Anal. Calcd. for C₁₃H₁₀NO₄Br (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.55; H, 3.22; N, 4.00. B. cis-Lactone VI, M.p. 187–189°, by Phthalation of cis-Bro-

moamino-lactone VIII.-To a solution of the lactone VIII (0.138 g., 0.5 mmole) and N-carboethoxyphthalimide (0.11 g., mmole), dissolved in dimethylformamide (0.7 ml.), was added tributylamine (0.5 mmole) diluted with dimethylformamide under ice-cooling. The reaction mixture was left for 0.5 hour under ice-cooling and 0.5 hour at room temperature. When water (5 ml.) was added crystals began to separate after some minutes. The crystals were collected and washed with water to yield 80 mg. of colorless crystals, m.p. 80-160°. After recrystallizations from ethyl acetate-petroleum ether there was obtained 21 mg. (13%) of the pure *cis*-lactone VI, m.p. 186–189°, undepressed on admixture with a sample of the same lactone VI prepared by route A.

Anal. Caled. for $C_{13}H_{10}NO_4Br$ (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.50; H, 3.39; N, 4.39.

C. trans-Lactone VII, M.p. 142-144° .--- By fractional crystallization from acetone-petroleum ether or from ethyl acetate-petroleum ether in the manner indicated in Chart I it was possible to obtain 0.148 g. (5%) of the *trans*-lactone VII, m.p. 142-144°.

Anal. Caled. for $C_{13}H_{10}NO_4Br$ (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.49; H, 3.29; N, 4.06.

Several attempts to convert the cis-lactone VI into allohvdroxysolution was then evaporated to dryness, 0.1 N HCl (1 ml.) was added to the residue, and the mixture was warmed for 10 minutes. The solution was filtered and the filtrate (ca. 15 ml.) was treated with 1.0 N NaOH at 50° with the final pH being set at 9.6 (pH-Stat). Subsequent assay by paper chromatography and electrophoresis failed to demonstrate the presence of hydroxyproline.

In another experiment, at room temperature, there was added to a suspension of the cis-lactone VI (0.081 g., 0.25 mmole) in dioxane (1 ml.) and water (0.3 ml.) a mixture of aqueous soda (0.163 mmole) and hydrazine (0.375 mmole) under stirring. The solution became clear immediately. After 2 days at room temperature the solution was treated with $1.0\ N$ NaOH in the pH-Stat in the same manner described above. The assay for hydroxyproline showed a very faint ninhydrin-positive spot on paper electrophoresis identical in position with a control spot of allohydroxyproline

N-Phthalamyl-L-leucine. A. From Phthaloyl-L-leucine.—To phthaloyl-L-leucine⁶ (0.261 g., 1 mmole), dissolved in methanol (3 ml.), was added 2.0 N NaOH (1.1 ml.). The solution was kept at 45° for 1.5 hours, evaporated and treated with 1.0 N VICI (9.0 ml.) HCl (2.2 ml.). After standing in a refrigerator for several hours the crystals were collected and washed with a small volume of cold water. Recrystallization from ethyl acetate-ether-petro-leum ether yielded 0.128 g. (46%) of colorless crystals, m.p. 139-140°, $[\alpha]^{23}$ D - 48.5° (c 2, ethanol).

Anal. Caled. for $C_{14}H_{17}NO_{5}$ (279.28): C, 60.20; H, 6.14; N, 5.02. Found: C, 60.14; H, 6.20; N, 4.99.

B. From Ethyl Phthaloyl-L-leucinate.-To a solution of Lleucine ethyl ester hydrochloride (0.978 g., 5 mmoles) in a mixture of chloroform (20 ml.) and triethylamine (0.7 ml.) was added N-carboethoxyphthalimide (1.096 g., 5 mmoles) under ice-cooling. After 5 hours the solution was washed with water, dilute hydrochloric acid and water, and dried over Na₂SO₄. The oily ethyl phthaloyl-L-leucinate (1.75 g.) failed to crystallize, even on prolonged standing. It was therefore saponified as described above. After recrystallization there was obtained 0.49 g. (35%) of colorless crystals, m.p. $138{-}139^{\circ}$, $[\alpha]^{23}p - 48.0^{\circ}$ (c 2, ethanol), identical in all respects with the material obtained under A.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ST. JOHN'S UNIVERSITY, NEW YORK, N. Y.]

Glutamic and Aspartic Anhydrides. Rearrangement of N-Carboxyglutamic 1,5-Anhydride to the Leuchs' Anhydride and Conversion of the Latter to Pyroglutamic Acid

BY J. KOVACS, H. NAGY KOVACS AND R. BALLINA **RECEIVED DECEMBER 31, 1962**

New rearrangements of glutamic acid derivatives are described. N-Carboxy-L-glutamic-1,5-anhydride (II), an intermediate in the hydrogenolysis of carbobenzoxy-L-glutamic anhydride (I), rearranges immediately on N-carboxy-L-glutamic anhydride (Leuchs' anhydride, III). Compound III is also obtained from N-carboxy-L-glutamic anhydride γ -benzyl ester (IV) by catalytic hydrogenation. Diazomethane treatment of III gave the known N-carboxy-L-glutamic anhydride γ -methyl ester. The Leuchs' anhydride III is unstable and slowly rearranges to L-pyroglutamic acid, probably through anhydride V. Unlike other N-carboxy anhydrides III violated only a small amount of polynerization experiments. The main product was pyroglutamic yielded only a small amount of polypeptide in polymerization experiments; the main product was pyroglutamic acid. Compound I gave glutamic acid hydrobromide and acetic anhydride with hydrogen bromide-acetic acid, indicating that glutamic anhydride hydrobromide (VII) is unstable in contrast to aspartic anhydride hydrobromide. The latter, however, is very reactive because of the inductive effect of the protonated amino group and is readily opened with alcohols almost exclusively in the α -position.

The free anhydride of aspartic acid has been used in the synthesis of α,β -poly-L-aspartic acid.¹ An attempted synthesis of glutamic-1,5-anhydride for the preparation of α, γ -polyglutamic acid has also been reported²; however, the catalytic hydrogenation of carbobenzoxy-L-glutamic anhydride yields pyroglutamic acid instead of the expected anhydride. Further study of aminodicarboxylic acid anhydrides and related compounds reported here led to the recognition of interesting new rearrangements of glutamic acid derivatives and to a convenient synthesis of aspartic acid α -esters and of isoasparagine.

(1) J. Kovacs, H. N. Kovacs, I. Konyves, J. Csaszar, T. Vajda and H. Mix, J. Org. Chem., 26, 1084 (1961).

(2) W. E. Hanby, S. G. Waley and J. Watson, J. Chem. Soc., 3239 (1950).

When N-carbobenzoxy-L-glutamic 1,5-anhydride³ (I) undergoes catalytic hydrogenation in absolute dioxane or ether, one mole of hydrogen is consumed without carbon dioxide evolution, and crystalline N-carboxy-L-glutamic anhydride (Leuchs' anhydride, III) may be isolated from the reaction mixture in 85% yield. Care was taken to exclude moisture in all procedures. Peaks at 5.50 and 5.66 μ in the infrared spectrum of N-carbobenzoxy-L-glutamic anhydride disappeared during the reduction, and absorption maxima at 5.37 and 5.57 μ characteristic of Leuchs' anhydrides⁴ appeared.

Crystalline N-carboxy-L-glutamic anhydride (III) was also obtained when N-carboxy-L-glutamic an-

(3) M. Bergmann and L. Zervas, Ber., 65, 1192 (1932).
(4) M. Idelson and E. R. Blout, J. Am. Chem. Soc., 79, 3948 (1957).

hydride γ -benzyl ester (IV) was hydrogenated.⁵ This material proved to be identical with the hydrogenation product of I, based on mixture melting point, rotation and infrared spectra.

These data seemed to be sufficient to establish the structure of the reduction product obtained from I. However, because of the extreme lability of III, the more stable methyl esters of the reduction products of I and IV were prepared for decisive comparison. Direct treatment of the hydrogenated product of I in dioxane with diazomethane yielded N-carboxy-L-glutamic anhydride γ -methyl ester which was identical with an authentic sample.²



The same N-carboxy-L-glutamic anhydride γ -methyl ester was obtained when the diazomethane procedure was applied to the hydrogenated solution of IV. In several experiments we were unable to isolate N-carboxy-L-glutamic anhydride γ -methyl ester; it polymerized unusually rapidly to poly- γ -methyl-L-glutamate.

We were unable to isolate N-carbomethoxy-L-glutamic anhydride, which would be expected⁶ from the diazomethane treatment of II. An authentic sample of the anhydride was prepared and its properties were found to be different from those of the methyl ester of III, with peaks at 5.50 and 5.67 μ , characteristic of N-acyl-glutamic 1,5-anhydrides.

It was found that N-carboxy-L-glutamic anhydride (III) rearranges very easily to L-pyroglutamic acid (VI) with carbon dioxide evolution. Evaporation of solvent at higher temperature following the hydrogenation of I or IV resulted in the formation of pyroglutamic acid. Similarly, when III was added to water, practically pure pyroglutamic acid was isolated together with a small amount of glutamic acid. Atmospheric moisture or wet solvents brought about this transformation.

Though Leuchs' anhydrides polymerize readily, our efforts to polymerize JII with pyridine or triethylamine were only partially successful. The main product was always pyroglutamic acid; the best yield of polyglutamic acid was about 20%. Similarly, when benzylamine in aqueous bicarbonate solution or

(5) The catalytic hydrogenation of N-carboxy-L-glutamic anhydride γ -benzyl ester was first reported by H. Tsuyuki, H. J. Von Kley and M. A. Stahmann, J. Am. Chem. Soc., **78**, 704 (1956), who used the oily NCA of free glutamic acid directly in the preparation of polyglutamyl bovine plasma albumin in bicarbonate solution.

sodium methoxide was used as initiator, only small amounts of biuret-positive materials were obtained.

According to scale models, the chair conformation of I and II with equatorial side chain seems to be stable.⁷ In this conformation, the favorable proximity of the N-carboxyl group to the α -carbonyl would explain the intramolecular rearrangement of II to III. The rearrangement of Leuchs' anhydride III to pyroglutamic acid (VI) may go through a seven-membered cyclic carbamic-carboxylic anhydride (V), which could be formed either by direct attack of the γ -carboxylate ion on the 2-carbonyl carbon of III or on the isocyanate which could be in equilibrium with Leuchs' anhydride.⁸ However, the possibility of equilibrium II \rightleftharpoons III and the formation of VI through the 1,5-glutamic anhydride after elimination of carbon dioxide from II cannot be completely excluded.

The formation of III from II supports the mechanism of the "bicarbonate effect" in which a Leuchs' anhydride intermediate has been proposed.⁹ The role of the γ -carboxyl group in this rearrangement affords an additional illustration¹⁰ of the importance of the side chain functional groups in peptide chemistry.¹¹

It was expected that the removal of the carbobenzoxy group from carbobenzoxy-L-glutamic anhydride (I) with hydrogen bromide in acetic acid would yield stable L-glutamic anhydride as an "N-protected hydrobromide" (VII). Unexpectedly, 45% to 57% of Lglutamic acid hydrobromide crystallized from the reaction mixture, even under rigorously anhydrous conditions. From the mother liquor, acetic anhydride was isolated as 2,4-dichloroacetanilide¹² in an amount approximately equivalent to that of glutamic acid hydrobromide. Some L-pyroglutamic acid was also isolated.



The instability of the L-glutamic anhydride hydrobromide (VII) under the above reaction conditions may be attributed to the presence of the positively charged amino group on the α -carbon atom, which considerably increases the reactivity of the anhydride carbonyl(s) mainly by a strong inductive effect. Therefore, it would be reasonable to assume that anhydride hydrobromide VII reacts with acetic acid¹³ (7) A chair conformation for glutaric anhydride was chosen by R. J. Le

(7) A chair conformation for glutaric annythe was chosen by R. J. De Fevre and A. Sundaram, J. Chem. Soc., 4009 (1962), on the basis of the molar Kerr constant.

(8) (a) K. D. Kopple, J. Am. Chem. Soc., 79, 6442 (1957); (b) C. H. Bamford and H. Block, "Polyamino Acids, Polypeptides, and Proteins," Proceedings of an International Symposium held at the University of Wisconsin, 1961; edited by M. A. Stahmann, The University of Wisconsin Press, Madison, Wis., 1962, pp. 65-81.

(9) Th. Wieland, R. Lambert and H. U. Lang, Ann., 597, 181 (1955).

(10) J. Kovacs, ref. 8b, pp. 37-47.

(11) L. A. Cohen and B. Witkop, Angew. Chem., 73, 253 (1961); B. Witkop, Advan. Protein Chem., 16, 221 (1961).

 (12) The determination of acetic anhydride in the presence of acetic acid was based on an analytical method published by M. G. Edwards and K. F. P. Orton (J. Chem. Soc., 99, 1181 (1911)).

(13) When Leuchs' anhydrides are treated with hydrogen chloride in inert solvents, the corresponding amino acid chloride hydrochlorides are formed (K. D. Kopple and J. J. Katz, J. Am. Chem. Soc., **78**, 6199 (1956); M. Brenner and I. Photaki, *Helv. Chim. Acta*, **39**, 1525 (1956)); similar reaction between the glutamic anhydride hydrobromide and hydrogen bromide is also possible.

⁽⁶⁾ M. Frankel and E. Katchalski, ibid., 65, 1670 (1943).

to yield mixed anhydride(s) of acetic acid-glutamic acid hydrobromide. Further reaction of the mixed anhydride(s) with acetic acid yielded acetic anhydride and glutamic acid hydrobromide. Pyroglutamic acid can be formed either from the γ -mixed anhydride or by the intramolecular rearrangement of anhydride VII. The more likely reaction path from VII to pyroglutamic acid would be through the mixed anhydride of the γ -carboxyl (formula VIII) rather than a direct reaction between the amino group and the γ -carbonyl of the



glutamic anhydride hydrobromide which according to scale models are not in favorable positions.

It is known that the pyroglutamic acid ring opens easily with alcoholic hydrogen chloride giving the γ ester of glutamic acid.¹⁴ If analogous reaction were to take place with acetic acid-hydrobromic acid,¹⁵ the insoluble glutamic acid hydrobromide could be expected through VIII and VII. However, an attempt to open the ring of pyroglutamic acid with this reagent resulted in the precipitation of "L-pyroglutamic acid hydrobromide" from solution.¹⁶ This hydrobromide was stable enough to be isolated and characterized by analysis, but it slowly lost hydrogen bromide on standing in a desiccator. From the hydrobromide, the L-pyroglutamic acid was regenerated on treatment with triethylamine.

Unlike L-glutamic anhydride hydrobromide, the five-membered ring aspartic anhydride hydrobromide was stable in acetic acid-hydrobromic acid; no acetic anhydride was obtained from the reaction mixture. However, the increased reactivity of this anhydride hydrobromide was demonstrated by its ease of reaction with alcohols. Furthermore, because of the strong inductive effect of the protonated amino group, the anhvdride ring was opened predominantly in the α -position. The ratio of the α - and β -ester was determined in the case of the methyl ester. The presence of at least 95% of the α -ester was indicated by electrophoretic¹⁷ and chromatographic analysis of the mixture of asparagine and isoasparagine, obtained upon the conversion of the crude ester with ammonia. In the absence of the inductive effect, the nucleophilic attack is nearly equally distributed between the α - and β carboxyl groups as in the polymerization of the free aspartic anhydride to α,β -poly-L-aspartic acid. Similarly, reaction of the free anhydride with glycine ethyl ester gave roughly equivalent amounts of α - and β dipeptides together with a series of oligopeptides of aspartic acid.

These results suggested a convenient and economical method of obtaining α -esters from aspartic anhydride hydrobromide, from which isoasparagine could easily be prepared. Aspartic anhydride hydrobromide was prepared in 81% yield directly from carbobenzoxy-aspartic acid by successive treatment with acetic anhydride and dry hydrogen bromide. An attempted synthesis of aspartic anhydride hydrobromide from aspartic acid hydrobromide and acetic anhydride yielded,

(14) R. B. Angier, et al., J. Am. Chem. Soc., 72, 74 (1950).

(16) Protonation probably occurred predominantly at the oxygen of the amide group of pyroglutamic acid as was demonstrated in the case of several amides in the literature; *e.g.*, A. Berger, *et al.*, J. Am. Chem. Soc., **81**, 62 (1959); R. Huisgen, *et al.*, Ber, **90**, 1432, 1437 (1957).

however, N-acetyl-L-aspartic anhydride¹⁸ in excellent yield. Aspartic acid α -esters (benzyl, ethyl, and methyl esters in the *D*- and *L*-series) were obtained according to a general procedure from the aspartic anhydride hydrobromide with excess alcohol and the free esters precipitated from the reaction mixture with triethylamine. After recrystallization, isomer-free α ester were obtained in high yield, owing to the low proportion of the undesired β -isomers initially formed. Direct treatment of α -methyl aspartate with liquid ammonia gave isoasparagine (D and L) quantitatively. Electrophoretic¹⁷ and chromatographic analysis indicated that the crystallized product (88% yield) was free of asparagine.

Alternative preparation of α -benzyl L-aspartate by controlled cleavage of dibenzyl L-aspartate with hydrogen bromide in acetic acid was also studied; however, less satisfactory yields were obtained.¹⁹

Experimental²⁰

N-Carboxy-L-glutamic Anhydride (III). (a) By Catalytic Hydrogenation of Carbobenzoxy-L-glutamic 1,5-Anhydride (I).— A solution of 1 g. (0.0038 mole) of N-carbobenzoxy-L-glutamic anhydride³ (I), m.p. 92-94°, $[\alpha]^{29}D - 44.62°$ (c 10.25 in AcOH) in 50 ml. of dry dioxane was treated with hydrogen in the presence of 0.2 g. of 10% palladium-charcoal catalyst. Scrupulous care was taken to assure anhydrous conditions by using anhydrous solvents and gases during this procedure. The hydrogen was passed through a phosphorus pentoxide tube and the catalyst was prepared as follows: The 10% palladium-charcoal catalyst was washed first with acetic acid, then with dioxane and pre-hydrogenated in dioxane. When the hydrogen uptake stopped, the catalyst was collected and washed 5-6 times with dioxane. These operations were carried out in a carbon dioxide stream to assure a dry atmosphere. After the calculated amount of hydrogen had been absorbed, the hydrogenation apparatus was flushed with carbon dioxide and the catalyst filtered. The filtrate was concentrated to dryness *in vacuo* using an oil-pump and the bath held below 20°, while carbon dioxide passed through the capillary. The residue was triturated with a small amount of dry ether and filtered. The product was dried *in vacuo* at room temperature; yield 548 mg. (83%), m.p. 64-65° dec. Melting points of different preparations were between 64 and 85°. Recrystallization of a sample from ether-petroleum ether raised the m.p. to 92-94° dec.

The hydrogenation was also carried out in 0.33% ethereal solution, using the same precautions to avoid moisture. After the catalyst had been filtered, the product was immediately precipitated from the solution by the addition of an equal volume of dry hexane. The crystalline material was collected by filtration; m.p. 90–93°, yield 434 mg. (68%) from 1 g. of anhydride, the initial bath temperature 85°. During melting gas evolution was observed, followed by solidification and melting again at $145-150^{\circ}$. Some preparations melted exactly at 160° , which is the melting point of L-pyroglutamic acid.²¹ When the reduced product was precipitated from the ether solution with unpurified dry petroleum ether, after a few minutes a violent carbon dioxide evolution took place and L-pyroglutamic acid separated from the even, after a week, the melting points and $1-15^{\circ}$ within 2-3 days; how-ever, after a week, the melting points increased and finally reached the m.p. of L-pyroglutamic acid, $158-160^{\circ}$. When a sample was put into a small amount of water, carbon dioxide sample was put interval a small allocation of the sample gave $[\alpha]^{26}$ D -28.57° (c 1.05 in dry dioxane). The disappearance of peaks at 5.5() and 5.66 μ in the infrared spectrum of carbobenzoxy-Lglutamic anhydride were followed during the reduction by taking samples at 50 and 100% hydrogen uptake. At 50% hydrogen uptake, the 5.50 and 5.66 μ peaks were present only as shoulders at both sides of the new peak at 5.57 μ . A new peak at 5.37 μ also appeared. When the reduction was completed, there was a new peak at 4.26 μ , indicating the presence of some carbon di-

(20) Melting points are uncorrected.

(21) E. Abderhalden and K. Kautzsch, Z. physiol. Chem., 64, 447 (1909-1910); 68, 487 (1910).

⁽¹⁵⁾ K. G. Wyness, J. Chem. Soc., 2934 (1958).

⁽¹⁷⁾ C. Ressler, J. Am. Chem. Soc., 82, 1641 (1960).

⁽¹⁸⁾ C. C. Barker, J. Chem. Soc., 453 (1953). The present procedure is a simplified route to N-acetyl-L-aspartic anhydride and to N-acetyl-L-aspartic acid through it hydrolysis in the usual manner.

⁽¹⁹⁾ This reaction is similar to that by which α -benzyl L-glutamate (H. Sachs and E. Brand, J. Am. Chem. Soc., **75**, 4610 (1953)) and α -benzyl L-aspartate (P. M. Bryant, R. H. Moore, P. J. Pimlot, and G. T. Young, J. Chem. Soc., 3868 (1959)) can be prepared using hydrogen iodide.

oxide as a result of decomposition, in addition to the peaks at 5.37 and 5.57 $\mu.$

Anal. Caled. for C₆H₇O₅N: N, 8.09. Found: N, 8.35.

(b) By Catalytic Hydrogenation of N-Carboxy-L-glutamic Anhydride γ -Benzyl Ester² (Leuchs' Anhydride) (IV).—A solution of 1 g. (0.0038 mole) of N-carboxy-L-glutamic anhydride γ -benzyl ester (IV, m.p. 92°), in 50 ml. of dioxane was hydrogenated under the same conditions described in procedure a. The crystalline residue obtained after evaporation of the solvent weighed 555 mg. (84%). When the hydrogenation was carried out in 0.4% ether solution and the reduction product precipitated with an equal volume of hexane, 486 mg. (from 1 g., 74%) of a pure crystalline material was obtained, m.p. 92–94° dec. (carbon dioxide evolution, 85°, preheated bath). The substance was identical with that obtained with procedure a, on the basis of mixture melting point, infrared spectrum and specific rotation; $[\alpha]^{26} D - 29.52°$ (c 1.05 in dry dioxane).

Anal. Calcd. for C₆H₇O₅N: N, 8.09. Found: N, 8.00.

Rearrangement of N-Carboxy-L-glutamic Anhydride (III) to L-Pyroglutamic Acid (VI).—To 2 ml. of a dioxane solution containing 548 mg. (0.003 mole) of N-carboxy-L-glutamic anhydride (m.p. 92-94°) obtained by the reduction of the Leuchs' anhydride IV, 1 ml. of water was added. After violent carbon dioxide evolution ceased, the solvent was removed under reduced pressure. The crystalline product, 404 mg. (98.8%), m.p. 148-150°, was triturated with 7 ml. of warm dioxane. There was obtained undissolved crude glutamic acid, 35 mg. (8.6%), which after filtration and drying, melted at 178-180° and gave identical infrared spectrum with that of glutamic acid. From the dioxane solution, 319 mg. (79%) of crystalline L-pyroglutamic acid was precipitated on addition of ether; m.p. 158-160°, $[\alpha]^{26} D - 10.7^{\circ} (c 5 in water).^{21}$

When the reduction product of 0.003 mole of carbobenzoxy-Lglutamic anhydride was treated with water and worked up as described above, 400 mg. (98.8%) of crude L-pyroglutamic acid was obtained, m.p. 148-150°. This product also contained 34 mg. (8.5%) of glutamic acid. From the dioxane solution 254 mg. (64%) of L-pyroglutamic acid was recovered, m.p. 158-160°, $|\alpha|^{26}$ D -11.3° (c 5 in water). The infrared spectra of both compounds were identical with that of an authentic sample, with peaks at 5.85 and 6.10 μ , but no amide II bands.

The Methylation of the Hydrogenated Products of N-Carbobenzoxy-L-glutamic Anhydride (I) and of N-Carboxy-L-glutamic Anhydride γ -Benzyl Ester (IV).—The ether solution of the hydrogenated product of 450 mg. (0.0017 mole) of N-carbobenzoxy-L-glutamic anhydride was treated with dry diazomethane gas until the yellow color persisted. The solvent was immediately removed in vacuo and the crystalline residue, m.p. 89-90° was dissolved in a mixture of ether and chloroform (6:1). On addition of petroleum ether, 175 mg. (54.7%) of N-carboxy-L-glutamic anhydride γ -methyl ester crystallized from the solution; m.p. $98-99^\circ$, no depression on admixture with an authentic sample; $[\alpha]^{26}$ -24.95° (c 5.85 in ethyl acetate) (lit.² $[\alpha]^{26}$ -24° (c 7.51 in ethyl acetate), infrared spectrum superimposable on that of an authentic sample. From the mother liquor, 57 mg. (23.5%) of methyl polyglutamate was isolated. In several experiments, N-carboxy-L-glutamic anhydride γ -methyl ester could not be isolated because of its extremely fast polymerization. When the hydrogenation was carried out in dioxane and the filtered solution was treated with diazomethane, in addition to the γ -methyl ester of the Leuchs' anhydride and methyl polyglutamate, some methyl pyroglutamate was also isolated, indicating that N-carboxy-L-glutamic anhydride decomposed prior to methylation. The methyl pyroglutamate remained in the ether solution after removal of the two major components and was identified by comparison of its infrared spectrum with that of an authentic sample prepared from pyroglutamic acid with diazomethane.

Similarly, the unisolated reduction product of 978 mg. (0.003 mole) of N-carboxy-L-glutamic anhydride γ -benzyl ester in ether solution was treated with diazomethane. The ether solution was concentrated to 15 ml., 2 ml. of chloroform was added and the warm solution was filtered, yielding 201 mg. (38%) of methyl polyglutamate. On addition of petroleum ether to the filtrate, 281 mg. (40%) of N-carboxy-L-glutamic anhydride γ -methyl ester was obtained; m.p. 98-99°, no depression on admixture with an authentic sample; $|\alpha|^{26}$ D - 25.06° (c 8.3 in ethyl acetate); infrared spectrum identical with that of an authentic sample. When the methylation procedure was carried out with recrystallized N-carboxy-L-glutamic anhydride, 71% methyl ester was obtained.

N-Carbomethoxy-L-glutamic Anhydride.—To a solution of 14.7 g. (0.1 mole) of L-glutamic acid in 50 ml. of 4 N sodium hydroxide, 19 g. (0.2 mole) of methyl chloroformate in 15 ml. of ether was added in small portions at -10° . The stirred reaction mixture was kept slightly alkaline with additional 4 N sodium hydroxide. The water phase, after extraction with ether, was acidified with 5 N hydrochloric acid. The N-carbomethoxyglutamic acid was extracted with ether and after drying with MgSO₄ it was concentrated to dryness under reduced pressure, yielding 4.5 g. (22%) of oily material. N-Carbomethoxyglutamic acid (3.6 g.) was stirred with 7 ml. of acetic anhydride at room temperature until it completely dissolved. The solvent was removed under reduced pressure, the crystalline residue was triturated with dry ether and was filtered yielding 2.5 g. (67%) of N-carbomethoxy-L-glutamic anhydride. Recrystallization from dioxane-ether gave pure anhydride, m.p. $105-107^{\circ}$, $[\alpha]^{26}D$ -56° (c 6 in ethyl acetate); strong peaks at 5.50 and 5.67 μ in the infrared spectrum, characteristic of 1,5-anhydrides.

Anal. Caled. for $C_7H_9O_8N$: (187.16): C, 44.92; H, 4.85; N, 7.48. Found: C, 45.06; H, 5.01; N, 7.61.

Polymerization Experiments with N-Carboxy-L-glutamic Anhydride. (a).-The hydrogenated product of 529 mg. of carbobenzoxy-L-glutamic anhydride (0.002 mole) in 150 ml. of ether solution was concentrated to dryness and the residue was dissolved in 2 ml. of dry pyridine. After standing at room temperature for 4 days, a hygroscopic material was obtained on addition of ether. After filtration, this precipitate was dissolved in 1 ml. of water and the turbid solution was acidified with 2 drops of 4 N hydrochloric acid; upon standing, 27 mg. of polyglutamic acid separated from the solution. The mother liquor was taken to dryness under reduced pressure, the residue was washed with chloroform, acetone and water and then an additional 36 mg. (a total of 63 mg., 22.5%) of water-insoluble polypeptide was obtained. The acetone solution was evaporated to dryness under reduced pressure; the residue was dissolved in 2 ml. of water and passed through a cation exchange resin (Dowex 50 WX 8; 3×1 cm.). Concentration of the effluent in vacuo gave 102 mg. (39.3%) of L-pyroglutamic acid, m.p. 145–148°. Recrystallization from dioxane-ether raised the m.p. to 158–159°; its infrared spectrum was identical with that of an authentic sample.

(b).—When 135 mg. (0.000785 mole) of N-carboxy-L-glutamic anhydride, prepared from N-carboxy-L-glutamic anhydride γ -benzyl ester, was allowed to stand with 0.11 ml. (0.000785mole) of dry triethylamine in 1 ml. of ether, 27 mg. (27%) of a strong biuret-positive substance was isolated which gave an identical infrared spectrum with that of polyglutamic acid prepared under (a). This polyglutamic acid did not contain Nterminal amino group according to the Van Slyke amino-nitrogen determination. Its infrared spectrum (0.5% in KBr pellet) gave peaks at 6.2μ , with shoulders at 6.04 and 6.15μ , and at 6.45μ with a shoulder at 6.55μ .

Similarly, the reduction product of 1.5 g. (0.0057 mole) of carbobenzoxy-L-glutamic anhydride in dioxane, when treated with 2% excess of triethylamine for 6 days, yielded 148 mg. (20.5%) of polyglutamic acid.

(c).—To a solution of 152 mg. (0.818 mmole) of N-carboxy-L-glutamic anhydride in 3 ml. of dry dioxane, 49.3 mg. (0.91 mmole) of sodium methylate in 0.235 ml. of methanol was added. Immediately, gummy material separated from the reaction mixture, which was allowed to stand at room temperature for 4 days. After addition of water followed by acidification, polyglutamic acid did not separate; the presence of peptides, however, was indicated by the positive biuret reaction.

indicated by the positive biuret reaction. (d).—N-Carboxy-L-glutamic anhydride (56 mg., 0.32 mmole) was dissolved in 1 ml. of ice-cold saturated aqueous solution of sodium bicarbonate and then 0.98 mg. (0.009 mmole) of benzylamine was added to the reaction mixture. After standing at room temperature for 2 months, the reaction mixture was acidified, the solvent was evaporated *in vacuo* and the residue was triturated with water; 3 mg. (7%) of waterinsoluble polypeptide was obtained.

Reaction of Carbobenzoxy-L-glutamic Anhydride with Hydrogen Bromide in Acetic Acid.—To 7.5 ml. of a solution of hydrogen bromide in glacial acetic acid (containing 625 mg., 0.05 mole, of hydrogen bromide per ml.), 5 g. of carbobenzoxy-L-glutamic anhydride (0.019 mole) was added while the temperature was kept at 0°. Carbon dioxide evolution ceased in a few minutes after the substance went into solution and this was followed by immediate separation of crystalline material. After standing at room temperature for an hour, the glutamic acid hydrobromide was filtered with exclusion of moisture and washed with glacial acetic acid, benzene, ether and finally with acetone; yield 2.23 g. (51.5%); after recrystallization from methanol-ether, it melted at 212-214° and showed no depression on admixture with an authentic sample; their infrared spectra were also identical; $|\alpha|^{36} 20.2°$ (c 3 in water). An authentic sample, which was prepared from L-glutamic acid with hydrogen bromide, gave $[\alpha]^{36} D 20.0°$ (c 3 in water). When the reaction was carried out with hydrogen bromide-acetic acid containing acetic anhydride, to assure anhydrous conditions, glutamic acid hydrobromide was isolated in the same yield.

The mother liquor obtained on filtration of the glutamic acid hydrobromide was taken to dryness *in vacuo*. The oily residue, 1.724 g., after washing with ether, benzene and acetone to remove benzyl bromide, yielded L-pyroglutamic acid (728 mg., 30%); m.p. 156-158°, no depression on admixture with an authentic sample; their infrared spectra were also identical; $[\alpha]^{26}D - 11.2^{\circ}$ (c 5 in water).

In order to determine the amount of acetic anhydride which was formed during the reaction, 5 g. of carbobenzoxy-L-glutamic anhydride was treated with hydrogen bromide-acetic acid as described above. When the reaction was completed, the reaction mixture without filtration of the separated crystals was distilled *in vacuo* using a Dry Ice-cooled receiver. The residue yielded 2.84 g. of glutamic acid hydrobromide (65.5%, m.p. 198-204°) equivalent to 1.27 g. of acetic anhydride. The distillate was diluted with 5 ml. of ether and neutralized with 3.5 ml. of triethylamine in 5 ml. of ether; then 2.5 g. (0.015 mole) of 2,4-dichloroaniline in 5 ml. of ether was added.¹² After standing at room temperature for 3 days the crystalline material was col-lected and washed with ether giving 117 g. of tricthylamine lected and washed with ether, giving 1.17 g. of triethylamine hydrobromide. The filtrate was evaporated, the residue was dissolved in ether and filtered again; an additional 0.621 g. of triethylamine hydrobromide was collected. The ethereal solution was evaporated, the residue dissolved in hot benzene and on addition of dry petroleum ether 5.06 mg. (0.0024 mole) of acetyl-2,4-dichloroaniline was obtained; m.p. 142-145°, no depression on admixture with an authentic sample.¹² This amount was equivalent to 253 mg. of acetic anhydride.

In order to determine the loss of acetic anhydride during these procedures, the following control experiment was carried out: A mixture of 1 ml. of acetic anhydride (1.09 g., 0.0106 mole), 2 g. of glutamic acid, 2 g. of benzyl bromide and 7.5 ml. of hydro-gen bromide-acetic acid (626.5 mg./ml., 0.05 mole) was distilled under the same conditions described above. To the distillate 10 ml. of ether, 8.5 ml. of triethylamine in 5 ml. of ether and 2 g. to introduction of the provided and the second sec

This experiment indicated an approximate loss of 79% of acetic anhydride. The corrected amount of acetic anhydride obtained from the carbobenzoxyglutamic anhydride reaction was 1.21 g., in good agreement with the calculated 1.27 g. Dichloroacetanilide could not be isolated when acetic acid-hydrobromic acid and 2,4-dichloroaniline were allowed to stand for 3 days,

without the addition of acetic anhydride. L-Pyroglutamic Acid Hydrobromide.—L-Pyroglutamic acid (3 g., 0.023 mole) was dissolved in 5 ml. of hydrobromic acid-acetic acid at 60°. After standing at room temperature for 1 hour a crystalline substance separated from the reaction mixture which after filtration was washed with dry ether and dry acetone to yield 1.3 g. of L-pyroglutamic acid hydrobromide, m.p. 120-125° dec. In the infrared spectrum there are strong peaks at 3.15, 5.68, 5.95, 6.70 and 8.45 μ ; strong peaks in the spectrum of L-pyroglutamic acid are at 2.85, 5.80, 6.05, 8.10 μ and a triplet at 6.80, 6.93, 7.03 μ , which is characteristic of and can be used for the detection of pyroglutamic acid in mixtures of glutamic acid-pyroglutamic acid.

Anal. Calcd. for C5H8O3NBr: Br, 38.53. Found: Br, 38.87, 37.91.

Preparations standing in a desiccator for a longer period of time gradually lost hydrobromic acid, indicating the instability of this salt. When 0.75 g. of L-pyroglutamic acid hydrobromide was treated with the calculated amount of triethylamine (0.57 ml.) in 3 ml. of glacial acetic acid solution, on addition of ether free L-pyroglutamic acid and triethylamine hydrobromide precipitated from the reaction mixture. Triethylamine hydrobromide was removed with chloroform, leaving 46% L-pyroglutamic acid, m.p. 158-160°, infrared spectrum identical with that of an authentic sample.

Preparation of L-Aspartic Anhydride Hydrobromide from Carbobenzoxy-L-aspartic Acid.—A solution of 6.24 g. (0.023 mole) of N-carbobenzoxy-L-aspartic acid in 7 ml. of acetic anhydride was allowed to stand at room temperature for 3 hours. Glacial acetic acid (5 ml.) was added to the reaction mixture which was then saturated at 0° with anhydrous hydrogen bromide with the exclusion of moisture. Crystalline L-aspartic anhydride hydro-bromide precipitated. Crystallization was brought to completion by the addition of absolute ether. The crystalline material was collected, washed with a mixture of dry ether-acetic anhydride (10:1), finally with a mixture of dry ethel-acteric anydrike phorus pentoxide; yield 5 g. (81%), m.p. 166–169°, $[\alpha]^{30}$ D -21.4 (c 2.1 in dimethylformamide). The infrared spectrum had peaks at 5.35 and 5.61 μ (in Nujol), which are typical for a five-membered anhydride ring.²² However, both bands had shoulders at 5.39 and 5.56 μ .

Anal. Caled. for C4H6O3NBr: Br, 41.1. Found: Br, 41.5, 41.6.

When 1 g. (0.005 mole) of L-aspartic anhydride hydrobromide was placed in 5 ml. of 62.4% hydrogen bromide (0.038 mole) in

glacial acetic acid and allowed to stand for 3 days, no acetic anhydride was obtained from the reaction mixture using the procedure described earlier in connection with the reaction of carbobenzoxy-L-glutamic anhydride and hydrogen bromide in glacial acetic acid.

D-Aspartic anhydride hydrobromide was obtained from N-carbobenzoxy-D-aspartic acid²³; 63.5% yield, m.p. 169–171°, $[\alpha]^{26}$ D 20.6° (c 1.7 in dimethylformamide). Found: Br, 41.24, 40.89

N-Carbobenzoxy-D-aspartic anhydride was obtained in 97% yield from N-carbobenzoxy-D-aspartic acid through the usual procedure³; m.p. 110–112°, $[\alpha]^{18}D$ 41.5° (c 2.25 in acetic acid).

Anal. Calcd. for C₁₂H₁₁O₅N: N, 5.64. Found: N, 5.82.

This compound was also used for the preparation of D-aspartic anhydride hydrobromide, giving 87% yield, m.p. $168-170^{\circ}$. Effect of the Protonated Amino Group on the Ring Opening of L-Aspartic Anhydride Hydrobromide.—The ratio of α - and β esters from the reaction of L-aspartic anhydride hydrobromide with methanol was determined by the following procedure: 197 mg. (0.001 mole) of L-aspartic anhydride hydrobromide was dissolved in 2 ml. of absolute methanol and the reaction mixture allowed to stand at room temperature for 2 days. The mixture was treated with 172 mg. (0.001 mole) of silver acetate in 5 ml. of water, the silver bromide precipitate was removed, and the fil-trate was treated with charcoal and filtered again. The filtrate was treated with hydrogen sulfide, then lyophilized and the resi-due was dissolved in 5 ml. of liquid ammonia. After evaporation of the ammonia, the residue was dissolved in 5 ml. of water, filtered (Ag_2S) and the solvent was removed by lyophilization, yielding a 120-mg. mixture of isoasparagine, asparagine and aspartic acid. Paper chromatography was used for semiquantitative determination of the ratio of these components in two sol-In butanol saturated with water-acetic acid vent systems. (4:1) 0.264 mg, of the above mixture gave two spots, a brown and a purple, with R_t values of 0.08 and 0.13, respectively, when developed with ninhydrin.²⁴ Under the same conditions, reference samples of asparagine and isoasparagine gave the same $R_{\rm f}$ values, 0.08 and brown for asparagine, 0.13 and purple for iso-asparagine. The intensity of the spot corresponding to asparagine in the mixture was less than that of 13.2 γ of pure asparagine. This indicated the presence of not more than 5% asparagine. In this solvent system, aspartic acid and isoasparagine have the same $R_{\rm f}$ values. In phenol saturated with water, asparagine and isoasparagine did not separate; both have about the same $R_{\rm f}$ values of 0.47, while aspartic acid has an $R_{\rm f}$ value of 0.22. The intensity of the ninhydrin-developed spot corresponding to aspartic acid in 0.264 mg. of the mixture was less than that of 10 γ of pure aspartic acid which could result from the hydrolysis of asparagine and isoasparagine (presumably in a ratio of 5:95) during these procedures. On the basis of these results, the anhydride ring was opened with methanol yielding at least 95% α -ester and only 5% β -ester.

To a stirred solution of 2.8 ml. (0.02 mole) of triethylamine in 10 ml. of dimethylformamide, 1.39 g. (0.01 mole) of glycine ethyl ester hydrochloride and 1.97 g. (0.01 mole) of L-aspartic an-hydride hydrobromide was added. After 12 hours, the filtered solution was concentrated to dryness in vacuo, the oily residue was triturated with ether and dried in vacuo. A chromatogram in butanol-water-acetic acid (4:1:1) indicated the presence of approximately equal amounts of α - and β -aspartyl glycine ethyl esters when compared with authentic samples.²⁵ The α -peptide had an R_t value of 0.36 and gave a purple color with ninhydrin; the β -peptide, 0.26 and light brown. In addition to this, several other spots, e.g., β -aspartylaspartic acid,²⁶ were also observed which resulted from the autoacylation reaction of aspartic anhydride molecules.

Preparation of *a*-Esters of Aspartic Acid from Aspartic Anhydride Hydrobromide .--- A general procedure was found to be applicable for the preparation of all the α -esters reported here. This is demonstrated below in the preparation of α -ethyl L-aspartate. L-Aspartic anhydride hydrobromide (1.84 g., 0.009 mole)

(23) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, J. Am. Chem. Soc., 82, 2268 (1960).

(24) A. J. Woiwod, J. Chromatog., 3, 279 (1960).

(25) A mixture of α - and β -L-aspartylglycine ethyl esters was prepared by catalytic hydrogenation of the reaction product of carbobenzoxy-L-aspartic anhydride and glycine ethyl ester.

(26) Ι. Fuzesi, Masters' Thesis, St. John's University, 1960; β-L-aspartyl-L-aspartic acid was prepared by catalytic hydrogenation of carbobenzoxy- β -L-aspartyl-L-aspartic acid α',β' -dibenzyl ester. The latter compound, m.p. 141-142°, was obtained from the reaction product of carbobenzoxy-Laspartic anhydride and dibenzyl L-aspartate by fractional crystallization using alcohol. The fractional extraction method of (W. J. LeQuesne and G. T. Young, J. Chem. Soc., 24 (1952)), could not be used here because the sodium salts of the dipeptide derivatives are not sufficiently soluble in water. β -L-Aspartyl-L-aspartic acid was an amorphous powder, $[\alpha]^{22}$ D 5.3° (c 5.0 in water), and gave a blue color when a paper chromatogram was developed with ninhydrin, which is characteristic for β -dipeptides.

⁽²²⁾ J. H. Golden and R. P. Linstead, J. Chem. Soc., 1732 (1958); L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958.

was dissolved in a large excess of absolute ethanol (10 ml.) and the reaction mixture was allowed to stand at room temperature for 3 hours after a clear solution was obtained. Triethylamine in an equivalent amount to the hydrogen bromide was then added to the reaction mixture. The ethanol-insoluble α -ethyl L-aspartate crystallized from the solution, which after filtration was washed with absolute ethanol giving crude ester, m.p. 179–181°. After recrystallization from water–ethanol, 1.3 g. (86.5%) of pure ester was obtained, m.p. 181–183°, $[\alpha]^{26}$ D 24.2° (c 2.1 in water).²⁷

Anal. Calcd. for C6H11O4N: N, 8.74. Found: N, 8.87.

 α -Ethyl D-aspartate was obtained in 75% yield, m.p. 181– 183°, $[\alpha]^{26}D - 23.6^{\circ}$ (c 2 in water). Found: N, 8.99. α -Methyl Aspartate. L-Isomer.—The crude ester was ob-tained in 83% yield, m.p. 169–172°.¹ After two recrystalliza-tions from water-alcohol, 67% pure α -ester was obtained, m.p. 181–189°. [α]²⁶ d3 3° (c 0.44 in water) 181-182°, [a]²⁶D 43.3° (c 0.44 in water).

Anal. Calcd. for C₅H₉O₄N: N, 9.58. Found: N, 9.32.

D Isomer.—The yield after two recrystallizations was 58%, m.p. 183–184°, $[\alpha]^{26}$ D -42.6° (c 0.5 in water). Found: N, 9.29

 α -Benzyl Aspartate. L-Isomer.—Preparation of this ester required 12 hours reaction time. After recrystallization from water, 63% pure α -ester was obtained, m.p. 174-175°, $[\alpha]^{2s}$ D -15.5° (c 5.1 in 1 N hydrochloric acid). It gave no depression on admixture with an authentic sample¹⁹ and with a sample which was prepared in 79% yield from α -benzyl-N-carbobenzoxy-L-aspartate²⁸ through the hydrobromic acid-acetic aid cleavage²⁹ followed by triethylamine treatment of the hydrobromide of

α-benzyl-L-aspartate in methanol solution. D-Isomer was obtained in 80% yield, m.p. 175–176°, $[\alpha]^{26}$ D $+16.0^{\circ}$ (c 4.1 in N hydrochloric acid).

Anal. Caled. for C₁₁H₁₃O₄N: N, 6.23. Found: N, 6.12.

Hydrobromic Acid Cleavage of Dibenzyl Aspartate.—Dibenzyl L-aspartate hydrochloride³⁰ (8.0 g., 0.023 mole) was dissolved in 12 ml. of 35% hydrobromic acid-acetic acid and was allowed to stand at room temperature for 24 hours. The solvent was evaporated in vacuo, the oily residue was covered with 5 ml. of methanol -10°, and the pH of the stirred reaction mixture was adjusted at . to 7 with triethylamine. The precipitate was filtered and washed with methanol and ether. After recrystallization from water, 1.36 g. (25%) of α -benzyl L-aspartate was obtained, m.p. 174–175°, which gave no depression on admixture with an authentic sample; $[\alpha]^{26}$ D -15.6° (c 4.2 in 1 N hydrochloric acid).

(27) The α -ethyl L-aspartate was also prepared through N-carbobenzoxy-L-aspartic anhydride by the method of LeQuesne and Young.22 They reported a m.p. of 181-183°; no specific rotation was given.

(28) M. Bergmann, L. Zervas and L. Salzmann, Ber., 66, 1288 (1933).

 (29) D. Ben-Ishai and A. Berger, J. Org. Chem., 17, 1564 (1952).
 (30) A. Miller, A. Neidle and H. Waelsch, Arch. Biochem. Bio hys., 56, 11 (1955).

Dibenzyl aspartate p-toluenesulfonate³¹ can also be used in place of the hydrochloride, yielding 29% of α -benzyl aspartate, m.p. 174–175°. Isoasparagine. L-Isomer.— α -Methyl L-aspartate (0.8 g.,

Isoasparagine. L-Isomer.— α -Metnyl L-aspartate (0.0 g., 0.0054 mole) was dissolved in liquid ammonia. The ammonia was allowed to evaporate at room temperature and the last traces were removed *in vacuo*. The residue was crystallized from wateralcohol; yield 87.5%, m.p. 212–214° dec., $[\alpha]^{26}$ D 15.4° (c 1.8 in 0.1 N hydrochloric acid).³ When it was examined by paper electrophoresis¹⁷ and paper chromatography, only one spot was obtained.

p-Isomer was obtained in 67% yield, m.p. 212–214° dec., $[\alpha]^{26}$ D – 15.0° (c 1.8 in 0.1 N hydrochloric acid). When aqueous ammonia was used instead of liquid ammonia, chromatographically pure isoasparagine was obtained in 64% yield.

Anal. Calcd. for C4H8O3N2·H2O: N, 18.66. Found: N, 18.55.

N-Acetyl-L-aspartic Anhydride from Aspartic Acid Hydrobromide and Acetic Anhydride.-L-Aspartic acid (4 g., 0.03 mole) was allowed to stand in 4 ml. of 35% hydrobromic acid-acetic acid for 0.5 hour. Then, 28 ml. of acetic anhydride was added to the reaction mixture and heated at 60° until a clear solution was obtained. The solution was concentrated to dryness and the crystalline residue triturated with dry ether and recrystallized from acetic anhydride giving flat plates, yield 2.9 g. (61%), m.p. 174-176°³²; $[\alpha]^{26}$ D -37.1° (c 1.4 in acetic acid). The infrared spectrum had strong peaks at 5.37 and 5.59 $\mu.$

Anal. Caled. for C₆H₇O₄N: C, 45.85; H, 4.49; N, 8.94. Found: C 45.95; H, 4.58; N, 9.21.

N-Acetyl-L-aspartic anhydride was hydrolyzed with water in the usual manner to an oily residue, which yielded upon standing 97% crvstalline N-acetyl-L-aspartic acid, m.p. 139-141°,³² 97% crystalline N-acetyl-L-aspartic acid, m.p. $[\alpha]^{26}$ 5.65° (c 2.39 in water).

The optical purity of the anhydride was investigated by hydrolyzing a 0.148-g, sample with 5 ml. of 6 N hydrochloric acid for The hydrolysate was evaporated to dryness and then 24 hours. the specific rotation of the resulting aspartic acid was compared with that of a sample of aspartic acid, treated with hydrochloric acid as above; the values, 24.3 and 25.7, respectively, indicated that practically no racemization occurred during the acetic anhydride treatment.32

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(31) L. Zervas, M. Winitz and J. P. Greenstein, J. Org. Chem., 22, 1515 (1957).

(32) C. C. Barker, J. Chem. Soc., 450 (1953); specific rotation is not reported.

[CONTRIBUTION FROM THE NATURAL PRODUCTS LABORATORY, RESEARCH TRIANGLE INSTITUTE, DURHAM, N. C., AND THE EASTERN REGIONAL RESEARCH LABORATORY, PHILADELPHIA 18, PENNA.]

Steroids. LXIX.^{1,2a,b} A Novel Michael Addition-Aldol Condensation Reaction between Acetone and Δ^{16} -12,20-Diketopregnenes

By Monroe E. Wall,³ Samuel Serota,⁴ Harold E. Kenney⁴ and G. Shuford Abernethy³

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The base-catalyzed reaction of acetone and 16-dehydro-12,20-ketopregnenes is described. Evidence is presented to show that the reaction path involves a typical Michael reaction between the carbanion derived from acetone and the steroid 16-carbon atom, followed by an intramolecular aldol condensation to produce a new ring The stereochemistry of this reaction sequence is discussed in detail, and it is shown that the substituents E. at C_{16} and C_{17} have, respectively, the 16α - and 17β -configurations.

We have discovered that 16-dehydro-12,20-diketopregnenes react with acetone in the presence of aqueous

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(3) Research Triangle Institute.

(4) Eastern Regional Research Laboratory.⁴



potassium hydroxide to give a novel cyclization product. The reaction does not occur with 12-desoxy- or 11-keto-

16-dehydropregnenes. In this paper we shall present

evidence to show that the best expression for the struc-

ture and stereochemistry of this cyclization product is