the resin washed with an additional 20 ml. of water and the combined filtrates acidified to *p*H 4 with glacial acetic acid and evaporated to dryness *in vacuo* at a bath temperature of 40–45°. The residue was evaporated with three 10-ml. portions of methanol and suspended in 10 ml. of ethanol containing a few drops of glacial acetic acid. The solids were collected by centrifugation and were re-extracted with three additional 5-ml. portions of ethanol. The residue was dried *in vacuo* over phosphorus pentoxide. The material was purified further by precipitation from water with ethanol. A white granular powder was obtained; yield 0.14 g. (63%); $[\alpha]^{26}D$ −20.6° (*c* 1.95, in 2 *N* HCl); R_f = 0.51 (Partridge). For analysis, the pentapeptide was dissolved in a small quantity of hot water, and separated in the form of small rosettes upon cooling of the solution. It was collected and dried at room temperature *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{25}H_{37}O_{11}N_5S(H_2O)$: C, 47.4; H, 6.2; N, 11.1. Found: C, 47.0; H, 6.4; N, 11.6.

PITTSBURGH, PA.

[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF PITTSBURGH, SCHOOL OF MEDICINE]

Studies on Polypeptides. IX. Synthesis of Peptides Containing Basic Amino Acid Residues, Related to Corticotropin and Intermedin

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The sequences histidyl-phenylalanyl-arginine and glutamyl-histidyl-phenylalanyl-arginine, have been shown to occur in the corticotropins and in the melanocyte-stimulating hormone. Peptides corresponding to these sequences have been synthesized and their homogeneity ascertained by paper chromatography and by quantitative determination of the amino acids liberated on acid hydrolysis. Leucine aminopeptidase converted both peptides into an equimolar mixture of the expected amino acids, demonstrating their stereochemical purity. The behavior, on paper chromatography, of the synthetic histidyl-phenylalanyl-arginine was identical with that of a peptide resulting from the enzymatic degradation of corticotropin-A. This provides unequivocal synthetic confirmation for the sequence his-phe-arg in this hormone. The synthesis of a series of compounds related to the two peptides is also described.

In previous communications,[2,3] we have described a synthesis of seryl-tyrosyl-seryl-methionyl-glutamic acid, the N-terminus of swine β-corticotropin, swine corticotropin-A, and sheep β-corticotropin.[4–6] A structural unit, common not only to these corticotropins but also to the melanocyte-stimulating principle of porcine pituitary glands,[7,8] possesses the sequence glutamyl-histidyl-phenylalanyl-arginine. In the present communication, we record a synthesis of this tetrapeptide. Prior to attempting the preparation of the entire peptide, methods were explored for preparing various smaller peptides and peptide derivatives embodying within their structures certain bond types occurring in the over-all sequence. The dipeptide, L-histidyl-L-phenylalanine, was prepared readily by the azide method *via* carbobenzoxy-L-histidyl-L-phenylalanine. Two routes to this substance were explored. In the first, the azide of carbobenzoxy-L-histidine[9] was coupled with the methyl ester of L-phenylalanine and the ensuing carbobenzoxy-L-histidyl-L-phenylalanine methyl ester was subjected to saponification. In the second, the triethylammonium salt of L-phenylalanine was treated with carbobenzoxy-L-histidine azide in a manner recently described.[3] The carbobenzoxy-L-histidyl-L-phenylalanine resulting from both approaches exhibited identical properties, and on catalytic hydrogenation afforded the crystalline free dipeptide. Recrystallization from water gave a product which was homogeneous, as revealed by paper chromatography. Acid hydrolysis converted the dipeptide into an equimolar mixture of histidine and phenylalanine.

The observation that the azide of carbobenzoxy-L-histidine lends itself to coupling in aqueous systems provided a key to a synthesis of L-histidyl-L-phenylalanylnitro-L-arginine. Acid-catalyzed decarbobenzoxylation in glacial acetic acid containing hydrogen bromide converted carbobenzoxy-L-phenylalanylnitro-L-arginine[10] into the hydrobromide of L-phenylalanylnitro-L-arginine, which gave the free peptide when treated with the ion exchanger Amberlite IR-4B in the acetate form. This dipeptide, as its triethylammonium salt, was then coupled with carbobenzoxy-L-histidine azide[9] to give carbobenzoxy-L-histidyl-L-phenylalanylnitro-L-arginine. The carbobenzoxylated tripeptide was sparingly soluble in water, but dissolved readily in dilute ammonia and was precipitated therefrom in crystalline form by glacial acetic acid. This property of the compound provided a convenient method of purification. Hydrogenation in glacial acetic acid in the presence of a palladium catalyst converted the carbobenzoxy derivative into the acetate salt of L-histidyl-L-phenylalanyl-L-arginine. This tripeptide salt was purified by precipitation from its ethanolic solution with ether, and exhibited a single ninhydrin-positive spot on paper in the Partridge[11] and the 2-butanol–ammonia systems.[12] Control strips sprayed with diazotized

(1) The authors wish to express their appreciation to the U. S. Public Health Service, the National Science Foundation, and Armour and Co. for generous support of this investigation.

(2) K. Hofmann and A. Jöhl, THIS JOURNAL, **77**, 2914 (1955).

(3) K. Hofmann, A. Jöhl, A. E. Furlenmeier and H. Kappeler, *ibid.*, **78**, 1636 (1956).

(4) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(5) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

(6) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

(7) J. I. Harris and P. Roos, *ibid.*, **178**, 90 (1956).

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(9) R. W. Holley and E. Sondheimer, *ibid.*, **76**, 1326 (1954).

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(11) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(12) J. F. Roland, Jr., and A. M. Gross, *Anal. Chem.*, **26**, 502 (1954).

p-bromoaniline developed single spots with R_f values identical to those observed with the ninhydrin technique. Quantitative amino acid analyses of an acid hydrolyzate of the peptide gave equimolar proportions of the expected amino acids. Digestion of corticotropin-A with pepsin, followed by trypsin, liberated from the hormone a tripeptide containing histidine, phenylalanine and arginine[13] in which histidine was shown to occupy the N-terminal position. A comparison (by paper chromatography) of this substance with our synthetic tripeptide demonstrated their identity.[14] Both peptides exhibited an R_f value of 0.42 in the Partridge system and travelled somewhat faster than methionine in 2-butanol–ammonia. This provides unequivocal synthetic confirmation for the sequence his-phe-arg in corticotropin-A. As far as we were able to ascertain, this tripeptide represents the first synthetic peptide containing both histidine and arginine. Exposure to hydrogen bromide in glacial acetic acid transformed carbobenzoxy-L-histidyl-L-phenylalanylnitro-L-arginine into the hydrobromide of L-histidyl-L-phenylalanylnitro-L-arginine which afforded the free tripeptide upon treatment with Amberlite IR-4B in the acetate form.

Since no synthetic peptides have been reported in the literature which contain glutamic acid linked through its α-carboxyl group to histidine, we turned to the preparation of peptides embodying this linkage. In view of the well-documented lability of the imidazole nucleus toward acyl chlorides and alkali,[15] the azide and mixed anhydride procedures were selected for introducing an α-glutaminyl or α-glutamyl residue, respectively, into two of the aforementioned peptides containing histidine. Carbobenzoxy-L-glutaminyl-L-histidyl-L-phenylalanine resulted in a 69% yield when an ethyl acetate solution containing carbobenzoxy-L-glutamine azide[16] was shaken with an aqueous solution of the triethylammonium salt of L-histidyl-L-phenylalanine. Similarly, the interaction of the azide of carbobenzoxy-L-glutamine with L-histidyl-L-phenylalanylnitro-L-arginine, under comparable experimental conditions, afforded (in a 61% yield) the expected carbobenzoxy-L-glutaminyl-L-histidyl-L-phenylalanylnitro-L-arginine. Using the Pauly reagent as the indicator, these acylated peptides were analyzed by paper chromatography, and both compounds produced a single spot: ninhydrin-positive materials were absent. In 1953, Sachs and Brand[17] prepared a series of peptides containing L-glutamic acid by the use of carbobenzoxy-γ-benzyl-L-glutamate. The mixed-anhydride method was employed to activate the α-carboxyl group, and amino acid benzyl esters served as coupling components. The coupling reactions were carried out in anhydrous solvents. We have now treated a mixed anhydride of carbobenzoxy-γ-benzyl-L-glutamate with the triethylammonium salt of L-histidyl-L-phenylalanylnitro-L-arginine and obtained carbobenzoxy-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanylnitro-L-arginine in a yield of 57%. Catalytic hydrogenation over a palladium catalyst in glacial acetic acid converted this compound into L-glutamyl-L-histidyl-L-phenylalanyl-L-arginine. Like some of the other more complex peptides reported in this paper, the tetrapeptide was obtained in the form of an amorphous powder which was purified by precipitation from its aqueous solution with ethanol. The material was reprecipitated until its paper chromatogram exhibited a single ninhydrin and Pauly positive spot. A sample of the compound was acid hydrolyzed, and the hydrolyzate subjected to quantitative amino acid analysis on paper. Equivalent quantities of the expected amino acids were found.

Of major importance was the behavior of two of the synthetic peptides toward leucine aminopeptidase.[18] Both histidyl-phenylalanyl-arginine and glutamyl-histidyl-phenylalanyl-arginine were separately incubated with this enzyme, and the ensuing digests analyzed for their amino acid composition by quantitative, paper chromatography. The hydrolyzates of both peptides contained the expected amino acids in equimolar ratios, demonstrating the stereochemical homogeneity of the two compounds.[14]

Experimental[19]

Carbobenzoxy-L-histidyl-L-phenylalanine Methyl Ester.—L-Phenylalanine methyl ester hydrochloride (500 mg.) was suspended in ether (8 ml.) and the suspension cooled in an ice-bath. Ice-cold 50% potassium carbonate (3 ml.) was added and the mixture was shaken with cooling. The ether layer was separated and dried over sodium sulfate at 0°. To this solution, containing methyl L-phenylalaninate, was added an ethyl acetate solution of carbobenzoxy-L-histidine azide (prepared from 606 mg. of the hydrazide[9]). The mixture was refrigerated for 12 hours, and the resulting precipitate was collected. Concentration of the mother liquors afforded additional quantities of crystals which were combined with the first crop. The substance was recrystallized from a mixture of methanol with ether; yield 0.7 g. (67%), m.p. 161–163°. A sample for analysis was dried over phosphorus pentoxide *in vacuo* at 100° for 12 hours.

Anal. Calcd. for $C_{24}H_{26}O_5N_4$: C, 64.0; H, 5.8; N, 12.4. Found: C, 64.6; H, 6.2; N, 12.8.

Carbobenzoxy-L-histidyl-L-phenylalanine. a. From the Methyl Ester.—Carbobenzoxy-L-histidyl-L-phenylalanine methyl ester (675 mg.) was dissolved in methanol (3 ml.) and 1 *N* sodium hydroxide (1.6 ml.) was added. The mixture was kept at room temperature for 30 minutes and was then neutralized by the addition of 1 *N* hydrochloric acid (1.6 ml.). The resulting crystals were collected, washed with water, and recrystallized from hot water. The compound was dried *in vacuo* over phosphorus pentoxide; yield 610 mg. (93%), m.p. 227–228°.

Anal. Calcd. for $C_{23}H_{24}O_5N_4$: C, 63.3; H, 5.5; N, 12.8. Found: C, 62.9; H, 5.9; N, 13.0.

b. From L-Phenylalanine.—L-Phenylalanine (1.98 g.) was dissolved in water (60 ml.) and triethylamine (1.84 ml.) was added. This solution was shaken at 5° (for 45 hours) with an ethyl acetate solution containing carbobenzoxy-L-histidine azide (prepared from 5.46 g. of the hydrazide[9]). Upon the addition of ice-cold 1 *N* sodium hydroxide, the heavy suspension divided into two clear layers which

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(14) We wish to express our appreciation to Dr. W. F. White of the Armour Laboratories for these determinations.

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(16) R. W. Holley and E. Sondheimer, THIS JOURNAL, **76**, 2816 (1954).

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(18) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955).

(19) The melting points are uncorrected. Optical rotations were determined in a Rudolph Precison Polarimeter, model 80, with model 200 photoelectric attachment.

were separated. The aqueous phase was washed with two portions of ethyl acetate, and was acidified with glacial acetic acid. The crystalline precipitate which resulted was collected, washed with ice-water, and dried *in vacuo* over phosphorus pentoxide; yield 3.0 g. (57%), m.p. 227–228°. The material was identical with the product resulting from the saponification of the methyl ester. A sample for analysis was recrystallized from water and dried *in vacuo* at 100°.

Anal. Calcd. for $C_{23}H_{24}O_5N_4$: C, 63.3; H, 5.5; N, 12.8. Found: C, 63.3; H, 5.4; N, 13.0.

L-Histidyl-L-phenylalanine.—Carbobenzoxy-L-histidyl-L-phenylalanine (1.6 g.) was hydrogenated in glacial acetic acid (20 ml.) over palladium for one hour. The catalyst was removed by filtration and the solution concentrated to a sirup *in vacuo* at a bath temperature of 40°. The dipeptide was precipitated by the addition of ethanol, and was recrystallized from 50% aqueous ethanol. The material was collected, washed with ethanol and ether, and dried *in vacuo;* yield 1.02 g. (92%), m.p. 255–258° dec., $[\alpha]^{27}D$ +33.8° (*c* 2.6 in 1 *N* HCl), R_f^{11} 0.50, R_f^{12} ileu$^-$.

Anal. Calcd. for $C_{15}H_{18}O_3N_4$: C, 59.6; H, 6.0; N, 18.5. Found: C, 59.9; H, 5.8; N, 18.7.

Carbobenzoxy-L-histidyl-L-phenylalanine Hydrazide.—Carbobenzoxy-L-histidyl-L-phenylalanine methyl ester (2.0 g.) was dissolved in hot methanol (20 ml.) and hydrazine hydrate (0.66 g.) was added. The mixture was kept at room temperature for 12 hours, the product was collected, dried over sulfuric acid *in vacuo*, and recrystallized from a mixture of dioxane and petroleum ether; yield 1.4 g. (70%), m.p. 201–202°.

Anal. Calcd. for $C_{23}H_{26}O_4N_6$: C, 61.3; H, 5.8; N, 18.7. Found: C, 60.9; H, 6.1; N, 18.3.

L-Phenylalanylnitro-L-arginine.—Carbobenzoxy-L-phenylalanylnitro-L-arginine[10] (5 g.) was dissolved in 2.5 *N* hydrogen bromide in glacial acetic acid (20 ml.), and the mixture was kept under nitrogen at 40° for 15 minutes and at room temperature for 45 minutes. The solvents were removed (bath temp., 40°), and the residue was precipitated by the addition of ether. The supernatant was decanted, and the gummy material washed by decantation with ether and dried *in vacuo* at room temperature over potassium hydroxide. The residue was then dissolved in water (40 ml.) and the solution was extracted with ethyl acetate. The ethyl acetate extract was discarded and Amberlite IR-4B (acetate form, 8 g.) was added to the aqueous phase. The suspension was shaken at room temperature for 30 minutes, when the supernatant was tested for bromide ions. Additional resin was added in small portions, with shaking, until the supernatant was bromide-free. The resin was removed by filtration, washed with water, and the combined filtrate and washings concentrated to a sirup *in vacuo* (bath temp., 50°). The dipeptide was precipitated by the addition of absolute ethanol. The material was collected, the mother liquors were concentrated to a small volume *in vacuo*, and additional material was precipitated by the addition of absolute ethanol. Both fractions were combined and dried *in vacuo* at room temperature; yield 3.2 g. (85%), m.p. 140-150° dec., $[\alpha]^{21}D$ +22.5° (*c* 8.9 in water), R_f^{11} 0.62, R_f^{12} Phe$^-$.

Anal. Calcd. for $C_{15}H_{22}O_5N_6(^1/_2H_2O)$: C, 48.0; H, 6.2; N, 22.4. Found: C, 48.1; H, 6.1; N, 22.6.

Carbobenzoxy-L-histidyl-L-phenylalanylnitro-L-arginine.—L-Phenylalanylnitro-L-arginine (11.0 g.) was dissolved in water (200 ml.) and triethylamine (4.6 ml.) was added. This solution was shaken for 24 hours at 5° with an ethyl acetate solution containing carbobenzoxy-L-histidine azide (prepared from 9.08 g. of the hydrazide[9]). An ethyl acetate solution containing additional azide (prepared from 4.5 g. of hydrazide) was added and shaking was continued for an additional 24 hours at 5°. Enough 1 *N* sodium hydroxide was added to the heavy emulsion to produce two clear layers, which were separated. The aqueous phase was re-extracted with two additional portions of ethyl acetate and was then acidified with glacial acetic acid to *p*H 4 to 5. The heavy precipitate was collected, washed with water, dissolved in 10% aqueous ammonia, and then reprecipitated with glacial acetic acid. The material was collected, washed with water, acetone and ether, and dried *in vacuo* over phosphorus pentoxide. For purification, the substance was again dissolved in hot 10% aqueous ammonia and precipitated in needles by addition of glacial acetic acid; yield 12.1 g. (63%), m.p. 222-223°, $[\alpha]^{26}D$ −13.8° (*c* 8.5 in glacial acetic acid), R_f^{12} Phe$^+$.

Anal. Calcd. for $C_{29}H_{35}O_8N_9$: C, 54.6; H, 5.5; N, 19.8. Found: C, 54.3; H, 5.1; N, 20.3.

L-Histidyl-L-phenylalanylnitro-L-arginine.—Carbobenzoxy-L-histidyl-L-phenylalanylnitro-L-arginine (2.5 g.) was dissolved in 2.5 *N* hydrogen bromide in glacial acetic acid (20 ml.) and the mixture was kept under nitrogen at 50° for 30 minutes and at room temperature for an additional 30 minutes. The solvents were removed *in vacuo* (bath temp. 30°) and the hydrobromide was precipitated by the addition of ether and washed by decantation with ether. The gummy mass was dried over potassium hydroxide *in vacuo* at room temperature and dissolved in water (20 ml.). The solution was extracted with three portions of ethyl acetate and Amberlite IR-4B (acetate form, 3 g.) was added to the aqueous phase. The suspension was shaken at room temperature for 30 minutes, when the supernatant was tested for bromide ions. Additional resin was added in small portions, with shaking, until the supernatant was bromide-free. The resin was removed by filtration, washed with water and the combined filtrate and washings evaporated to a sirup *in vacuo* (bath temp., 50°). The peptide was precipitated by the addition of ethanol, was collected, washed with ethanol, and dried at room temperature over phosphorus pentoxide; yield 1.6 g. (78%), m.p. 110° dec., $[\alpha]^{25}D$ +23.2° (*c* 1.9 in 1 *N* HCl), R_f^{11} 0.48; R_f^{12} ileu$^-$.

Anal. Calcd. for $C_{21}H_{29}O_6N_9(H_2O)$: C, 48.4; H, 6.0; N, 24.2; NH_2-N, 2.7. Found: C, 47.9; H, 6.0; N, 24.9; NH_2-N, 3.0.

L-Histidyl-L-phenylalanyl-L-arginine.—Carbobenzoxy-L-histidyl-L-phenylalanylnitro-L-arginine (400 mg.) was hydrogenated in glacial acetic acid (15 ml.) over a palladium catalyst for four hours. The catalyst was removed by filtration, the filtrate was evaporated to a sirup *in vacuo* (bath temp. 40°), and ether was added. The peptide acetate was collected and purified by precipitation from its solution in ethanol by ether. The material was dried *in vacuo* at 80° over phosphorus pentoxide; yield 290 mg. (89%), m.p., decomposes at 120–140°, $[\alpha]^{25}D$ +3.0° (*c* 2.8 in water), R_f^{11} 0.42, R_f^{12} Met$^+$.

Anal. Calcd. for $C_{23}H_{33}O_6N_8$: C, 53.4; H, 6.4; N, 21.6. Found: C, 53.6; H, 6.3; N, 21.5.

Carbobenzoxy-L-glutaminyl-L-histidyl-L-phenylalanine.—To a solution of L-histidyl-L-phenylalanine (604 mg.) in water (20 ml.) plus triethylamine (0.31 ml.) was added an ethyl acetate solution (approximately 50 ml.) of carbobenzoxy-L-glutamine azide (prepared from 936 mg. of the hydrazide[16]) and the mixture was shaken at 5° for 40 hours. Ice-cold 1 *N* sodium hydroxide (7 ml.) was then added to the emulsion and the resulting clear layers were separated. The aqueous layer was extracted with two additional portions of ethyl acetate, and was acidified to *p*H 4 to 5 with glacial acetic acid. The mixture was kept in the refrigerator for 12 hours, and the precipitate was collected, washed with water, and dried *in vacuo* at room temperature over phosphorus pentoxide; yield 830 mg. (69%), m.p. 208-210° dec., $[\alpha]^{24}D$ −31.0° (*c* 0.9 in 1 *N* HCl), R_f^{11} 0.90, R_f^{12} Phe$^+$. A sample for analysis was recrystallized twice from hot water.

Anal. Calcd. for $C_{28}H_{32}O_7N_6(2H_2O)$: C, 56.0; H, 6.0; N, 14.0. Found: C, 55.9; H, 5.6; N, 14.3.

Carbobenzoxy-L-glutaminyl-L-histidyl-L-phenylalanylnitro-L-arginine.—To a solution of L-histidyl-L-phenylalanylnitro-L-arginine (1.39 g.) in water (50 ml.) plus triethylamine (0.42 ml.) was added an ethyl acetate solution (approximately 150 ml.) containing carbobenzoxy-L-glutamine azide (prepared from 1.28 g. of the hydrazide[16]) and the mixture was shaken at 5° for eight hours; a heavy precipitate formed after some 40 minutes. Water (10 ml.) and ethyl acetate (20 ml.) were then added, and shaking was continued for 16 hours at 5°. Saturated, aqueous sodium bicarbonate solution was added to dissolve the precipitate, and the ethyl acetate layer was separated from the aqueous phase which was re-extracted with two additional portions of ethyl acetate. The water layer was now acidified with glacial acetic acid (to *p*H 4 to 5) and the resulting precipitate was collected, washed with water, and dried *in vacuo* at room temperature. For purification, the material was dissolved in 2.8% aqueous ammonia and reprecipitated by addition of glacial acetic acid. The final product was

washed with ice-water and dried *in vacuo* over phosphorus pentoxide at room temperature; yield 1.3 g. (61%), m.p. 170° dec., $[\alpha]^{29}D$ −4.7° (*c* 1.1 in glacial acetic acid), R_f^{11} 0.82, R_f^{12} Phe^+.

Anal. Calcd. for $C_{34}H_{43}O_{10}N_{11}(2H_2O)$: C, 50.9; H, 5.9; N, 19.2. Found: C, 51.2; H, 5.7; N, 19.0.

Carbobenzoxy-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanylnitro-L-arginine.—Carbobenzoxy-γ-benzyl-L-glutamate[20] (1.85 g.) was dissolved in dry dioxane (30 ml.), the solution was cooled until the dioxane partially solidified, when tri-*n*-butylamine (1.32 ml.) was added with stirring. Ethyl chloroformate (0.53 ml.) was then added and stirring was continued for 20 minutes. The mixture was completely frozen by cooling with an ice–salt-bath, and an ice-cold solution of L-histidyl-L-phenylalanylnitro-L-arginine (2.5 g.) in water (10 ml.) plus triethylamine (0.77 ml.) was gradually added with shaking and cooling. In order to maintain a clear solution, additional dioxane was added, and the mixture was stirred with cooling for 20 minutes and at room temperature for one hour. The solution was then acidified with glacial acetic acid (5 ml.) and evaporated to a small volume *in vacuo* (bath temp. 40–50°). The reaction product was precipitated by the addition of water, and the mixture was kept in the refrigerator for 12 hours. The product was collected, dried *in vacuo* over phosphorus pentoxide at room temperature, and purified by one precipitation from methanol with ether and by two precipitations from methanol with water. It was dried *in vacuo* over phosphorus pentoxide at 70° for 12 hours; yield 2.4 g. (57%), m.p. 185–187° dec., $[\alpha]^{29}D$ −2.4° (*c* 1.7 in glacial acetic acid), R_f^{11} 0.87, R_f^{12} Phe^+.

Anal. Calcd. for $C_{41}H_{48}O_{11}N_{10}(H_2O)$: C, 56.3; H, 5.8; N, 16.0. Found: C, 56.3; H, 5.5; N, 16.1.

L-Glutamyl-L-histidyl-L-phenylalanyl-L-arginine.—Carbobenzoxy-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-nitro-L-arginine (1.3 g.) was hydrogenolyzed over spongy palladium in glacial acetic acid (20 ml.) for 4.5 hours in a stream of hydrogen. Fresh catalyst was added at this point and the hydrogenation continued for an additional 4.5 hours. The catalyst was removed by filtration, the glacial acetic acid evaporated off (bath temp., 40–50°), and the product precipitated by addition of dry ethanol. The compound was dissolved in water (3 to 5 ml.), the solution filtered, concentrated to a volume of 1 ml. *in vacuo*, and absolute ethanol was added. The peptide was collected, washed with ethanol and dried *in vacuo* at room temperature; yield 810 mg. (93%), m.p. 200–205° dec., $[\alpha]^{26}D$ −5.0° (*c* 1.97 in water), R_f^{11} 0.32, R_f^{12} Arg^+. A sample for analysis was dried at 60° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{26}H_{37}O_7N_9$: C, 53.2; H, 6.3; N, 21.4; NH_2–N, 2.4. Found: C, 53.0; H, 6.8; N, 21.5; NH_2–N, 2.6.

Analytical Procedures.—The paper chromatograms were prepared by the descending technique on Whatman No. 1 paper. Histidine and histidine peptides were localized on the chromatograms by the Pauly reaction.[21] For quantitative determination of amino acids, samples of the various peptides and peptide derivatives (2–5 mg.) were hydrolyzed with double-distilled (from glass) 6 *N* hydrochloric acid, in sealed tubes for 20 hours at 110°. The hydrolyzates were evaporated to dryness *in vacuo* at room temperature over phosphorus pentoxide and potassium hydroxide pellets, and the residues were dissolved in water. Aliquots were used for amino acid determination according to the method of Fowder,[22] using the Moore and Stein ninhydrin reagent.[23]

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PITTSBURGH, PENNA.

[COMMUNICATION NO. 1853 FROM THE RESEARCH LABORATORIES, EASTMAN KODAK CO.]

The Reaction of Cystine and Lanthionine with Aqueous Calcium Hydroxide. The Identification of 2-Methylthiazolidine-2,4-dicarboxylic Acid

BY J. R. DANN, G. L. OLIVER AND J. W. GATES, JR.

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One of the products of the reaction between aqueous calcium hydroxide and cystine at room temperature has been identified as 2-methylthiazolidine-2,4-dicarboxylic acid, which was isolated as the diethyl ester. Proof of structure was carried out by comparison with synthetic 2-methylthiazolidine-2,4-dicarboxylic acid and by cleavage to a cysteine fragment and a pyruvic acid fragment. Lanthionine has also been found to form 2-methylthiazolidine-2,4-dicarboxylic acid in aqueous calcium hydroxide at room temperature.

Introduction

The instability of cystine in alkaline solutions is well known and has been studied and observed by many workers. Hoffman and Gortner[1] have surveyed the chemistry of cystine as known before 1922, and later work has included that of Andrews,[2a,b] Gortner and Sinclair,[3] Thor and Gortner,[4] Clarke and Inouye[5] and Lindstrom and Sandstrom.[6] Inorganic compounds including sulfides, sulfur, thiosulfate, sulfite, carbon dioxide and ammonia have been observed in the action of alkali upon cystine. Organic products which have been observed under various conditions are cysteine,[2a] alanine,[6] oxalic acid,[6] pyruvic acid,[5] uvitic acid,[6] uvitonic acid,[6] α-mercaptopropionic acid[6] and lanthionine.[7]

Discussion

In our laboratory, it had been observed that the action of aqueous calcium hydroxide upon L-cystine (I) at room temperature gave an acid II containing nitrogen and sulfur, which could be isolated in crude form by precipitating its calcium salt III in 80% ethanol. Attempts to purify this acid or to prepare derivatives of it for characterization had failed previously because of the great tendency of the crude acid or its salt to form intractable oils. This acid has now been identified as 2-methylthiazolidine-2,4-dicarboxylic acid (IV). This identification was made possible by the fact that the ethyl ester V of the unknown acid could be distilled

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