equiv., 132. Found: sapon. equiv., 67.4; neut. equiv., 130.5.

Summary

β-Propiolactone reacts with thionyl chloride or phosphorus pentachloride to give β-chloropropionyl chloride. With halogen acids it gives β-halogenopropionic acids. Acetyl chloride, acetic anhydride and acetic acid react with β-propiolactone under acid catalyzed conditions to give β-acetoxypropionyl chloride, β-acetoxypropionic anhydride and β-acetoxypropionic acid, respectively.

BRECKSVILLE, OHIO RECEIVED MAY 31, 1949

[CONTRIBUTION FROM LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

Pteroic Acid Derivatives. VI. Unequivocal Syntheses of Some Isomeric Glutamic Acid Peptides

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Previous communications in this series[1,2,3] have described the synthesis of all of the possible isomeric pteroyldiglutamic and pteroyltriglutamic acids. During the search for satisfactory methods of preparing the intermediates, *i. e.*, the various *p*-nitrobenzoylglutamic acid peptides, the use of *l*-2-pyrrolidone-5-carboxylic acid and its derivatives was investigated. It was soon noted that attempts to esterify 2-pyrrolidone-5-carboxylic acid using ethanol and hydrogen chloride resulted in cleavage of the pyrrolidone ring at the lactam linkage with the formation of diethyl glutamate. In the same manner *l*-2-pyrrolidone-5-carboxamide (I) when treated with ethanolic hydrogen chloride gave γ-carbethoxy-α-aminobutyramide (ethyl isoglutaminate) isolated as its hydrochloride (II). However, substantial amounts of ammonium chloride were also obtained due to the simultaneous alcoholysis of the primary amide group. A series of experiments demonstrated that the best yield (30%) was obtained when 1.3 moles of dry hydrogen chloride was used for each mole of 2-pyrrolidone-5-carboxamide. The ethyl isoglutaminate was *p*-nitrobenzoylated and the product converted to the corresponding hydrazide (IV) and azide (V) by standard methods. The reaction of *p*-nitrobenzoylisoglutamine azide (V), which was a crystalline solid, with each of the compounds ethyl isoglutaminate, diethyl glutamate and triethyl-γ-glutamylglutamate[4] produced, respectively, ethyl *p*-nitrobenzoylisoglutaminylisoglutaminate (VI), diethyl *p*-nitrobenzoylisoglutaminylglutamate (VII) and triethyl *p*-nitrobenzoylisoglutaminyl-γ-glutamylglutamate (VIII). A sample of VII was hydrolyzed in dilute sodium hydroxide to give a compound which was shown to be identical with the *p*-nitrobenzoyl-γ-glutamylglutamic acid prepared previously by other methods.[1,4]

The ease with which the pyrrolidone ring in I could be opened indicated that this method might also be employed for the synthesis of α-glutamyl derivatives. Such a synthesis would be feasible only if the presence of a substituent on the amide nitrogen of I would stabilize that linkage so as to permit the opening of the pyrrolidone ring without the simultaneous alcoholysis of the amide group. This proved to be the case. Ethyl *l*-2-pyrrolidone-5-carboxylate was converted to the corresponding hydrazide (IX) and azide (X) in the usual manner. The reaction of the azide with diethyl glutamate produced diethyl α-(2-pyrrolidone-5-carboxamido)-glutarate (XI). XI was then treated with ethanolic hydrogen chloride to give triethyl α-glutamylglutamate (XII) which without isolation was *p*-nitrobenzoylated to produce triethyl *p*-nitrobenzoyl-α-glutamylglutamate (XIII). The over-all yield of pure, recrystallized XIII from XI was 42%. This yield, in addition to the fact that the product (XIII) was quite pure, indicated that the substituted amide linkage in XI was quite stable under the conditions used.

A sample of *p*-nitrobenzoyl-α-glutamylglutamic acid prepared previously by another method[5] was then esterified. The resulting ester was shown by mixed melting point and optical rotation to be identical with the product (XIII) prepared by the method described above.

The series of reactions was further extended by treating the crude triethyl α-glutamylglutamate (XII) with 2-pyrrolidone-5-carboxylic acid azide (X) to obtain ethyl γ-(2-pyrrolidone-5-carboxamido) - N - (1,3 - dicarbethoxypropyl) - glutaramate (XIV). XIV was treated with ethanolic hydrogen chloride to give tetraethyl α-glutamyl-α-glutamylglutamate which was not isolated but was *p*-nitrobenzoylated to give tetraethyl *p*-nitrobenzoyl-α-glutamyl-α-glutamylglutamate (XV). XV was shown to be identical with the tetraethyl *p*-nitrobenzoyl - α - glutamyl - α - glutamylglutamate prepared previously by another method.[3]

The syntheses of the glutamic acid peptides described herein were carried out by unequivocal methods. Three of these compounds, XIII, XV

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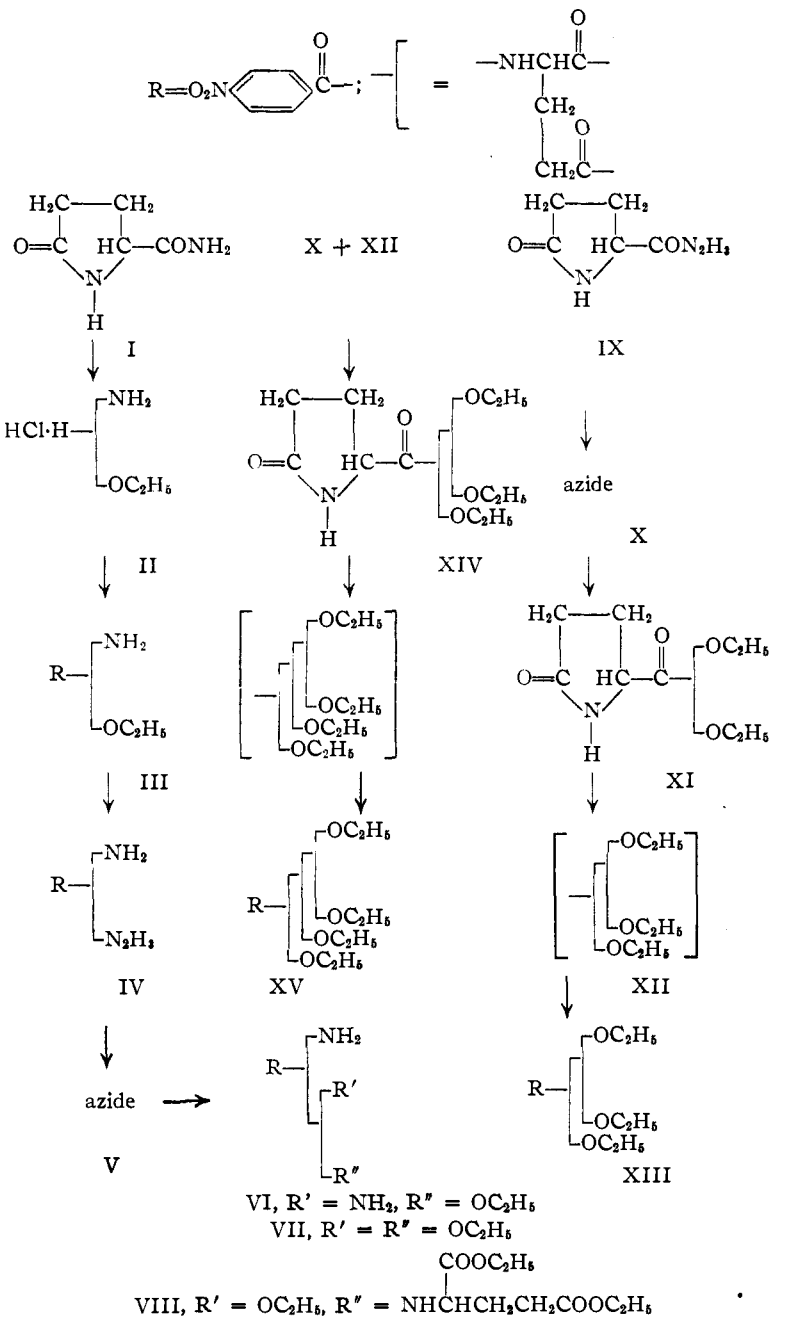
(1) Boothe, *et al.*, THIS JOURNAL, 71, 2304 (1949).
(2) Mowat, *et al.*, *ibid.*, 71, 2308 (1949).
(3) Semb, *et al.*, *ibid.*, 71, 2310 (1949).
(4) Boothe, *et al.*, *ibid.*, 70, 1099 (1948).
(5) Mowat, *et al.*, *ibid.*, 70, 1096 (1948).



and the free acid prepared from VII, have been shown to be identical with the corresponding peptides prepared previously by other methods. This constitutes final proof that the structures assigned to all the various *p*-nitrobenzoylglutamic acid peptides in papers I to V of this series were correct.

Experimental[6]

Action of Ethanolic Hydrogen Chloride on *l*-2-Pyrrolidone-5-carboxylic Acid.—A mixture of 10.0 g. of 2-pyrrolidone-5-carboxylic acid and 50 cc. of ethanol saturated with hydrogen chloride was refluxed over a steam-bath for one hour. It was then concentrated under vacuum to a sirup-like mass, 50 cc. of benzene was added and the solvent was again distilled off under vacuum. This material solidified after standing overnight and was then crystallized once from xylene and twice from an acetone–ether solution; yield 6.0 g., m. p. 113–114°. Elementary analyses and the optical rotation of this purified compound proved it to be diethyl glutamate hydrochloride; $[\alpha]_D$ +22.4° (*c* 4 in water), literature value $[\alpha]_D$ + 22.8°.[7]

Anal. Calcd. for $C_9H_{17}NO_4$·HCl: C, 45.10; H, 7.57; N, 5.85; Cl, 14.79. Found: C, 45.14; H, 8.03; N, 6.07; Cl, 15.01.

***l*-2-Pyrrolidone-5-carboxamide (I).**—*l*-2-Pyrrolidone-5-carboxamide has been prepared by the action of liquid ammonia on ethyl 2-pyrrolidone-5-carboxylate[8] and also by the action of alcoholic ammonia on diethyl glutamate.[9] However, in the preparation of large quantities of I the use of aqueous ammonia, as described below, is preferred.

Diethyl glutamate[10] (238.0 g.) was dissolved in 290 cc. of concentrated ammonium hydroxide (28% ammonia) and allowed to react at room temperature for five hours. It was then cooled in the refrigerator for one or two days. (Seeding or scratching the side of the vessel may be necessary to ensure crystallization.) The product was then collected, washed with ethanol and ether and dried for eight hours in an oven at 90° in order to remove water of crystallization; yield 112.0 g. (75%); m. p. 166–168°; $[\alpha]_D$ −42.25° (*c* 2 in water).

γ-Carbethoxy-α-aminobutyramide Hydrochloride (Ethyl Isoglutaminate Hydrochloride) (II).—One hundred grams of I was suspended in 675 cc. of U. S. P. absolute ethanol containing 37.5 g. (1.3 mole equivalents) of dry hydrogen chloride and the mixture refluxed for thirty to forty minutes. This was filtered hot to remove the precipitated ammonium chloride and then cooled well, filtered, the crystalline product washed with ethanol and ether and dried; yield 50.0 g. (30%), m. p. 190–193°. The material contains a small amount of ammonium chloride but is satisfactory for preparative purposes. A sample was recrystallized twice from ethanol; m.p. 197–198°; $[\alpha]^{26}_D$ +21.2° (*c* 2 in water).

Anal. Calcd. for $C_7H_{14}N_2O_3$·HCl: C, 39.90; H, 7.18; N, 13.30; Cl, 16.83. Found: C, 40.45; H, 7.38; N, 13.18; Cl, 16.69.

Ethyl *p*-Nitrobenzoylisoglutaminate (III).—Ethyl isoglutaminate hydrochloride (30.0 g.) was added to a solution of 400 cc. of ethyl acetate and 40 cc. of triethylamine and the mixture shaken until most of the starting material had reacted. The triethylamine hydrochloride was then filtered off and 30.0 g. of *p*-nitrobenzoyl chloride was added

(6) All melting points are corrected for the exposed stem of the thermometer.

(7) Knoop and Oesterlin, *Z. physiol. Chem.*, **170**, 204 (1927).

(8) Fischer and Bochner, *Ber.*, **44**, 1335 (1911).

(9) Abderhalden and Rossner, *Z. physiol. Chem.*, **152**, 281 (1926).

(10) Chiles and Noyes, THIS JOURNAL, **44**, 1798 (1922).

to the filtrate. The resulting reaction was slightly exothermic and a precipitate formed immediately. After standing two hours at room temperature and two hours at 5°, the solid was collected by filtration, washed with ethyl acetate and then several times with water to remove the *p*-nitrobenzoyl chloride–triethylamine reaction product. The remaining solid was washed with methanol and ether and dried; yield 39.5 g. (85%), m. p. 175–181°. This material is satisfactory for the next reaction.

A pure sample was obtained as follows: 1 g. of the product (III) was suspended in 35 cc. of sodium bicarbonate solution and shaken for some time. The solid was collected and dried and then dissolved in 15 cc. of glacial acetic acid. This was filtered and 70 cc. of water added to the filtrate. After cooling the resulting crystalline product was filtered off, washed and dried and recrystallized from 25 cc. of absolute ethanol; yield 0.65 g., glistening white platelets, m. p. 186–188°; $[\alpha]^{25}D$ + 11.75° (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{14}H_{17}N_3O_6$: C, 52.0; H, 5.30; N, 13.0. Found: C, 52.11; H, 5.55; N, 13.26.

p-Nitrobenzoylisoglutamine-γ-hydrazide (IV).—Ethyl *p*-nitrobenzoylisoglutaminate (14.0 g.) was suspended in 80 cc. of 100% hydrazine hydrate and stirred until the solid had slowly dissolved and the product had crystallized to form a thick paste. This stood five minutes more and then 25 cc. of ethanol was added. After shaking for fifteen minutes another 50 cc. of ethanol was added, the mixture was cooled for several hours and the product collected; yield 9.2 g. It was recrystallized once from 60 cc. of water; yield 7.7 g. (57%). The melting point on this material is poor due to the presence of water of crystallization. The one recrystallization from water is necessary in order to make the product satisfactory for use in subsequent reactions. A sample of this compound was recrystallized twice from absolute ethanol; m. p. 185–187°.

Anal. Calcd. for $C_{12}H_{15}N_5O_5$: C, 46.59; H, 4.89; N, 22.64. Found: C, 46.82; H, 5.43; N, 22.41.

p-Nitrobenzoylisoglutamine-γ-azide (V).—*p*-Nitrobenzoylisoglutamine-γ-hydrazide (8.0 g.) was suspended in a mixture of 80 cc. of water and 20 cc. of ethyl acetate. This was cooled in an ice-bath and 12 cc. of concentrated hydrochloric acid was added. Then at a temperature of 0–5° and with vigorous stirring a solution of 1.8 g. of sodium nitrite in 7 cc. of water was added dropwise. The azide began to crystallize out during the reaction. The mixture was stirred for about twenty minutes after the addition was completed. The crystalline product was then collected on a funnel, washed with water and a little ether and air-dried; yield 7.5–8.0 g. This compound was never characterized by melting point or analyses but was always used immediately in one of the succeeding reactions.

Ethyl p-Nitrobenzoylisoglutaminylisoglutaminate (VI).—Ethyl isoglutaminate hydrochloride (11.0 g.) was suspended in a solution of 200 cc. of ethyl acetate and 16 cc. of triethylamine. After stirring well to ensure complete reaction, the triethylamine hydrochloride was filtered off and to the resulting filtrate was added the azide (V) prepared from 8.0 g. of the hydrazide (IV) as described above. The mixture was stirred and the azide dissolved with the production of an intense purple color. Within a short time a crystalline product began to appear and the color faded. After standing ninety minutes at room temperature it was cooled well and the product collected, washed and dried. This was recrystallized from 550 cc. of water using Norite to clarify the solution; yield 6.3 g. (54%), m. p. 221–223°.

A portion of the material was recrystallized several times from water; m. p. 223–224°; $[\alpha]^{28}D$ + 8.5° (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{19}H_{25}N_5O_8$: C, 50.55; H, 5.58; N, 15.52. Found: C, 50.76; H, 6.19; N, 15.46.

Diethyl p-Nitrobenzoylisoglutaminylglutamate (VII).—The azide (V) prepared from 2.7 g. of the hydrazide (IV) as described above was suspended and partially dissolved in 75 cc. of ethyl acetate. To this was added an excess of diethyl glutamate (8 cc.) and the mixture was shaken. The azide dissolved and a crystalline product appeared. After reacting for ninety minutes at room temperature the mixture was cooled in an ice-bath. The product was then collected, washed with ethyl acetate and ether and dried; yield 2.5 g. (62%), m. p. softened at 160° and melted at 191–193°.

A portion (2.3 g.) of this material was recrystallized once from a solution of 325 cc. of water and 25 cc. of ethanol and a second time from 120 cc. of ethanol; yield 1.2 g.; fine hair-like crystals; m. p. 193–194°; $[\alpha]^{25}D$ + 8.75° (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{21}H_{28}N_4O_9$: C, 52.49; H, 5.87; N, 11.66. Found: C, 52.77, H, 6.06; N, 11.90.

A sample of VII (0.4 g.) was hydrolyzed by stirring in 1 *N* sodium hydroxide for two hours at 40–50°. The solution was neutralized to *p*H 2.0, evaporated to one-half its volume and cooled. The solid product was filtered off and recrystallized twice from water; m. p. 194–195°; the mixed melting point between this compound and the *p*-nitrobenzoyl-γ-glutamylglutamic acid prepared previously[4] was 194–195°.

Triethyl p-Nitrobenzoylisoglutaminyl-γ-glutamylglutamate (VIII).—Triethyl γ-glutamylglutamate hydrochloride[4] (7.6 g.) was suspended in 75 cc. of ethyl acetate and treated with 6 cc. of triethylamine to remove the hydrogen chloride. The mixture was filtered and to the filtrate was added the azide (V) prepared from 3.0 g. of the hydrazide (IV). This was shaken well and allowed to react for two hours at room temperature. It was then cooled to 5° and the resulting gelatinous precipitate collected, washed and dried; yield 4.2 g. (68%). A portion of the product (3.0 g.) was dissolved in 20 cc. of glacial acetic acid, treated with Norite and filtered. To the filtrate was added slowly 100 cc. of water. The resulting solid was collected, washed and dried and then recrystallized from 15 cc. of glacial acetic acid; yield 1.1 g., m. p. 193–194°; $[\alpha]^{27}D$ + 4.5° (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{28}H_{39}N_5O_{12}$: C, 52.75; H, 6.17; N, 10.97. Found: C, 53.08; H, 6.40; N, 10.71.

***l*-2-Pyrrolidone-5-carboxylic Acid Hydrazide (IX).**—Ethyl *l*-2-pyrrolidone-5-carboxylate[10] (216.0 g.) was dissolved in 500 cc. of U. S. P. absolute ethanol containing 70 cc. (1 mole equivalent) of 100% hydrazine hydrate.[11] The solution was warmed to 40° and then allowed to stand at room temperature for one day after which it was placed in the refrigerator for twenty-four hours. The product was then filtered off, washed with ethanol and ether and dried; yield 175 g. This was recrystallized from 1 l. of ethanol; yield 150 g. (77%), m. p. 112–114°. A portion was crystallized again from ethanol; white needle-like crystals, m. p. 114–115°; $[\alpha]^{28}D$ −11.75° (*c* 2 in water).

Anal. Calcd. for $C_5H_9N_3O_2$: C, 41.96; H, 6.34; N, 29.37. Found: C, 42.16; H, 6.12; N, 29.41.

Diethyl α-(2-Pyrrolidone-5-carboxamido)-glutarate (XI).—A solution of 75 g. of the hydrazide (IX) in 125 cc. of water was cooled in an ice-salt-bath. To this was added 95 cc. of concentrated hydrochloric acid. Then with vigorous stirring a solution of 33 g. of sodium nitrite in 75 cc. of water was added while keeping the temperature below 5°. The azide (X) which was formed was very soluble in water and could not be extracted with the usual organic solvents.

Without any extractions the original azide solution was added slowly to a mixture of 161 g. of diethyl glutamate and 200 g. of sodium bicarbonate in 400 cc. of water while keeping the temperature between 5 and 10°. It was warmed to 20°, stirred for an hour and then allowed to stand one day. The excess sodium bicarbonate was filtered off and potassium carbonate added until two layers separated. This was seeded and cooled overnight. The crystalline product was collected, washed with ether and dried; yield 88 g. (very crude). It was suspended in 500 cc. of absolute ethanol, brought to reflux and filtered to

(11) The use of excess hydrazine must be avoided since it causes the cleavage of the pyrrolidone ring to produce some glutamic acid dihydrazide.

remove inorganic salt. The filtrate was evaporated to 75 cc. and 350 cc. of ether was added. After cooling, the crystalline product was collected and recrystallized from 500 cc. of ethyl acetate; yield 30.5 g. (20%), m. p. 129–131°. A portion was crystallized again from ethyl acetate; m. p. 132–134°; $[\alpha]^{29}D$ − 40.3° (*c* 4 in water).

Anal. Calcd. for $C_{14}H_{22}N_2O_6$: C, 53.49; H, 7.06; N, 8.92. Found: C, 53.65; H, 6.79; N, 9.15.

Triethyl *p*-Nitrobenzoyl-α-glutamylglutamate (XIII).—Four grams of XI was suspended in 15 cc. of U. S. P. absolute ethanol containing 0.6 g. of dry hydrogen chloride. This was refluxed for one hour and then evaporated under reduced pressure to a sirup. The sirup was dissolved in 35 cc. of ethyl acetate containing 2.0 g. of triethylamine. The resulting triethylamine hydrochloride was filtered off and 4.3 g. of *p*-nitrobenzoyl chloride was added to the filtrate. After standing at room temperature for two hours the mixture was cooled well and the product collected and washed with ethyl acetate. The solid was then washed thoroughly with water, dried well, and recrystallized from 35 cc. of ethanol; yield 2.7 g. (42%), m. p. 146–148°. A sample was recrystallized from absolute ethanol; m. p. 148–149°; $[\alpha]^{28}D$ + 2.76° (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{23}H_{31}N_3O_{10}$: C, 54.21; H, 6.13; N, 8.25. Found: C, 54.27; H, 6.31; N, 8.39.

A sample of *p*-nitrobenzoyl-α-glutamylglutamic acid prepared by a previously described method[5] was esterified with ethanolic hydrogen chloride and the crystalline product recrystallized once from ethanol and once from ethyl acetate; m. p. 147–149°; $[\alpha]^{26}D$ + 3.25 (*c* 2 in glacial acetic acid). A mixed melting point between this material and XIII was 148–149°.

Ethyl γ-(2-Pyrrolidone-5-carboxamido)-N-(1,3-dicarbethoxypropyl)-glutaramate (XIV).—A mixture of 23.3 g. of XI and 80 cc. of absolute ethanol containing 3.5 g. of dry hydrogen chloride was refluxed on the steam-bath for one hour. The solution was filtered through a Celite pad and the filtrate was evaporated to a thick sirup under reduced pressure. This was dissolved in ethyl acetate and evaporated to dryness again. The sirup, triethyl α-glutamylglutamate hydrochloride, was dissolved in 40 cc. of water containing 30 g. of sodium bicarbonate. To the solution was added 50 cc. of a second solution containing the azide (X) prepared from 7.25 g. of 2-pyrrolidone-5-carboxylic acid hydrazide (IX) as described above. This was stirred for three hours at room temperature during which time a crystalline product appeared. It was cooled in the refrigerator, collected in a funnel and dried; yield 24.8 g. The crude product was suspended in 150 cc. of absolute ethanol which was brought to reflux and filtered to remove inorganic salts. The alcohol was evaporated under reduced pressure to give a sirup which was redissolved in 50 cc. of ethanol. After the addition of 50 cc. of ether and strong cooling a crystalline product appeared; yield 5.3 g. This was recrystallized from 45 cc. of absolute ethanol; yield 3.8 g. (11%), m. p. softens at 117°, melts at 133–135°.[12]

(12) If XIV is placed in the melting point bath at 125° it melts entirely. But if it is repeatedly frozen and melted again at 125° it becomes a solid which melts at 133–135°.

Anal. Calcd. for $C_{21}H_{33}N_3O_9$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.47; H, 6.34; N, 8.95.

Since the m. p. of this compound and XI were almost the same a mixed m. p. determination was made; m. p. 103–115°; they are not identical.

Tetraethyl *p*-Nitrobenzoyl-α-glutamyl-α-glutamylglutamate XV.—A mixture of 3.5 g. of XIV and 15 cc. of absolute ethanol containing 0.34 g. of dry hydrogen chloride was refluxed for one hour. The solution was evaporated to a sirup under reduced pressure, redissolved in ethyl acetate, evaporated again and finally dissolved in 30 cc. of ethyl acetate. This was treated with 1.5 cc. of triethylamine, filtered and 3.5 g. of *p*-nitrobenzoyl chloride was added to the filtrate. The mixture was allowed to stand at room temperature for two hours and cooled in the refrigerator overnight. The product was collected, washed well with water, dried and then recrystallized from 15 cc. of absolute ethanol; yield 1.5 g. (29%), m. p. 113–115°. This was recrystallized from ethanol; m. p. 114–115°, literature value, m. p. 115° (uncor.).[3]

Anal. Calcd. for $C_{30}H_{42}N_4O_{13}$: C, 54.04; H, 6.35; N, 8.40. Found: C, 54.29; H, 6.90; N, 8.42.

As has been described by Semb, *et al.*, this compound can be obtained in two forms having different melting points. The product described above is the lower melting form. A portion of the compound was recrystallized several times from an ethanol–water solution containing enough water to allow crystallization while the solution was still warm. It was seeded each time with some of the high-melting form.[3] The product melted at 147–148° and a mixed melting point with some of the material prepared previously by another method[3] was 146–148°.

Acknowledgment.—The microanalyses were carried out by Mr. Louis Brancone and staff.

Summary

A method is described for the conversion of ethyl *l*-2-pyrrolidone-5-carboxylate and some of its derivatives into glutamic acid compounds by opening the pyrrolidone ring with ethanolic hydrogen chloride.

By the use of this procedure the unequivocal synthesis of a number of *p*-nitrobenzoylglutamic acid peptides has been accomplished. Several of these peptides have been compared with previously prepared compounds to provide final proof of the structure of all of the isomeric *p*-nitrobenzoyldiglutamic and *p*-nitrobenzoyltriglutamic acids.

PEARL RIVER, NEW YORK RECEIVED JUNE 2, 1949