40°. The residue, on trituration with dry ethyl acetate, gave 13.5 g. (66%) of a white to yellowish substance which decomposed at 153–155°.

Anal. Calcd. for $C_{12}H_{12}O_5NCl$: N, 4.9; Cl, 12.5. Found: N, 4.6; Cl, 11.6.

N$^{\alpha}$-Benzyl-α-*dl*-asparagine (III). (A) From Solid II.—II (8 g.) was added to 30 ml. of a 30% aqueous ammonium hydroxide solution, cooled in a freezing mixture and stirred. After the solution had been evaporated almost to dryness on a water-bath, a small quantity of absolute methanol was added and evaporation continued to remove water quantitatively. The brownish residue was triturated with absolute methanol, yielding a chloride-free white crystalline substance. The compound, recrystallized from water (5.1 g. (81%), m.p. 180°), gave a red biuret reaction.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.0; H, 6.2; N, 12.0.

(B) Without Isolation of II.—A solution of II in dioxane was prepared from 11.2 g. of I in 250 ml. of dioxane, as described above; the excess phosgene but not the solvent was removed *in vacuo* at room temperature (2.5–3 hours). Slightly more than one equivalent of gaseous ammonia (dried over soda lime) was bubbled into the cooled solution, and a white crystalline mass settled out immediately. The material was filtered, washed with ether and dried in a vacuum desiccator. On trituration with absolute methanol, 6.8 g. (60%) of III was obtained, identical with the substance prepared by method (A).

α-*dl*-Asparagine (V).—To 2.3 g. of III dissolved in 40 ml. of acetic acid (50%) in an hydrogenation bottle was added 0.3 g. of a palladium chloride-on-carbon (Norite) catalyst (30%). The hydrogenolysis was carried out in a Parr low pressure apparatus for three hours at 60–70°.[7] The catalyst was then filtered off and the solvent removed *in vacuo*. The residue was dissolved in water and on addition of absolute methanol, the substance crystallized in fine long needles, 1.2 g. (80%), which gave a pronounced red biuret color.

Anal. Calcd. for $C_4H_8O_3N_2 + H_2O$: N, 18.7. Found: N, 18.7.

The purple spot (R_f 0.41) obtained by chromatography with phenol–water as the mobile phase, conformed exactly to the spot produced by an authentic sample of *dl*-α-asparagine.

N$^{\alpha}$,N'$^{\alpha}$-Dibenzyl-α-*dl*-asparagine (IV).—To a cooled solution of II in dioxane, prepared from 5 g. of I in 100 ml. of dioxane as above, was added 5.2 g. of benzylamine. A white crystalline material precipitated at once. After the reaction mixture had stood overnight at room temperature, the precipitate, consisting of IV and benzylamine hydrochloride, was filtered off. It was washed several times with ether to remove traces of dioxane and finally recrystallized from water to free it from benzylamine hydrochloride. The globular crystals, 5 g. (70%), m.p. 173°, gave a red biuret reaction.

Anal. Calcd. for $C_{18}H_{20}O_3N_2$: C, 69.2; H, 6.5; N, 9.0. Found: C, 69.2; H, 6.5; N, 8.9.

N'$^{\alpha}$-Benzyl-α-*dl*-asparagine (VI).—IV (8 g.) was dissolved in 20 ml. of glacial acetic acid and 0.5 g. of catalyst added. Hydrogenolysis was complete after about 5 hours. Most of the material which adhered to the catalyst was extracted with cold formic acid and, on evaporation of this solvent *in vacuo*, 5.1 g. (90%) of VI was obtained. This recrystallized from water in feather-like crystals, m.p. 235°, which gave a red biuret reaction.

(7) All hydrogenations were performed at 60–70° in the same apparatus with different quantities of the same catalyst.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.5; H, 6.2; N, 12.7.

N-Benzyl-α-*dl*-aspartylglycine Ethyl Ester (VII).—To an ice-cooled solution of II in dioxane (from 9 g. of I in 200 ml. of solvent) was added 9 g. of freshly distilled glycine ethyl ester, prepared according to Emil Fischer[8]; the reaction mixture was kept overnight in an ice-box. The precipitate, consisting of VII and glycine ethyl ester hydrochloride, was then filtered off and VII isolated by recrystallization from ethanol, 8.2 g., m.p. 140°; evaporation of the dioxane gave an additional 0.4 g. (total yield 70%). Recrystallization from ethanol gave needles, m.p. 146°.

Anal. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.2; H, 6.5; N, 9.1. Found: C, 58.2; H, 6.5; N, 9.1.

N-Benzyl-α-*dl*-aspartylglycine (XI).—VII (3.5 g.) was dissolved in 24 ml. of 1 *N* sodium hydroxide solution and kept at room temperature for two hours. On acidification with hydrochloric acid, XI precipitated out; after cooling, it was filtered off; 3 g. (95%), m.p. 180°. Recrystallization from ethanol gave needles joined at center, m.p. 187°.

Anal. Calcd. for $C_{13}H_{16}O_5N_2$: N, 10.0. Found: N, 9.9.

α-*dl*-Aspartylglycine (XII).—To 2.8 g. of XI dissolved in 100 ml. of 35% acetic acid was added 0.3 g. of catalyst. After 4 hours of hydrogenolysis, the catalyst was filtered off and the solution evaporated to dryness *in vacuo*. The residue, on recrystallization from a small quantity of water, yielded rhombic plates, 1.2 g. (63%), m.p. 155°, which gave a positive ninhydrin and red biuret reaction. Chromatography with phenol–water as the mobile phase gave a purple spot (R_f value 0.17); see ref. 6.

Anal. Calcd. for $C_6H_{10}O_5N_2 + H_2O$: C, 34.6; H, 5.8; N, 13.5; N (Van Slyke), 6.7. Found: C, 34.7; H, 5.8; N, 13.3; N (Van Slyke), 6.6.

α-*dl*-Aspartylglycine Ethyl Ester (IX).—To 6 g. of VII dissolved in 100 ml. of glacial acetic acid was added 0.5 g. of catalyst. After 4 hours of hydrogenolysis, the catalyst was filtered off and the solution evaporated to dryness *in vacuo*. The residue was recrystallized from ethanol, and the substance which was recovered in almost quantitative yield, m.p. 175–176°, gave positive nonhydrin and biuret reactions.

Anal. Calcd. for $C_8H_{14}O_5N_2$: N (Van Slyke), 6.4. Found: N (Van Slyke), 6.4.

N-Benzyl-β-*dl*-aspartylglycine Ethyl Ester (VIII).—Freshly distilled glycine ethyl ester (4.6 g.) was added to 6 g. of solid II suspended in 150 ml. of dry toluene in a 500-ml. glass-stoppered flask. While the flask was shaken for six hours, the sticky brownish material (II) changed into a white crystalline mass. This was filtered off, washed with ether and recrystallized from ethanol to remove glycine ethyl ester hydrochloride; 2.5 g. (39%), m.p. 194°. Recrystallization from ethanol yielded hexagonal plates, m.p. 201°. A large quantity of acetone was added to the alcoholic mother liquor; on standing overnight in an ice-box, the α-isomer 0.2 g., m.p. 140°, precipitated out.

Anal. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.2; H, 6.5; N, 9.1. Found: C, 58.0; H, 6.6; N, 9.2.

β-*dl*-Aspartylglycine Ethyl Ester (X).—VIII (2 g.) was dissolved in 40 ml. of glacial acetic acid and 0.2 g. of catalyst added. Hydrogenolysis was complete in 4 hours. The reaction mixture was filtered off while still hot (to prevent crystallization of X) and the solvent evaporated *in vacuo*. The substance, which melted at 238° on recrystallization from glacial acetic acid, was obtained in an almost quantitative yield. It gave a positive ninhydrin, but a negative biuret reaction.

Anal. Calcd. for $C_8H_{14}O_5N_2$: N (Van Slyke), 6.4. Found: N (Van Slyke), 6.6.

β-*dl*-Aspartylglycine (XIII).—VIII (3 g.) was dissolved in 21 ml. of 1 *N* sodium hydroxide solution. After two hours at room temperature, the solution was acidified with hydrochloric acid to congo red paper; the N-benzyl-β-*dl*-aspartylglycine did not precipitate. The solution was evaporated to dryness *in vacuo*, the residue redissolved in 50 ml. of 50% acetic acid and subjected to hydrogenolysis for 4 hours, after the addition of 0.2 g. of catalyst. The latter was filtered off and the solution evaporated to dryness *in vacuo*. Recrystallization from a small quantity of water gave convex lens-like crystals of XIII, 0.7 g. (35% over-

(8) E. Fischer, *Ber.*, **34**, 433 (1901).

all), m.p. 156°. This substance gave a positive ninhydrin, but a negative biuret reaction. Chromatography gave a blue spot (R_f value 0.17); *cf.* ref. 6.

Anal. Calcd. for $C_6H_{10}O_5N_2 + H_2O$: C, 34.6; H, 5.8; N, 13.5; N (Van Slyke), 6.7. Found: C, 34.9; H, 5.8; N, 13.2; N (Van Slyke), 6.4.

N-Benzyl-β-*dl*-aspartyl-*dl*-alanine Ethyl Ester (XIV). (A) From II in Dioxane Solution.—A cooled solution of II in dioxane was prepared from 9 g. of I in 200 ml. of dioxane; 10 g. of *dl*-alanine ethyl ester was added and the reaction mixture kept overnight at room temperature. No precipitate formed owing to the solubility of both *dl*-alanine ethyl ester hydrochloride and XIV in dioxane. The solution was evaporated to dryness *in vacuo;* the residue was recrystallized from a small quantity of ethanol and kept in an ice-box overnight. Recrystallization from ethanol yielded 4.1 g. (31.5%), m.p. 197°.

Anal. Calcd. for $C_{16}H_{22}O_5N_2$: N, 8.7. Found: N, 8.4.

(B) From Solid II.—A suspension of 13 g. of solid II in 200 ml. of dry toluene was shaken with 13 g. of *dl*-alanine ethyl ester in a 500-ml. glass-stoppered flask for 6 hours. The reaction mixture was filtered and the precipitate washed with ether and recrystallized from ethanol; 5.5 g. (37.5%) of XIV was obtained, which was identical with the substance prepared by method (A).

β-*dl*-Aspartyl-*dl*-alanine Ethyl Ester (XV).—XIV (3 g.) was dissolved in 50 ml. of glacial acetic acid, 0.2 g. of catalyst added and hydrogenolysis carried out for 4 hours. After filtration and evaporation of the solvent, the residue was recrystallized from ethanol. The substance, melting at 218°, was obtained in an almost quantitative yield. It gave a positive ninhydrin, but a negative biuret reaction.

Anal. Calcd. for $C_9H_{16}O_5N_2$: N, 12.0; N (Van Slyke), 6.0. Found: N, 11.9; N (Van Slyke), 6.1.

β-*dl*-Aspartyl-*dl*-alanine (XVI).—XIV (1 g.) was dissolved in 7 ml. of 1 *N* sodium hydroxide solution. After two hours, the solution was acidified with hydrochloric acid, but no precipitate formed. It was evaporated *in vacuo* to dryness and redissolved in 50 ml. of 50% acetic acid; after the addition of 0.2 g. of catalyst, hydrogenolysis was carried out for 4 hours. After filtration and evaporation of the solution *in vacuo* to dryness, the residue was recrystallized from water to yield 0.19 g. (30%) of short needles, m.p. 232°, which gave a positive ninhydrin, but a negative biuret reaction. Chromatography gave a blue spot (R_f value 0.28).

Anal. Calcd. for $C_7H_{12}O_5N_2$: C, 41.1; H, 5.9; N, 13.7; N (Van Slyke), 6.8. Found: C, 41.1; H, 5.8; N, 13.7; N (Van Slyke), 6.8.

JERUSALEM, ISRAEL

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Application of a Quantitative Method of Peptide Analysis to the N-Terminal Sequence of Lysozyme[1]

BY DOROTHY DE FONTAINE[2] AND SIDNEY W. FOX[3]

RECEIVED FEBRUARY 4, 1954

The microbiological phenylthiohydantoin subtractive method of peptide analysis has been applied to the terminal aminoid tripeptide residue of chicken egg lysozyme, without discernible alteration of the rest of the molecule. The sequence is shown to be L-lysyl-L-valyl-L-phenylalanyl-, which includes the configurational designations for the first time. Further evaluation of the quantitative method of peptide analysis is presented. Various assay media and microbes for the determination of lysine have been compared; one combination has given in this work significantly greater precision than two others.

A stepwise N-terminal method, representing a modification of the original Abderhalden–Brockmann[4] and Edman[5] methods has been applied with microbiologically quantitative results to synthetic peptides[6] and to ACTH preparations.[7] Some of these modifications have also been useful in qualitative structural studies of natural peptides.[8,9] In this paper are presented the results of study of applicability of the subtractive procedure to lysozyme, for which structural information is available, particularly from an investigation by Schroeder, with the DNP technique.[10] The molecular weight of lysozyme is sufficiently high (*ca.* 14,700)[11] to provide a severe test of the method.

(1) Journal Paper No. J-2466 of the Iowa Agricultural Experiment Station, Project 863, supported by Grant No. G-3025(C) from the National Institutes of Health, Public Health Service.

(2) Taken in part from the M.S. Thesis of Dorothy De Fontaine, 1952.

(3) To whom inquiries should be directed.

(4) E. Abderhalden and H. Brockmann, *Biochem. Z.*, **225**, 386 (1930).

(5) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(6) S. W. Fox, T. L. Hurst and K. F. Itschner, THIS JOURNAL, **73**, 3573 (1951).

(7) S. W. Fox, T. L. Hurst and C. Warner, *ibid.*, **76**, 1154 (1954).

(8) W. Landmann, M. P. Drake and J. Dillaha, *ibid.*, **75**, 3638 (1953).

(9) W. Landmann, M. P. Drake and W. F. White, *ibid.*, **75**, 4370 (1953).

(10) W. A. Schroeder, *ibid.*, **74**, 281 (1952); **74**, 5118 (1952).

(11) C. Fromageot and M. B. de Garilhe, *Biochim. et Biophys. Acta*, **4**, 509 (1950).

Materials and Methods

Lysozyme.—Lysozyme was a thrice recrystallized material[12] furnished by Dr. Joseph F. Foster. Electrophoretic analysis at *p*H 7.7 (phosphate–sodium chloride buffer $\gamma/2 = 0.20$) indicated this material was nearly homogeneous. There was observed in the electrophoretogram a trace (estimated 1–2%) component which moved slightly ahead of the main boundary. The descending boundary was somewhat asymmetric but showed no trace of the fast component. For some of the determinations of lysine, Armour lysozyme Lot 003 L1, was used.

Aminoid Treatment.—The procedure previously described[7] was followed with the following modifications: phenyl isothiocyanate (PTC) treatment was terminated after 4 hr. of incubation, selective fission of the treated product with dioxane–hydrogen chloride in a hydrogen chloride atmosphere was discontinued after 6 hr., and 6 *N* aqueous hydrochloric acid was used for total hydrolysis of protein.

Assays.—Standard curves covered the range of 0–50 γ of L-amino acid. The *p*H of the medium was adjusted in each case to 6.8 ± 0.1. Sixteen-hour inoculum cultures were used in the assays which were incubated for 72 hr. at 37° except for glycine assays which were incubated for 48 hr.

(12) G. Alderton, W. H. Ward and H. L. Fevold, *J. Biol. Chem.*, **157**, 43 (1945); R. H. Forsythe, Ph.D. thesis, Iowa State College, 1949.