sium phthalimide[7b] showed no depression and the infrared spectra were identical.

Ethyl α-benzamido-γ-dibenzylaminobutyrate (XX). Ethyl α-benzamido-γ-iodobutyrate[6] (3.65 g., 0.01 mole) was dissolved in 200 ml. of ether and a solution of 4 g. (0.02 mole) of dibenzylamine in 50 ml. of ether was added with cooling. The mixture was left at room temperature for 4-5 days, and the precipitated dibenzylamine hydroiodide was filtered off. Further amounts of the salts were removed by repeated concentration of the solution, addition of ethyl acetate, and filtration. After final concentration, the semisolid residue was purified by washing with portions of water, then with petroleum ether until crystallization. The ester XX (2.8 g., 65%) melted at 80-82°.

Anal. Calcd. for $C_{27}H_{30}N_2O_3$: N, 6.5; OC_2H_5, 10.5. Found: N, 6.4; OC_2H_5, 10.8.

The same ester (XX) was obtained in an analogous reaction with ethyl α-benzamido-γ-bromobutyrate.[6]

α-Benzamido-γ-dibenzylaminobutyric acid (XXI). The ester XX (1.1 g., 0.025 mole) was dissolved in 50 ml. of methanol containing 25 ml. of 0.5% aqueous sodium hydroxide and the solution refluxed for 6 hr. The methanol was removed *in vacuo*, and the remaining cooled solution acidified with hydrochloric acid. The semisolid precipitate was recrystallized from benzene yielding 0.6 g. (60%) of the acid XXI, m.p. 147°.

Anal. Calcd. for $C_{25}H_{26}N_2O_3$: N, 7.0. Found: N, 6.8.

α-Amino-γ-dibenzylaminobutyric acid (XXII). The ester XX (1.1 g.) was refluxed in 50 ml. of 12% hydrochloric acid for 5 hr., during which time it dissolved completely. The solution was cooled, filtered from benzoic acid, washed with ether, and the solvent was evaporated *in vacuo*. The solid hygroscopic residue was dissolved in water, the solution made alkaline with aqueous sodium bicarbonate and the α-amino acid XXII (0.55 g., 73%), precipitated; recrystallized from water, m.p. 188-190° dec.

Anal. Calcd. for $C_{18}H_{22}N_2O_2$. C, 72.5; H, 7.4; N (Kjeldahl), 9.4; N (Van Slyke), 4.7. Found. C, 72.8; H, 7.6; N (Kjeldahl,) 9.2; N (Van Slyke), 4.6.

1-Benzyl-3-benzamidopyrrolidin-2-one (XXIII). The ester XX (2.15 g.) was dissolved in 100 ml. of ethanol, 3 g. of 5% palladium on charcoal was added and the mixture hydrogenated at 60-70° for 12 hr. (3 atm.). The catalyst was filtered off, the solvent evaporated and the oily residue crystallized from ethanol-water. Recrystallization from benzene-petroleum ether yielded 1.2 g. (80%) of the pyrrolidinone XXIII, m.p. 160-161°.

Anal. Calcd. for $C_{18}H_{18}N_2O_2$. C, 73.5; H, 6.1; N, 9.5. Found. C, 73.9; H, 6.4; N, 9.3.

α-Benzamido-γ-benzylamino-N-benzylbutyramide (XXIV). Ethyl α-benzamido-γ-iodobutyrate[6] (3.6 g., 0.01 mole) was dissolved in 50 ml. of benzene, benzylamine (10.7 g., 0.1 mole) was added and the solution was allowed to stand at room temperature for 1 week. Precipitated benzylamine hydroiodide was filtered off, and the filtrate evaporated *n vacuo*. The residue was recrystallized three times from ethanol-water to give the amide XXIV (2 g. 50%), m.p. 165°.

Anal. Calcd. for $C_{25}H_{27}N_3O_2$. C, 75.0; H, 6.7; N, 10.5. Found. C, 74.5; H, 6.8; N, 9.9.

The same amide (XXIV) was obtained in an analogous reaction with ethyl α-benzamido-γ-bromobutyrate.[6]

JERUSALEM, ISRAEL

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Crystalline Complexes of Amino Acids with Alkyl Titanates

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Glycine dissolves on heating in solutions of a titanate ester in an alcohol. A crystalline complex containing the amino acid and titanium ester separates from the cold solution. On further standing, the solution deposits crystals of diketopiperazine. Dipeptides also dissolve in the titanate ester-alcohol solution, and yield a substituted diketopiperazine corresponding to the dipeptide employed.

In preliminary studies of the yellowing of wool by ultraviolet light[2] it was observed that pretreatment of wool with an alkyl titanate, for example, tetra-*n*-butyl titanate, protects wool from yellowing. It was proved that protection was not due to screening of ultraviolet light from wool by the titanate. Other observations suggested that the titanates may react with proteins, peptides, and amino acids. In this investigation, reaction of titanates with amino acids is confirmed by preparation of crystalline complexes of several alkyl titanates with glycine, alanine, and phenylalanine. These complexes were found to lose titanate on standing at room temperature, with conversion of the amino acid moiety to cyclic anhydrides.

Several amino acids were found to react with alkyl titanates in the presence of alcohol. Glycine is typical of these. It is insoluble in butyl titanate but becomes soluble when ethyl alcohol is present. From a solution prepared by warming a mixture of glycine, butyl titanate, and ethyl alcohol, present in the molar ratios 1:2:4 (a glycine concentration of about 8%), crystals deposit on cooling to room temperature. The crystals contain both titanium esters and glycine and appear usually as rhomb-shaped plates. Similar crystals can be obtained from less concentrated solutions of glycine and by precipitation with hexane. Recrystallization is inconvenient because of hydrolysis from atmospheric moisture, which proceeds in a manner similar to that of the alkyl titanates. The crystals of complex are birefringent and exhibit well defined X-ray diffraction patterns differing from those of the constituents. Analysis of the complex indicates an equimolar ratio of glycine and titan-

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(1) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) H. P. Lundgren, *Proc. Intern. Wool Textile Research Conf., Melbourne*, 1955C, pp. 374-395.

TABLE I

RATIOS OF TITANIUM TO AMINO ACID IN REACTANTS AND IN COMPLEX

Ratios of Reactants				Ratios in Product		
Moles	Amino acid	Moles	Alkyl titanate	Amino acid, mol.	Titanium, mol.	Alkoxy groups, mol.
1.0	Glycine	3.7	Butyl	0.98	1.00	
1.0	Glycine	2.0	Butyl	0.97	1.00	
1.0	Glycine	4.2	Isopropyl	1.07	1.00	
1.0	Glycine	2.4	2-Ethylhexyl	0.98	1.00	
1.0	Alanine	2.0	Butyl	1.00	1.00	
1.0	Phenylalanine	2.0	Butyl	1.00	1.00	
1.0	Glycine	2.3	Butyl	1.03	1.00	2.79
1.0	Glycine	1.5	Butyl	0.97	1.00	2.98

ium. Decomposition of the oven-dried complex by hydrolysis with water liberates the amino acid from the titanate. Similarly, the amino acid is liberated by solution of complex in hydrochloric acid and precipitation of titanium as hydroxide on addition of ammonium hydroxide or other base.

The ability of alkyl titanates to undergo alcoholysis[3] and to catalyze ester interchange suggests that mixed esters—for example, ethyl butyl titanate—might be required for complex formation. This requirement is eliminated, however, in the case of glycine, by complex formation with butyl titanate in butyl alcohol.

The choice of alkyl titanate is important. Methyl titanate is a relatively insoluble solid. Ethyl, propyl, isopropyl and butyl titanates complex readily at room temperature; of these, isopropyl is least satisfactory because of rapid hydrolysis. Exchange of alkyl groups with added alcohol takes place rapidly with isopropyl titanate. Exchange also occurs with 2-ethylhexyl titanate and methyl alcohol although the other reactions of this titanate decrease in activity, as anticipated, with increase in molecular weight. Stearyl titanate is a waxy solid and its mixture in ethyl alcohol is a poor solvent for amino acids. For complex formation butyl titanate was usually chosen, but for complex formation followed by additional reaction 2-ethylhexyl titanate was used because it frequently gave a greater yield.

The choice of alcohol is of less importance. Absolute methyl alcohol offers a lower heating temperature and is difficult to handle because of the ease of hydrolysis of the corresponding titanate. Isopropyl alcohol appears to be the only alcohol that can be used with isopropyl titanate without interchange of titanate. Of the various butyl alcohols, tertiary is poorest in forming complexes. Isobutyl alcohol with isopropyl titanate forms tetraisobutyl titanate which crystallizes at room temperature. Dodecyl alcohol is effective as a solvent. Such variations as 2-methoxyethanol, trichloroethanol, and cyclohexanol appear to offer no special advantage. Polyhydroxy compounds tend to form insoluble precipitates, probably with considerable cross-linking.

Other amino acids which exhibit good solubility with butyl titanate in ethyl alcohol include alanine, leucine, phenylalanine, tyrosine, cysteine, methionine, and proline. Those which show moderate solubility include histidine dihydrochloride, glutamic acid, and serine. Cystine shows little or no solubility. Arginine and lysine hydrochlorides are only slightly soluble; addition of sodium hydroxide to the latter increases solubility but the solution is unstable and continually deposits lysine and titanate. Similarly to solutions of glycine, complexes crystallize from solutions of alanine, phenylalanine, and leucine.

Table I shows the complex to consist of an equimolar ratio of titanium to amino acid, and shows this ratio to be independent of the mixing proportions or the alkyl group in the titanate. In each example a crystalline product formed; it was separated, the amino acid recovered by hydrolysis with water, and the insoluble residue ashed. The complex prepared from butyl titanate and glycine was similarly analyzed with an accompanying determination of alkyl groups (as alcohol following hydrolysis), and the ratios of alcohol, glycine, and titanium were found to be 3:1:1. The complex is probably formed by interchange and liberation of one alkyl group from the titanate, the added alcohol assisting by a solvent effect.

Peptides found to dissolve in titanate–ethyl alcohol include glycylglycine, glycylalanine, glycylvaline, triglycine, and glutathione. Glycylglycine dissolves with heating or slowly with stirring at room temperature. Triglycine is only about half as soluble as glycine, and tetraglycine gives no indication of solution, complexing, or alteration.

The special case of the interaction of glutathione with titanate was studied in detail, and the properties of the resulting substance have been described.[2] The peptide dissolved readily in titanate–ethyl alcohol, but the complex was so stable that hy-

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[3] F. Bischoff and H. Adkins, *J. Am. Chem. Soc.*, 46, 257 (1924).

driodic acid was required to separate the titanium. The product was titrated in acid to show a new inflection near *p*H 5.2, and alkaline titration in formaldehyde indicated the absence of an amino group. Ninhydrin test for an *alpha* amino acid was negative. However, amino nitrogen by the Van Slyke method was similar to glutathione. Nitroprusside test was positive after sodium cyanide reduction but became negative after acid hydrolysis. Paper chromatography of the hydrolyzate showed spots corresponding to glutamic acid, glycine, and cysteic acid. It is assumed that titanate treatment modified the amino group, making it unavailable except under the conditions of the Van Slyke reaction.

Solutions of glycine in titanate-alcohol, on standing several weeks at room temperature, deposit small quantities of crystalline glycine anhydride (2,5-piperazinedione), identified by its melting point, refractive indices, elementary analyses, and X-ray diffraction pattern. Normally there is no other product except complex. Butyl titanate in dodecyl alcohol and 2-ethylhexyl titanate in isobutyl alcohol produce greater yields, the latter about 45%.

Other titanate-free material, in small amounts, has resulted from addition of sodium glycinate or glycine hydrochloride to glycine complex and evaporation of a solution of glycine complex. The material is biuret-positive but fractionation does not yield a linear peptide. One product appears to be a cyclic anhydride of more than two amino acid residues.

EXPERIMENTAL

All melting points are uncorrected. The chemicals used were commercially available materials. The amino acids were the optically active L-isomers, except the following, which were optically inactive: glycine, alanine, leucine, phenylalanine, methionine, serine, and lysine.

Preparation of glycine complex. A mixture of 1.00 g. of glycine (0.0133 mole) and 9.05 g. of tetra-*n*-butyl titanate (0.0266 mole) in 41 ml. of absolute ethyl alcohol was heated to boiling on a steam bath with constant stirring in an open flask (with forced draft to prevent introduction of moisture) until the glycine completely dissolved (about 30 min.). The flask was closed immediately with a stopper provided with a capillary for introduction of air. After standing 4 days at room temperature the crystals were centrifuged, washed twice with 30-ml. portions of commercial hexane, and air dried; yield, 1.69 g. of partially hydrolyzed product. The determination of titanium in this and the following preparations was based on the method outlined by Treadwell and Hall,[4] who state "When titanium is unaccompanied by other cations in this group, it is best to precipitate it with ammonia. The precipitate of titanium hydroxide or hydrated titanium dioxide is not dissolved in an excess of ammonia and is easily converted to titanium dioxide by ignition." The partially hydrolyzed product was therefore hydrolyzed to completion by grinding in an agate mortar and heating to constant weight in an oven at 105°. This heated material (230.6 mg.) was stirred for 1 hr. with 35 ml. of water, centrifuged, and again extracted with concd. ammonium hydroxide. The insoluble residue was ignited and weighed as titanium dioxide; yield, 120.3 mg. The combined extracts were dried and weighed; yield, 110.3 mg. This product was identified as glycine by m.p. (240° dec.) and m.p. of a mixture with an authentic sample (238°, dec.). Phthaloylglycine, prepared by fusing the product with phthalic anhydride, melted at 192°; an authentic sample and mixture melted at 192°.

Quantitative composition of glycine complex. Two different preparations of glycine complex were analyzed for glycine, titanium, and alkoxy groups, the latter determined as alcohol following hydrolysis. In the first of these (a) the complex was derived from butyl titanate and butyl alcohol, and therefore contained only butoxy groups; this alcohol was determined by oxidation. In the second (b) the complex was derived from butyl titanate and ethyl alcohol, and theoretically could contain either alkoxy or both; this was determined by quantitative gas chromatography. The ratios found are reported in Table I.

(a) A mixture of 4.25 g. of glycine (0.0566 mole) and 45 g. of butyl titanate (0.132 mole) in 24 ml. of butyl alcohol was heated 13 min. to dissolve the bulk of the glycine. The undissolved glycine was separated by centrifuging hot and resuspending the upper layer of crystals that formed during the centrifuging. This mixture was heated briefly to give a clear solution, and allowed to stand overnight. The crystalline product was centrifuged, washed twice with 70-ml. portions of hexane, and dried in a vacuum; yield, 5.13 g. For analysis 294 mg. was added to 125 ml. of water, the mixture distilled, and 100 ml. of distillate collected. Aliquots of the distillate were oxidized with dichromate[5] and the yield of butyl alcohol calculated to be 179.8 mg. (2.43 mmoles). The undistilled residue was separated into glycine and titanium dioxide; yield of glycine 67.0 mg. (0.892 mmole), yield of titanium dioxide, 69.5 mg. (0.870 mmole).

(b) A mixture of 2.00 g. of glycine (0.0267 mole) and 13.6 g. of butyl titanate (0.0400 mole) in 11 ml. of absolute ethyl alcohol was heated 15 min. to effect complete solution. On standing overnight the solution crystallized to solidification. Fifteen milliliters of commercial hexane were stirred in, the mixture centrifuged, and the supernatant discarded. The hexane washing was continued with three portions of 35 ml. The bulk of the precipitate was transferred to a distilling flask with 15 ml. of hexane and dried by distillation under reduced pressure. The dried crystals were hydrolyzed, and the resulting alcohols separated, by steam distillation. The refractive index of the distillate was determined and a portion taken for quantitative gas chromatography. The chromatographic analysis was confirmed by duplicating the mixture and the refractive index; yield of butyl alcohol 0.84 g. (11 mmoles), yield of ethyl alcohol 1.40 g. (30 mmoles). The undistilled residue was separated into glycine and titanium dioxide; yield of glycine 1.02 g. (14 millimoles), yield of titanium dioxide 1.12 g. (14 mmoles).

Vapor pressure and cryoscopic measurements on solutions of glycine complex. Because of the instability of the complex, highly variable results were obtained; consequently the values do not correlate with the minimal molecular weight as determined by composition studies. To avoid interaction and possible exchange, the solvent used to determine molecular weight should be that used in the preparation of the complex, and the complex should have no unrelated alkoxy groups. Attempts to prepare such a complex from glycine and *t*-butyl alcohol, glycine and isopropyl alcohol, and glycine and cyclohexanol gave products of questionable crystallinity and low solubility. Vapor pressure measurements on tributoxyglycyl-titanium complex in butyl alcohol were invariably higher than that indicated by the minimal molecular weight (341) established by the composition studies. Solutions of this complex in 2-methoxyethanol

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(4) F. P. Treadwell and W. T. Hall, *Analytical Chemistry*, Vol. II, Wiley, New York, 1942, p. 158.

(5) L. M. Christensen and E. I. Fullmer, *Anal. Chem.*, **7**, 180 (1935).

are not representative of a normal complex since they do not show typical hydrolysis on addition of water. Two separate molecular weight determinations on tributoxyglycyl-titanium complex, by cryoscopic measurements on 1.2% solutions in cyclohexanol, gave values of 163 and 161, approximately half the minimal molecular weight.

Preparation of alanine complex. A mixture of 1.00 g. of alanine (0.0112 mole) and 7.66 g. of butyl titanate (0.0225 mole) in 92 ml. of absolute ethyl alcohol was heated 10 min. as for glycine complex. The solution was allowed to stand overnight at room temperature, 24 hr. at −12°, and overnight at room temperature. The crystals were washed with hexane and air dried; yield, 1.08 g. of partially hydrolyzed product. A portion was separated into its constituents as in the comparable glycine preparation. Treatment of 143.8 mg. of heated material yielded 67.7 mg. of titanium dioxide and 75.4 mg. of alanine. The alanine was identified by conversion to phthaloylalanine, m.p. 162.5–163°. Phthaloylalanine prepared from an authentic specimen of DL-alanine melted at 162.5–163°. A mixture of the two products also melted at 162.5–163°. Gabriel[6] reported the m.p. of phthaloylalanine to be 160–162°.

Preparation of phenylalanine complex. A mixture of 2.00 g. of phenylalanine (0.0121 mole) and 8.16 g. of butyl titanate (0.0240 mole) in 22 ml. of absolute ethyl alcohol was heated 23 min. in a centrifuge bottle. A small amount of material remained undissolved. To separate this, the centrifuge bottle was capped, centrifuged hot, and the supernatant gently stirred to carry with it the light, loose crystals that separated during the centrifuging. After decanting this supernatant, the loose crystals redissolved on heating, giving a clear solution. Crystallization occurred at room temperature; the yield was augmented by storage at −12° overnight. The preparation was thereafter treated like the glycine complex; yield, 2.42 g. of partially hydrolyzed product. Treatment of 253.1 mg. of heated material yielded 81.1 mg. of titanium dioxide and 168.1 mg. of phenylalanine. The phenylalanine was identified by conversion to phthaloylphenylalanine, m.p. 174–175°. Phthaloylphenylalanine prepared from an authentic sample of DL-phenylalanine melted at 174–175°. A mixture of the two products also melted at 174–175°. Billman and Harting[7] reported a m.p. of 174–175° for phthaloyl-DL-phenylalanine.

Preparation of complexes of other amino acids. No general procedure was established for the demonstration of complex formation except analysis of a crystalline product showing molar proportions of amino acid and titanium. However, solubility of the amino acid in titanate-alcohol mixtures appeared to be a reliable index to the binding of titanate, and some pattern for the use of this solvent mixture evolved. A molar proportion of titanate per mole of amino acid constituted a poor solvent regardless of added alcohol. Two moles of titanate were adequate for hot concentrated solution with 4 moles of alcohol. For maximum solubility of a given weight of amino acid, with a fixed amount of titanate, the amount of alcohol passes through an optimum; thus it appears that excess titanate and alcohol form a solvent determining solubility of the product. This solvent was considered to be in excess when crystallization was not spontaneous from two moles of titanate, and the excess solvent was decreased by repetition with smaller amounts of titanate and alcohol. Attempts were made to show that water-soluble amino acids became water-insoluble after titanate-alcohol treatment. No evidence was obtained that a bond survived after water hydrolysis, whether the hydrolysis took place before or after separation from the original solvent mixture. Leucine and phenylalanine preparations derived from mixtures containing titanate in excess but insufficient for complete solubilization showed incomplete conversion. Some solutions developed yellow or red discolorations on long standing. Although cystine is insoluble, a solution of cysteine gave no precipitation on standing for 6 months. Various commercial preparations of butyl titanate showed distinct differences in their complexing characteristics. Mixtures of methyl alcohol and butyl titanate, usually stable, deposited methyl titanate on heating with glycine. Phthaloylglycine dissolved very readily. Copper glycinate did not dissolve or alter.

Peptide complexes. Glycylalanine dissolved to make a 1% solution in a 1:3 (vol.) mixture of butyl titanate-ethyl alcohol. Glycylglycylglycine treated in the same manner as glycylalanine gave a 0.5% solution. Reduced glutathione (γ-glutamylcysteinylglycine) treated in the same manner as glycylalanine gave a 1% solution. Contrary to the behavior of the amino acid complexes, the solutions of glycylglycylglycine and glutathione gave products still bound to titanium after addition of water.

Treatment of tetraglycine. Tetraglycine was prepared from glycylglycylglycine and chloroacetyl chloride by the method of Fischer.[8] A mixture of 0.35 g. tetraglycine and 35 ml. of a 1:3 (vol.) mixture of butyl titanate–ethyl alcohol was heated 30 min. with no obvious dissolving. The mixture was allowed to stand at room temperature 24 days and the residue then centrifuged, washed with hexane, dried, and weighed; yield, 0.32 g. of tetraglycine (containing a small amount of ash). A mixture of 0.30 g. of this preparation, 4.5 ml. butyl titanate, and 1.55 ml. of ethyl alcohol was heated 20 min. with no obvious dissolving. Five milliliters of ethyl alcohol was added and the mixture reheated for 10 min., again with no obvious dissolving. After standing 7 days the residue was centrifuged, washed with hexane, dried, and weighed; yield, 0.30 g. of tetraglycine.

Conversion of glycine complex to glycine anhydride. A mixture of 0.50 g. of glycine and 12.5 ml. of 2-ethylhexyl titanate in 37.5 ml. of isobutyl alcohol was heated on a steam bath as indicated for glycine complex. After 30 min. a clear solution resulted. Precipitation began in 24 hr. at room temperature and was allowed to continue for 2 weeks. The precipitate was centrifuged, washed with ethyl alcohol, hexane, and dried, yield 0.17 g. The product was identified as glycine anhydride by negative ninhydrin and biuret tests, a strongly positive diketopiperazine test,[9] and m.p. (317°). A similar experiment substituting 12.5 ml. of butyl titanate and 37.5 ml. of dodecyl alcohol yielded 0.07 g. of glycine anhydride, identified as above and including a mixed melting point with an authentic sample of glycine anhydride.

Conversion of glycylglycine to glycine anhydride. A mixture of 1.00 g. of glycylglycine and 25 ml. of butyl titanate in 75 ml. of ethyl alcohol was heated on a steam bath until the glycylglycine dissolved. The solution was allowed to stand 1 week at room temperature. A precipitate formed, and was separated by centrifuging, washing with hexane, and drying; yield, 0.56 g. The product contained a little ash, gave a negative ninhydrin test and decomposed at 312–318°. It was crystallized twice from hot water with a similar treatment of glycine anhydride for comparison. The losses for both substances for each crystallization were comparable (0.12–0.13 g.). Final yield of 2X crystalline unknown was 0.21 g. The known and unknown gave identical melting points and mixed melting point. They were compared by microscopic examination of their optical characteristics and found to be identical.

Anal. Calcd. for $C_4H_6N_2O_2$: C, 42.1; H, 5.31; N, 24.6. Found: C, 42.2; H, 5.33; N, 24.7.

Conversion of glycylglycine to glycine anhydride at room temperature. A mixture of 1.00 g. of glycylglycine and 25 ml. of butyl titanate in 75 ml. of ethyl alcohol was placed in a flask closed with a stopper provided with a capillary opening. The mixture was stirred constantly for 5 days using

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(6) S. Gabriel, *Ber.*, **38**, 634 (1905).

(7) J. H. Billman and W. F. Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948).

(8) E. Fischer, *Ber.*, **37**, 2486 (1904).

(9) E. Brand and M. Sandberg, *J. Biol. Chem.*, **70**, 381 (1926).

magnetic stirring with an air space between the flask and driving unit; the temperature fluctuated slightly above room temperature, and at the finish was 26.5°. During the stirring the preparation was observed to enter a period of solution followed by a period of precipitation. The crystalline precipitate was centrifuged, washed with hexane, and dried; yield, 0.14 g. The preparation was extracted with water at room temperature, the extracts combined, and recrystallization effected by refrigeration, yield, 0.07 g. This was identified as glycine anhydride by microscopic examination of its optical characteristics.

Conversion of glycylvaline complex to glycylvaline anhydride. A mixture of 0.50 g. of glycylvaline and 12.5 ml. of butyl titanate in 37.5 ml. of ethyl alcohol was heated until the glycylvaline dissolved (a few minutes). The solution remained clear for several weeks at room temperature and was unchanged by overnight storage at $-12°$. Thereafter the solution gradually deposited long slender needles. The crop was permitted to grow for a month and was then centrifuged. An attempt to wash the precipitate with ethyl alcohol, to avoid possible precipitation of complex with hexane, caused the precipitate to dissolve. The precipitate was recovered by adding water to hydrolyze residual butyl titanate, drying, and extracting the residue with ethyl alcohol. The ethyl alcohol extract was dried and extracted with water to clarify it, and the water extract dried; yield, 0.12 g. The product contained no ash, gave negative ninhydrin and biuret tests, a positive diketopiperazine test, and melted at 240–242° with sublimation and slight decomposition (reported[10] m.p. of glycylvaline anhydride, 245°). Glycylvaline melted with decomposition at 228–233°; m.p. of the mixture was 228–240° with partial decomposition and some sublimation.

Anal. Calcd. for $C_7H_{12}N_2O_2$: C, 53.8; H, 7.75; N, 17.9. Found: C, 53.9; H, 7.57; N, 17.8.

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(10) P. Bergell, *Hoppe-Seyler's Z. physiol. Chem.*, **97**, 300 (1916).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Preparation of Benzyl and *p*-Nitrobenzyl Esters of Amino Acids[1]

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The use of an azeotropic procedure to prepare the benzyl and *p*-nitrobenzyl esters of several amino acids is described Included are the *p*-nitrobenzyl esters of N^ϵ-tosyl-L-lysine, N^ϵ-(*p*-nitrobenzyloxycarbonyl)-L-lysine and L-histidine, compounds of difficult accessibility utilizing previously published methods.

The synthesis of peptides frequently involves the coupling of an *N*-carbobenzoxyamino acid or peptide with an amino acid or peptide ester. When methyl or ethyl esters are used, a saponification step is required to uncover the carboxyl group of the coupled product.[3] It is often convenient and sometimes mandatory to avoid this saponification step. In such cases the benzyl ester has been used to cover the carboxyl group.[4] The benzyl ester can be removed concomitantly with the carbobenzoxy group by hydrogenolysis[4] or with less facility by the use of hydrogen bromide in acetic acid.[5] Improved methods of preparation have made the benzyl esters of amino acids more generally available for peptide synthesis.[6] Recently Maclaren, Savige and Swan[6f] employed an azeotropic distillation method to prepare *S*-benzyl-L-cysteine benzyl ester. Carbon tetrachloride was used to form the azeotrope, and *p*-toluenesulfonic acid was used to catalyze the esterification. We are reporting our experience in the preparation of several benzyl esters of amino acids in which a similar procedure was used with the exception that benzenesulfonic acid[6a] instead of *p*-toluenesulfonic acid was used as the catalyst. The condensed azeotrope was passed through a bed of silica gel or anhydrous calcium sulfate to remove the water before being returned to the pot. The use of commercial preparations of

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[3] For a review of the subject see: J. S. Fruton, *Advances in Protein Chem.*, **5**, 1 (1949); M. Goodman and G. W. Kenner, *Advances in Protein Chem.*, **12**, 466 (1957).

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