The Action of Ammonia and Other Bases on γ-Methyl and γ-Ethyl L-Glutamate

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Ammonolysis of the γ half-esters of glutamic acid might be expected to provide a convenient synthesis of glutamine. However, aqueous ammonia has been shown[1,2] to convert γ-ethyl L-glutamate to L-pyrrolidonecarboxylic acid. A study of the effect of ammonia, under various conditions, and of other bases on γ-methyl and γ-ethyl L-glutamate is reported here.

The esters were treated with methanolic, ethanolic, aqueous or liquid ammonia, and progress of the reactions followed by paper chromatography. From both compounds the main product was always L-pyrrolidonecarboxylic acid, isolated in 70–90% yield. Minor amounts of glutamine were formed also but, in every case, the cyclization appeared to be far more rapid than the competing ammonolysis. An indication of the relative rates of the two reactions is given by the following comparison. A solution of γ-methyl L-glutamate in methanolic ammonia contained only a trace of uncyclized ester after standing for four hours, whereas complete conversion to the amide of the homologous β-methyl, L-aspartate under the same conditions required over 40 hours. In the latter case cyclization does not occur.

The formation of glutamine from the γ-half esters of L-glutamic acid appeared most pronounced when liquid ammonia was used. A 3% yield of the compound was isolated after liquid ammonia treatment of γ-methyl L-glutamate. No evidence of selective catalysis of the glutamine-producing reaction could be obtained when either sodium amide or various ammono acids were added to the medium.

Other experiments showed that conversion of the γ-half esters to L-pyrrolidonecarboxylic acid is readily effected by bases other than ammonia[3] and that cyclization occurs even under mildly alkaline conditions.

Experimental

γ-Methyl L-Glutamate.—To γ-methyl L-glutamate hydrochloride[2] (19.75 g., 0.1 mole) dissolved in methanol (150 cc.) was added with stirring 10 *N* ammonia (10 cc., 0.1 mole) to give, after recrystallization from aqueous ethanol, the free ester, 14.7 g. (92%), m.p. 175° dec.

Cyclization of γ-Methyl L-Glutamate.—The ester, 5 g., dissolved readily in methanol (150 cc.) saturated with dry ammonia. Samples of the solution withdrawn at intervals examined by paper chromatography showed, after four hours, only a trace of unreacted ester. The solution was evaporated, ethanol added, evaporated again and the residue taken up in *N* aqueous hydrochloric acid (31 cc.). Addition of 300 cc. of acetone precipitated ammonium chloride. The filtrate was evaporated, the residue taken up in hot methyl ethyl ketone and carbon tetrachloride added until precipitation was complete. The crystalline product, 3.89 g., had m.p. 157–159°. Recrystallization by the same procedure gave L-pyrrolidonecarboxylic acid, 3.82 g. (95%), m.p. 159–160.5°, $[\alpha]^{20}D$ −11.7° (*c* 4 in water), R_f 0.71 (phenol/water).

The same compound was isolated (yields in parentheses) after dissolution of γ-methyl L-glutamate in the following: ammonia-saturated ethanol (72%), 0.88 sp. gr. aqueous ammonia (80%)[4]; anhydrous liquid ammonia (76%), the equivalent amount of *N* aqueous sodium hydroxide (87%), the equivalent amount of 0.52 *N* methanolic sodium methoxide (80%).

An aqueous solution of γ-methyl L-glutamate continually brought to *p*H 9 by the addition of *N* aqueous ammonia, dropped to lower *p*H values after each addition until, after six hours, one equivalent of the base had been consumed.

Cyclization of γ-Ethyl L-Glutamate.—The ester, prepared according to Coleman,[2] was treated with ammonia under the conditions described for γ-methyl L-glutamate. Reaction was somewhat slower, but in each case the product was identical and the yield similar to that obtained using the methyl ester.

L-Glutamine.—Paper chromatography gave evidence of glutamine formation from both esters in the presence of ammonia, liquid ammonia treatment producing the strongest spot with the R_f value of the amide. γ-Methyl L-glutamate, 1.61 g., was dissolved in 30 cc. of liquid ammonia, and the solvent allowed to evaporate overnight. The residue, dissolved in 5 cc. of water, was run onto an alumina column, 10 × 1.8 cm., developed with water, and the eluate collected in 10-cc. fractions. These were examined by paper chromatography. Fractions 5–13 contained only glutamine. Evaporation over sulfuric acid gave 0.05 g. (3%) of L-glutamine, m.p. 180–181°. A mixture with a sample isolated from red beet[5] melted at 180–182°.

Reaction with liquid ammonia in the presence of sodium amide, ammonium chloride, ammonium bromide, ammonium nitrate and ammonium acetate gave no evidence of enhanced glutamine formation.

The Reaction of Ammonia with β-Methyl L-Aspartate.—β-Methyl L-aspartate hydrochloride,[2] 5 g., was dissolved in 100 cc. of ammonia-saturated ethanol, and the solution examined at intervals by paper chromatography. After three hours only the ester was detectable, after 18 hours both the ester and asparagine were present and after 42 hours only asparagine. Removal of the solvent, dissolution in 30 cc. of hot water and addition of 150 cc. of methanol gave asparagine monohydrate, 2.94 g. (72%), R_f 0.41 (phenol/water).

Paper Chromatography.—Paper chromatograms were run using aqueous phenol as the mobile phase. The developing agents were ninhydrin (0.1% w./v. in *n*-butyl alcohol) and brom phenol blue (0.4% w./v. in water made just alkaline with sodium hydroxide).

(1) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **221**, 51 (1933).

(2) D. Coleman, *J. Chem. Soc.*, 2294 (1951).

(3) However, the γ-acid hydrazide of glutamic acid has been prepared from γ-ethyl L-glutamate by the action of aqueous hydrazine; *cf.* J. A. Roper and H. McIlwain, *Biochem. J.*, **42**, 485 (1948).

(4) The appearance of glutamic acid on paper chromatograms of the ammonia solution indicated some hydrolysis of the ester.

(5) H. B. Vickery and G. W. Pucher in H. E. Carter's "Biochemical Preparations," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 44.

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Analogs of Benadryl

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This paper describes the preparation of basic ethers of the general formula $(C_6H_5)_2C(R)OCH_2CH_2X$ in which R represents hydrogen, methoxy or β-diethylaminoethoxy, and in which the basic radical (X) is diethylamino, morpholino, piperidino, 1-hexamethylenimino or 4-methyl-1-hexamethylenimino. In addition, α-phenyl-α-(2-pyridyl)-β′-(1-hexamethylenimino)-diethyl ether and the corresponding β′-(4-methyl-1-hexamethylenimino) compound were obtained.

Diphenylbromomethane reacted with β-(1-hexa-

(1) The Wm. S. Merrell Company Fellow.