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Peptide Synthesis *via* Amino Acid Active Esters. II.[1] Some Abnormal Reactions during Peptide Synthesis

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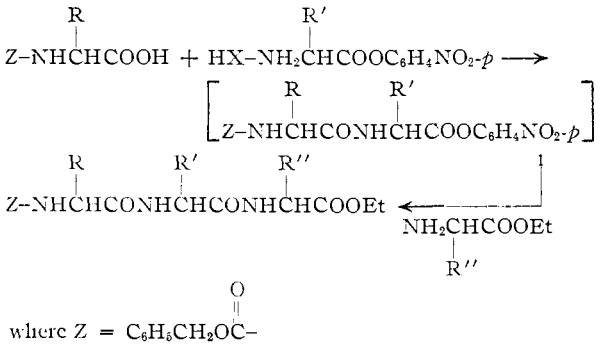
In the course of our work on peptide synthesis *via* amino acid active esters, some interesting side reactions were noted. When benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester was aminolyzed or hydrolyzed, benzyloxycarbonylglycyl-L-prolyldiketopiperazine was obtained as a side product. Appreciable yields of the dipeptide benzyloxycarbonyldiglycine ethyl ester formed as a side product during the synthesis of benzyloxycarbonyltriglycine ethyl ester. Another diketopiperazine derivative, 1-(N-benzyloxycarbonylglycyl)-2,5-dibenzyl-3,6-dioxopiperazine, was isolated in a small yield from the alkaline hydrolysis of benzyloxycarbonylglycyl-L-phenylalanine-*p*-nitrophenyl ester. Possible mechanisms for these side reactions are discussed. Two polymorphic crystalline modifications have been noted for benzyloxycarbonylglycyl-L-phenylalanine-*p*-nitrophenyl ester, each differing in extent and type of hydrogen bonding. Infrared spectral correlations are presented.

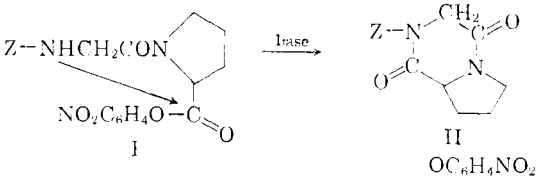
The search for improved methods of peptide synthesis is actively continuing.[4a-h] The newer approaches have involved refinements in the blocking and coupling techniques.[5-8] Unfortunately the synthesis of complicated peptides remains a laborious process. We have developed an approach which involves amino acid active esters as key intermediates.[1] With this method, a tripeptide can be synthesized directly from three components at the amino acid level, thus eliminating manipulations at the intermediate dipeptide level:



While examining the scope of this technique (see previous paper[1]) several unexpected reactions were discovered. In this paper we present our findings about these unexpected reactions. These side reactions are not necessarily inherent in this new method but may occur in other syntheses involving activated peptide derivatives as well.

In contrast to the satisfactory yields generally encountered when using amino acid active esters,[1] the preparation of benzyloxycarbonyl-L-glycyl-prolylglycine ethyl ester[9] afforded only small amounts of product. During the first phase of this synthesis in which benzyloxycarbonylglycine was coupled with proline *p*-nitrophenyl ester an intense yellow color (due to *p*-nitrophenoxide ion) developed. The expected dipeptide intermediate benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester (I) could be isolated in only 22% yield.

Two possible side reactions which would produce *p*-nitrophenol as a by-product were considered as being responsible for the poor yield. The first of these involved the dimerization of proline *p*-nitrophenyl ester to give its diketopiperazine. Although proline derivatives have a strong tendency to form such compounds,[10-12] the mode of addition of reactants as well as the extremely rapid acylation by the blocked amino acid made this appear unlikely. An alternate possibility involved a base-catalyzed intramolecular displacement on the ester carbonyl by the nitrogen of the glycyl residue.



Support for the latter hypothesis came unexpectedly from a separate study of the racemization-hydrolysis of this dipeptide ester I.[13] In this work, benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester (I) was subjected to mild alkaline hydrolysis in an aqueous dioxane medium and both the rates of *p*-nitrophenol formation and change in optical rotation were observed. The kinetic data thus obtained indicated a rapid liberation of *p*-nitrophenol followed by still another slower reaction. By stopping the reaction after liberation of the phenol was complete, and isolating the neutral fraction, it was possible to obtain the intermediate benzyloxycarbonylglycyl-L-prolyldiketopiperazine

(1) Paper I, M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).

(2) Union Carbide Plastics Co., Bound Brook, N. J.

(3) Submitted by K. C. Stueben in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Polytechnic Institute of Brooklyn.

(4) (a) M. Goodman and G. W. Kenner, *Adv. in Protein Chem.*, **12**, 465 (1957); (b) P. Edman, *Ann. Rev. Biochem.*, **28**, 69 (1959); (c) *Annual Reports*, **56**, 304 (1959); (d) R. Schwyzer, *Ann. Rev. Biochem.*, **29**, 183 (1960); (e) T. Wieland, *Angew. Chem.*, **71**, 417 (1959); (f) A. H. Cook and G. Harris, "Progress in Organic Chemistry," Butterworths, London, Vol. IV, 1958, p. 140; (g) H. Tuppy, *Naturwiss.*, **46**, 35 (1959); (h) Third European Peptide Symposium, Basel, 1960; *Chimia*, **14**, 366 (1960).

(5) F. Weygand and W. Steglich, *Chem. Ber.*, **92**, 313 (1959).

(6) G. C. Stelakatos, D. M. Theodoropoulos and L. Zervas, *J. Am. Chem. Soc.*, **81**, 2884 (1959).

(7) G. W. Anderson and R. Paul, *ibid.*, **80**, 4423 (1958).

(8) F. Michael and H. Hunke, *Chem. Ber.*, **92**, 309 (1959).

(9) Isolated and identified by hydrolysis to the carboxylic acid.

(10) J. Kapfhammer and K. Matthes, *Z. physiol. Chem.*, **223**, 43 (1934).

(11) E. L. Smith and M. Bergmann, *J. Biol. Chem.*, **153**, 627 (1944).

(12) E. Abderhalden and H. Nienburg, *Fermentforsch.*, **13**, 573 (1933).

(13) A study of the base-catalyzed racemization of amino acid and peptide derivatives will be published elsewhere.

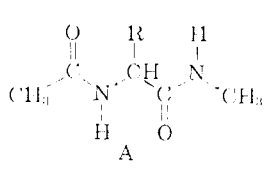
(II) in pure form. The structure of this compound was established by chemical analysis, molecular weight, infrared data and, finally, conversion to a known compound. Hydrogenolysis converted the intermediate to the known glycyl-L-prolinediketopiperazine in satisfactory yield.

Subsequent experiments showed that benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester was partly converted to the substituted diketopiperazine (II) simply by treatment with catalytic amounts of triethylamine. In view of these observations, it would appear advisable to exercise caution if it is necessary to use similarly constructed proline derivatives in peptide synthesis.

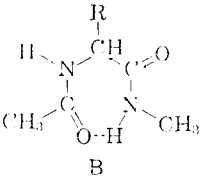
The ease with which this cyclization takes place can be ascribed to the fact that the rigid proline ring holds the reacting groups in close proximity. Substituting another amino acid in place of proline lessens the possibility for ring closure as evidenced by the fact that benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester can be prepared in good yield by the same procedure.[1]

The compound, benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester, has been found to exist in two polymorphic forms. In the course of crystallizing a large sample of this dipeptide active ester (III) from a mixture of ethyl acetate and petroleum ether, a low melting fraction, m.p. 108.5–109.8° (IIIa), was isolated. The expected product has a melting point of 146°. As can be seen from Table I, the infrared spectrum of this low melting material was completely different from that of the high melting form III. Further recrystallizations from ethyl acetate caused compound IIIa to yield appreciable quantities of III, m.p. 144–145°, indicating a close relationship between these two substances. Confirmation that these materials were in fact identical in all other respects was established by elemental analysis and identical solution spectra in chloroform (Table I).

The existence of several different hydrogen bonded forms of peptide-like compounds in solution has been demonstrated by Mizushima and co-workers.[14-15] Using infrared techniques to study the N–H stretching portion of the spectra of a series of acetyl amino acid derivatives, these workers were able to establish the presence of both the extended (A) and folded (B) forms in chloroform solution. In the solvents used (chloroform



A



B

and carbon tetrachloride) the bonded N–H absorption was in the 3330–3360 cm.$^{-1}$ region while the free N–H was found at 3420–3460 cm.$^{-1}$. With this background in mind the N–H stretching and carbonyl regions of the infrared were considered for the two polymorphic forms of Z-gly-phe-*p*$NO_2C_6H_4$. These data are shown in Table I. In addition, all spectra have peaks at 1760–1780 cm.$^{-1}$ for the carbonyl of the nitrophenyl ester.

TABLE I

INFRARED STRETCHING FREQUENCIES FOR POLYMORPHIC BENZYLOXYCARBONYL-GLYCYL-L-PHENYLALANINE *p*-NITROPHENYL ESTER

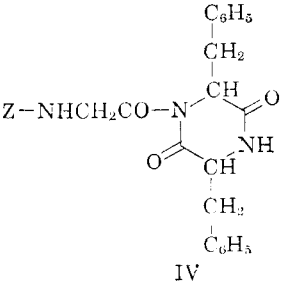
Cpd.	Medium	Free N–H	Bonded N–H	Carbonyl region		
III	Nujol	.....	3330(m-s)	....	1685(s)	1664(s)
IIIa	Nujol	3430(w)	3310(m)	1725(s)	1692(s)	1660(s)
III and	$CHCl_3$[a]	3430(m)	3330(m)	1725(s)	1692(s)	....
IIIa	Dioxane	.....	3320(m)	1728(s)	1692(s)	....

[a] 4.6%. [b] 15% solution.

The high melting substance III has no free N–H and in contrast to IIIa shows only the two lower frequency carbonyl bands. It would appear therefore that III is a completely hydrogen bonded form. The lower melting modification, on the other hand, possesses both free and bonded N–H and carbonyl peaks. The optical activities of compounds III and IIIa are identical[1] [$[\alpha]^{23}D$ −12.3° (*c* 2, ethanol)].

The carbonyl oxygen of the nitrophenyl ester is probably too electron poor to participate in any effective hydrogen bonding. Thus, we suggest that the presence of the 1725 cm.$^{-1}$ absorption band must be due to the non-bonded benzyloxycarbonyl group. (Additional work must be carried out before any further conclusions can be drawn as to the structures involved here.)

Although no evidence has been found for intramolecular cyclization with benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (III), this compound does undergo other unexpected reactions[10] on treatment with base. Upon removal of the dioxane, used as co-solvent, in the alkaline hydrolysis of this active ester a trace amount of solid separated. This neutral fragment (IV) constituted about 3% by weight of the starting ester III and was devoid of nitro groups. Its infrared spectrum indicated the presence of N–H and four different carbonyl groups. Treatment of this material with a solution of hydrogen bromide in acetic acid liberated a gas and a water-soluble hydrobromide showing that a benzyloxycarbonyl group was present in the molecule. Acid hydrolysis of IV followed by paper chromatography of the 2,4-dinitrofluorobenzene derivatives indicated the presence of two phenylalanine residues per glycine. These data, taken together with elemental analysis and molecular weight, were consistent with the structure



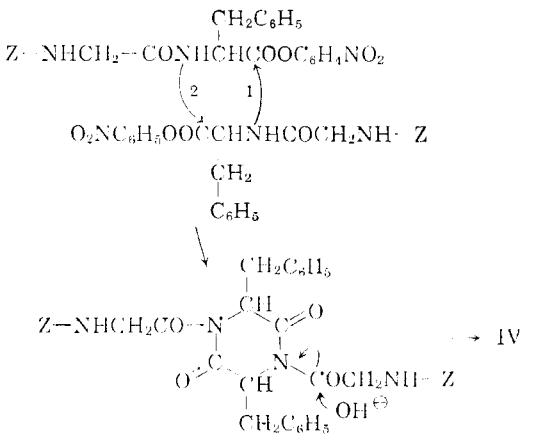
IV

(14) M. Tsuboi, T. Shimanouchi and S. Mizushima, *J. Am. Chem. Soc.*, **81**, 1406 (1959).

(15) S. Mizushima, T. Shimanouchi, M. Tsuboi and T. Arakawa, *ibid.*, **79**, 5357 (1957).

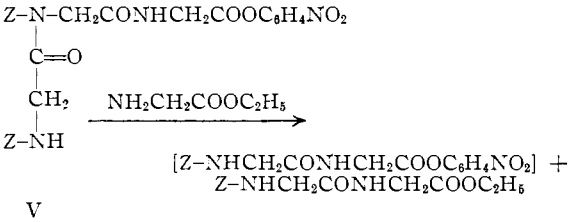
Initially, it was thought that this compound may have been present in the starting ester as an impurity. Its formation might be explained by reaction of the benzyloxycarbonylglycine–carbodiimide adduct with phenylalanine diketopiperazine (formed from phenylalanine *p*-nitrophenyl ester). Several attempts to bring about this very reaction were unsuccessful, however, apparently because of the extreme insolubility of the diketopiperazine. A more remote possibility was that some L-phenylalanyl-L-phenylalanine *p*-nitrophenyl ester formed during the preparation of the starting ester and was subsequently acylated by benzyloxycarbonylglycine. Hydrolysis of the resultant tripeptide might then be accompanied by some intramolecular displacement to give compound IV.

Evidence that the substituted diketopiperazine IV was a true side product rather than an impurity was obtained by examining samples of ester prepared by entirely different routes. Separate samples of benzyloxycarbonylglycylphenylalanine isolated from the hydrolysis above and from reaction of benzyloxycarbonylglycine acid chloride with phenylalanine were converted to the *p*-nitrophenyl ester by reaction with tris-(*p*-nitrophenyl) phosphite.[16] Both of these esters give rise to comparable amounts of the unknown on hydrolysis. A route which would explain the formation of IV as a side product of the hydrolysis involves an intermolecular attack of the amide nitrogen on the active ester followed by ring closure and partial cleavage by base



Still another side reaction was encountered during the synthesis of benzyloxycarbonylglycyldiglycine ethyl ester *via* the amino acid active ester technique. Using a mixed anhydride to effect the first coupling, an over-all yield of 31% of the tripeptide was obtained. The experimental details of this reaction are contained in a previous paper.[1] At first it was suspected that the active ester had dimerized or even polymerized, a result which might not be unexpected since the lower steric requirements of the glycine residue permit far more rapid acylations to occur.[17] Such an interpretation would not appear to be reasonable, however, since the analogous preparation of benzyloxycarbonyl-L-leucylglycyl-L-leucine methyl ester proceeded in 76% yield.[1] In this latter case the initial coupling should take place more slowly thus increasing the opportunities for diketopiperazine formation. A possible explanation to this problem was provided in a paper by Kopple and Renick.[18] These workers found that when benzyloxycarbonylglycine was coupled with glycine ethyl ester *via* the mixed anhydride a large portion of the product was the N-acylamide resulting from acylation on the benzyloxycarbonyl-blocked nitrogen of the product.

No such side reaction was reported for carbodiimide couplings and so this reagent was used with anticipation of an improved yield but resulted instead in an even poorer yield (17%) of the desired product. Most interesting of all was the fact that a 22% yield of the dipeptide benzyloxycarbonylglycylglycine ethyl ester also could be isolated. It is not likely that this compound resulted from a reaction between the third component (glycine ethyl ester) and unreacted acid carbodiimide adduct since the latter rearranges to an acyl-urea on standing.[19,21] Such acylureas have been shown to be unreactive as acylating agents.[20] A more plausible explanation for the formation of the diglycine peptide derivative involves the N-acylamide (V) as the intermediate which then reacts with glycine ethyl ester as



In the Experimental section of this paper we describe the isolation of the benzyloxycarbonyl glycylglycine ethyl ester.

Experimental[22]

Benzyloxycarbonylamino Acids.—The benzyloxycarbonyl amino acids were prepared by the method of Bergmann and Zervas,[23] with precautions to prevent the formation of complexes.[24]

Attempted Preparation of Benzyloxycarbonylglycyl-L-prolylglycine Ethyl Ester and its Hydrolysis.—To a magnetically stirred solution of benzyloxycarbonyl-glycine (0.63 g., 0.0030 mole) in 25 ml. of acetonitrile was added L-proline *p*-nitrophenyl ester hydrobromide (0.95 g., 0.0030 mole) which was prepared previously,[1] followed by 0.42 ml. of triethylamine at ice-bath temperatures. To this was added N,N'-dicyclohexylcarbodiimide (0.65 g., 0.0031 mole). The reaction was allowed to proceed for 1 hour during which the reaction solution became very yellow, showing that *p*-nitrophenoxide was liberated. Following an additional reaction period of 4 hours at room temperature a drop of glacial acetic acid was added to stop the reaction. The co-product dicyclohexylurea was removed by filtration and

(16) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

(17) R. Schwyzer, M. Feuer and B. Iselin, *ibid.*, **38**, 83 (1955).

(18) K. D. Kopple and R. J. Renick, *J. Org. Chem.*, **23**, 1565 (1958).

(19) H. G. Khorana, *Chem. Revs.*, **53**, 145 (1953).

(20) H. G. Khorana, *Chemistry & Industry*, 1087 (1955).

(21) H. G. Khorana, *J. Chem. Soc.*, 2081 (1952).

(22) All melting points are corrected. Analyses were carried out by Schwarzkopf Laboratories, Woodside, Long Island, N. Y.

(23) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(24) M. Goodman and K. C. Stueben, *J. Org. Chem.*, **24**, 112 (1959); W. Grassmann and E. Wünsch, *Ber.*, **91**, 462 (1958); E. P. Grommers and J. F. Arens, *Rec. trav. chim.*, **78**, 558 (1959).

glycine ethyl ester hydrochloride (0.42 g., 0.0030 mole), together with triethylamine (0.50 ml., 0.0033 mole), was added. The reaction was allowed to proceed for 18 hours at room temperature. After extraction with dilute acid, water and dilute base, the crude benzyloxycarbonylglycyl-L-prolylglycine ethyl ester was obtained as an oil (1.2 g., 71%). All attempts to crystallize this material failed, as was reported by Rydon and Smith.[25]

The tripeptide ester (0.50 g., 0.0013 mole) was hydrolyzed in 5 ml. of methanol using two portions of *N* sodium hydroxide (0.7 ml.). After a suitable length of time, water (50 ml.) was added and unsaponifiable material extracted with ethyl acetate. The aqueous layer was acidified, extracted with ethyl acetate which was dried and removed under reduced pressure, yielding the desired product, benzyloxycarbonylglylcyl-L-prolylglycine, as a yellow oil. This was crystallized from ethyl acetate–petroleum ether to yield 0.25 g., m.p. 135–138° (reported[26] 144–145°). Extensive attempts to crystallize the unsaponifiable product failed. The nature of this product is probably best explained by the cyclization reaction described for benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester.

Benzyloxycarbonylglycyl-L-proline.—The procedure of Bergmann, *et al.*,[27] was followed with certain modifications. A solution of benzyloxycarbonylglycine chloride was prepared by allowing benzyloxycarbonylglycine (9.2 g., 0.044 mole) to react with phosphorus pentachloride (10.1 g., 0.048 mole) in 200 ml. of anhydrous ether at 0°. After 1 hour, the ethereal solution was washed with ice-water and then dried with anhydrous magnesium sulfate in the cold. This solution was placed in a dropping funnel equipped with a cooling jacket. The solution was added dropwise with stirring to a solution of L-proline (5.00 g., 0.0435 mole) in 25 ml. of 2 *N* sodium hydroxide over a 20-minute period. The separate dropwise addition of 20 ml. of 2 *N* sodium hydroxide kept the *p*H between 8 and 10 during the reaction. After an additional 15 minutes stirring at ice temperatures, the reaction mixture was acidified and extracted with ethyl acetate three times. Evaporation of the dried extract gave 11 g. of an oil which solidified on triturating with a small amount of ether. Filtration yielded 6.2 g. of crystals, m.p. 120–130°. This material was recrystallized from hot water once and then from ethyl acetate to give 2.0 g. (15%), m.p. 152–154.5°. Further crystallizations were carried out until a constant melting point, 155.5–157°, and rotation, $[\alpha]^{21}D$ −77.5° (*c* 2, $CHCl_3$), were obtained (lit.[27] m.p. 156°, no rotation is given).

Benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester (I) was prepared by the same procedure used for the preparation of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester.[1] The oil which was obtained was crystallized with difficulty from ethyl acetate–petroleum ether to give a 22% yield, m.p. 100–102°. Further recrystallizations from ethanol gave a good recovery of product, m.p. 104–105°, $[\alpha]^{21}D$ −102.8° (*c* 2.6, EtOAc).

Anal. Calcd. for $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83. Found: C, 58.72; H, 4.83; N, 10.17.

This product was also obtained from the carboxylic acid *via* reaction with tris (*p*-nitrophenylphosphite)[16]; yield 84%.

Isolation of Intermediate II in the Hydrolysis of Benzyloxycarbonylglycyl-L-proline *p*-Nitrophenyl Ester.—A solution of benzyloxycarbonylglycyl-L-proline *p*-nitrophenyl ester (0.500 g., 0.00117 mole) in 0.3 molar buffer[28] (Veronal, *p*H 8 containing 64% dioxane) was prepared at 25°. The *p*H was maintained to ±0.03 unit by use of a *p*H meter and microburet containing 8 *M* sodium hydroxide. After 20 minutes the reaction was stopped by addition of 2 *N* hydrochloric acid and dilution with chloroform. Repeated extraction of the chloroform solution with 5% bicarbonate was then carried out until the yellow color no longer persisted. After drying, the solvent was removed *in vacuo* and the solid residue crystallized from ethyl acetate–petroleum ether to yield beautiful needles, 0.21 g., m.p. 110–111.5°, $[\alpha]^{25}D$ −109.5 ± 0.3° (*c* 1, EtOAc) of 4-(benzyloxycarbonyl) - L - 3,6 - dioxo - 1,2 - pyrrolidinopiperazine. The infrared spectrum showed bands at 1720(s) cm.$^{-1}$ for urethan carbonyl and 1660(s) cm.$^{-1}$ for amide carbonyl.

Anal. Calcd. for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.6; N, 9.7; mol. wt., 288.3. Found: C, 62.61; H, 5.7; N, 9.88; mol. wt., 282 (isothermal distillation).

Hydrogenolysis of Intermediate from Hydrolysis of Benzyloxycarbonylglycyl-L-proline *p*-Nitrophenyl Ester.—A solution of the intermediate (0.05 g., 0.173 mmole) in 5 ml. of ethanol containing 2 drops of 2 *N* hydrochloric acid was hydrogenated at atmospheric pressure in the presence of 30% palladium-on-charcoal for 3.5 hours. The mixture was diluted with ethanol and the catalyst filtered off. The filtrate was concentrated to a few ml. *in vacuo* whereupon 5 mg. of crystalline glycyl-L-proline anhydride, m.p. 213.5–215.5°, separated out. Addition of petroleum ether to the filtrate gave an additional 13 mg., m.p. 212–213°, making the total yield 68%. The combined crops had an optical rotation of $[\alpha]^{28}D$ −185° (±1) (*c* 0.5, H_2O)[29] (lit.[30] m.p. 213°, $[\alpha]^{20}D$ −217° (*c* 7.4, H_2O)).

Preparation of Benzyloxycarbonylglycyl-L-phenylalanine *p*-Nitrophenyl Ester (III).—The preparation of this ester was carried out as described previously.[1] Following the recrystallization of a large batch (27 g.) of III from ethyl acetate it was found that the melting point was much broader than usual (125–143°). The second crop (4.7 g.), obtained by adding petroleum ether, had m.p. 110–139°. Subsequent recrystallizations of this second crop ultimately yielded material (0.4 g.) which melted cleanly at 108.5–109.8° (IIIa). The infrared spectrum of this material in the solid state was markedly different from that of the expected product. However, as a 4.6% solution in chloroform, this substance gave a spectrum which was superposable with the solution spectrum of an authentic sample of III melting at 143–144.5°.

Furthermore, the 0.4 g. of compound melting at 108.5–109.8° (when crystallized once again from ethyl acetate) gave rise to a large percentage of material (0.26 g.) having m.p. 144–145°. Addition of petroleum ether yielded the low melting crop, m.p. 110.5–112°, which had the same analysis as compound III.

Anal. Calcd. for $C_{25}H_{23}N_3O_7$: C, 62.88; H, 4.85. Found: C, 63.29; H, 5.13.

Isolation of 1-(N-Benzyloxycarbonylglycyl)-2,5-dibenzyl-3,6-dioxopiperazine (IV) from the Hydrolysis of Benzyloxycarbonylglycyl-L-phenylalanine *p*-Nitrophenyl Ester.—A sample of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (3.57 g., 0.00750 mole) was dissolved in 145 ml. of dioxane and treated with 150 ml. of 0.1 *M* sodium hydroxide (15 mmoles) at room temperature. After 45 minutes the clear solution was concentrated to half volume under reduced pressure and the *p*H adjusted to between 8 and 9 with additional base. The solid which precipitated out during the concentration was then removed by filtration to give 0.121 g. of material, m.p. 102–104°. By fractional crystallization from ethyl acetate–petroleum ether, four fractions of similar melting point range (*ca.* 110–116°) were obtained. The central fractions were combined and recrystallized from ethyl acetate to yield the unknown, 32 mg., m.p. 175–176°.

Anal. Calcd. for $C_{28}H_{27}N_3O_5$: C, 69.26; H, 5.61; N, 8.65; mol. wt., 485. Found: C, 69.40; H, 5.61; N, 8.75; mol. wt., 485 (Rast).

The infrared spectrum has an NH stretching band at 3310-(ms) and an NH deformation band at 1535(s). It has carbonyl stretching bands at 1720(s), 1695(s) and 1655(s) cm.$^{-1}$. Treatment of this compound with saturated hydrogen bromide in acetic acid resulted in the evolution of a gas and an ether-insoluble hydrobromide, m.p. 253° (Kofler).

Quantitative Paper Chromatography on Hydrolysis of the Product from the Hydrolysis of Benzyloxycarbonylglycyl-L-phenylalanine *p*-Nitrophenyl Ester.—A sample of 0.90 mg. of the above solid and 0.5 ml. of concentrated hydrochloric acid was placed in a sealed tube and heated at 100–

(25) H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 3642 (1956).

(26) N. C. Davis and E. L. Smith, *J. Biol. Chem.*, **200**, 373 (1953).

(27) M. Bergmann, L. Zervas, H. Schleich and F. Leinert, *Z. physiol. Chem.*, **212**, 72 (1932).

(28) The details for the preparation of this buffer will appear elsewhere.

(29) The lower rotation of the isolated diketopiperazine as compared to that reported may be due to concentration effects or the presence of some racemate in the benzyloxycarbonyl derivative. Diketopiperazines racemize readily in alkali; *cf.* A. Newburger "Advances in Protein Chemistry," Vol. 4, Academic Press, Inc., New York, N. Y., 1948, p. 339.

(30) E. Fischer and G. Reif, *Ann.*, **363**, 118 (1908).

110° for 24 hours. After cooling, the contents of the tube were rinsed into a flask with distilled water and evaporated to dryness *in vacuo*. The contents were redissolved in water and evaporated a second time. The residue was then taken up in a small amount of water and treated with an excess of dinitrofluorobenzene reagent (prepared by mixing a solution of 0.4 g. of sodium bicarbonate in 5 ml. of water with a solution of 0.4 g. of dinitrofluorobenzene in 10 ml. of ethanol[31] for 2.5 hours with vigorous stirring). After removing most of the ethanol under reduced pressure, the solution was diluted with 5% bicarbonate solution and extracted repeatedly with ether to remove excess reagent. Acidification of the aqueous layer followed by ether extraction yielded an ether solution of the dinitrophenylene derivatives which, after concentration, was used to spot the paper for the chromatographic separation. The paper was eluted for 15 hours with a solvent system of toluene, chloroethanol, pyridine and 0.8 *M* ammonia (5:3:1.5:3).[31] Only two spots were evident; the faster one was the larger. The paper was dried and the spots cut out and extracted separately with 4.0 ml. of water. After heating in a 55–60° water-bath for 15 minutes after a fivefold dilution, the optical density at 360 mμ was determined. The solution from the fastest spot (phenylalanine) had an optical density of 0.407 while the other (glycine) had 0.172, corresponding to a glycine:phenylalanine ratio of 1:2.36.

Attempts to Synthesize 1-(N-Benzyloxycarbonylglycyl)-2,5-dibenzyl-3,6-dioxopiperazine.—A number of unsuccessful attempts was made to monoacylate L-phenylalanine anhydride ("L-diketopiperazine") with benzyloxycarbonylglycine. It is felt that many of these reactions failed because of the insolubility of the diketopiperazine. The following combinations and conditions were tried: (1) Z-glyOH + "L-diketopiperazine" + carbodiimide in acetonitrile at room temperature; (2) above reactants in dimethyl sulfoxide at 45–50° for 2 hours; (3) Z-glyOH + "L-diketopiperazine" + tetraethyl pyrophosphite in dioxane at reflux, 3 hours; the dioxane was then replaced with higher boiling chlorobenzene (b.p. 132°) and refluxed one additional hour; (4) Z-gly acid chloride + product of "L-diketopiperazine" with one part of sodium hydride; ether–benzene solvent at 0° for 0.5 hour, then room temperature for 3 hours; (5) Z-gly acid chloride + "L-diketopiperazine" in pyridine at 0° for 0.5 hour, then room temperature for 5 hours.

Preparation of Benzyloxycarbonylglycylglycine Ethyl Ester *via* the Hypothetical Intermediate Diacylamide.—Using the procedure previously described for the preparation of tripeptide derivatives,[1] benzyloxycarbonylglycine (0.63 g., 0.0030 mole), glycine *p*-nitrophenyl ester hydrobromide (0.83 g., 0.0030 mole) and glycine ethyl ester hydrochloride (0.42 g., 0.0030 mole) (added after 16 hours) were allowed to react. The reaction mixture was worked up in the usual way.[1] Evaporation of the neutral extract gave a residue which was fractionally crystallized from aqueous ethanol and ethyl acetate–petroleum ether (b.p. 30–60°) solvent combinations. The initial crops consisted of 0.16 g. of high and broad range melting materials. After these, a crop of 0.18 g. (17%) of benzyloxycarbonylglycyl diglycine ethyl ester was obtained, m.p. 143–149°. Finally, crops amounting to 0.19 g. (22%), m.p. 64–77°, were obtained. Recrystallization of these final crops gave product, m.p. 77–79° (lit.[32] m.p. 82.5 or 86–87°), of benzyloxycarbonylglycylglycine ethyl ester. Mixed melting point determinations, microchemical analysis and the infrared spectrum confirmed the structure of this material.

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(31) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(32) O. Süs, *Ann.*, **572**, 105 (1951), reports 82.5–83° while G. W. Anderson and R. W. Young, *J. Am. Chem. Soc.*, **74**, 5307 (1952), give 86–87°.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y., AND THE ROCKEFELLER INSTITUTE, NEW YORK, N. Y.]

Conformational Aspects of Polypeptides. III.[1] Synthesis of Oligomeric Peptides Derived from γ-Methyl Glutamate

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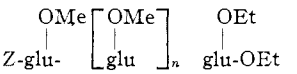
The syntheses of optically pure oligomeric peptides derived from γ-methyl glutamate containing between two and eleven residues are described. The evidence to date relating the physical properties of the oligomeric peptides to conformational structure is presented.

Introduction

In order to study the critical range for intramolecular hydrogen bonding in polypeptides,[1,4] it was necessary to synthesize oligomeric peptides using the most applicable methods available for coupling amino acid and peptide derivatives. Each peptide derivative was prepared in a high degree of chemical and optical purity since conformations of low molecular weight peptides described in this and earlier papers[1,4] are probably sensitive to small irregularities of the configuration of amino acids, and size of the peptide chain. In this paper we wish to describe the synthesis and techniques employed for the preparation of a homologous series of oligomeric peptides derived from γ-methyl L-glutamate using unequivocal methods. A general representation[5] is



where *n* is an integer between zero and nine inclusively.

Peptides derived from glutamic acid were prepared because of the fact that many correlations between conformational structure and physical

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(2) Submitted by Edward E. Schmitt to the faculty of the Polytechnic Institute of Brooklyn, 1961, in partial fulfillment of the requirements for the Ph.D. degree.

(3) The Rockefeller Institute, New York, N. Y.

(4) M. Goodman and E. E. Schmitt, *J. Am. Chem. Soc.*, **81**, 5507 (1959).

(5) The abbreviations used follow a modified Brand–Edsall scheme [*cf.* E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947)]. Z refers to the benzyloxycarbonyl group. The symbols above the "glu" term denote the methyl or ethyl esters of the γ-position [*cf.* M. Goodman and G. W. Kenner, *Adv. in Protein Chem.*, **12**, 465 (1957)].