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Preparation of γ-Alkylamides of Glutamic Acid

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When pyrrolidonecarboxylic acid is treated with aqueous methylamine, a product is obtained which corresponds in elementary composition to a methylated glutamine.

The possibility that this product is a methylamine salt of pyrrolidonecarboxylic acid is eliminated by the following findings: (1) distillation with excess magnesium oxide in vacuum at 35–40° for thirty minutes does not liberate methylamine, whereas the recovery from methylamine hydrochloride under the same conditions is 50–60%. (2) Determination of amino nitrogen by the method of Van Slyke (shaking five minutes) yields abnormally high values (90% of the total nitrogen) such as are characteristic of glutamine.[1] Pyrrolidonecarboxylic acid fails to react in the Van Slyke apparatus; methylamine under the same conditions yields values corresponding to 40% of the theoretical nitrogen content.

The position of the methyl group in the new product is shown by (1) the strongly positive ninhydrin reaction (sarcosine tested for comparison reacted negatively), and (2) the fact that after hydrolysis with hydrochloric acid distillation with calcium hydroxide yields methylamine rather than ammonia. It is clear that the methyl group of the new product is bound to the amido- and not to the α-amino group. The substance is thus a γ-methylamide of glutamic acid.

In a similar manner, using ethylamine, the ethyl derivative has been prepared. This, too, in contrast to ethylamine hydrochloride, fails to liberate ethylamine on distillation in vacuum with excess magnesium oxide, and yields ethylamine rather than ammonia on hydrolysis with acid and distillation with calcium hydroxide. The ethyl derivative, like the methyl, gives a strong positive ninhydrin reaction. The Van Slyke nitrogen value corresponds to about 88% of the total nitrogen content.

Both substances show a specific rotation approaching that ascribed in the literature to natural glutamine. The melting point of methylglutamine is higher than that of glutamine; the melting point of ethylglutamine is higher than that of the methyl derivative.

(1) Chibnall and Westall, *Biochem. J.*, **26**, 122 (1932).

It has been reported some time ago[2] that when pyrrolidonecarboxylic acid is treated at room temperature with aqueous ammonia, a product is obtained whose melting point, nitrogen content, and amino and carboxyl titration values correspond to glutamine. Further investigation has shown that the product obtained is in fact an ammonium salt of pyrrolidonecarboxylic acid: when distilled *in vacuo* with magnesium oxide, the material evolved ammonia in theoretical yield; in the Van Slyke apparatus, on the other hand, only very slight amino nitrogen values were recorded. Variation of the conditions of amination has not been found to affect the nature of the obtained product.

The abnormally high Van Slyke values of our alkyl derivatives and of glutamine itself require further elucidation. According to Plimmer,[3] amides in acetic acid solution occur in the form of the C(OH)=NH tautomer. Chibnall and Westall[1] argue from this that glutamine is different from the other amides and occurs in acetic acid solution as the $CONH_2$ tautomer whose amido NH_2 group reacts with nitrous acid. This suggestion is rendered untenable by the finding that the alkyl amido derivatives of glutamic acid here described, which contain no primary amido group, nevertheless yield abnormally high Van Slyke values. It seems possible to explain this property by the assumption that under the action of nitrous acid the α-amino group of glutamine is first replaced by an OH group, that the γ-hydroxy acid amide thus formed closes to form a lactone ring with attendant liberation of ammonia or alkyl amine, and that the latter then react with the nitrous acid. It is known that γ-hydroxy acid amides decompose readily into ammonia and lactone. Investigation of their behavior in the Van Slyke apparatus would appear to be desirable.

Experimental

WITH COLLABORATION OF S. GERTNER

Preparation and Recrystallization of Pyrrolidonecarboxylic Acid.—*l*-(+)-Glutamic acid was converted into a clear bubble-free fluid by heating 25-g. portions in small round flasks in an oil-bath at a bath temperature of 180–

(2) N. Lichtenstein, *Enzymologia*, **7**, 383 (1939).

(3) Plimmer, *J. Chem. Soc.*, **127**, 2651 (1925).

185°. The hot molten mass was poured into a porcelain mortar, and, when set, was scratched from the porcelain surface with a pointed rod. To recrystallize, the *l*-pyrrolidonecarboxylic acid ($[\alpha]^{28}D$ −11.4°) is dissolved in one and one-half times its weight of water by warming gently in a water-bath. The solution is filtered and the filtrate is concentrated in a vacuum desiccator over sulfuric acid at room temperature to a semi-solid crystalline mass. The latter is then filtered under reduced pressure. The crystals are washed with a little cold water and dried in the vacuum desiccator. From the pooled filtrate and wash water, an additional one or two crystal fractions may be obtained. The entire treatment, as shown by titration according to Linderstrøm-Lang, does not open the pyrrolidone ring. The optical activities of the different crystal fractions were essentially the same as those of the raw product.

γ-Methylamide of Glutamic Acid.—A 2.5-g. portion of pyrrolidonecarboxylic acid which had been recrystallized from water was treated with 30 cc. of 17% aqueous solution of methylamine for ten days at 37° in a sealed glass tube. At the end of this time, the fluid was transferred to a crystallizing dish and placed in a vacuum desiccator over sulfuric acid at room temperature, maintained for several hours under low vacuum and afterwards concentrated under full vacuum to a sirup. To prevent undue local increase in temperature, the bottom of the sulfuric acid container was separated from the floor of the desiccator by a layer of cotton wool. The sirup, which should be further dried in the desiccator for several days, was rubbed up with 20–25 cc. of absolute alcohol, and allowed to stand with the latter in a corked bottle in the ice box overnight. The colorless crystalline precipitate was separated by filtration under reduced pressure, washed with cold absolute alcohol, and dried at room temperature over sulfuric acid in vacuum; yield 0.5 g.; m. p. 192° (uncor.); $[\alpha]^{26.5}D$ +6.45°. *Anal.* Calcd.: C, 44.97; H, 7.56; N, 17.50. Found: C, 45.04; H, 7.56; N (Kjeld.), 17.40; NH_2-N (Van Slyke), 15.75. The ninhydrin reaction was strongly positive. The absence of methylamine salt was shown as follows: 100 mg. of the material was dissolved in water and distilled over 1 g. of magnesium oxide for one-half hour at 35–40° *in vacuo* into standard acid, using the apparatus recommended for determination of ammonia in protein hydrolyzates in Bertho-Grassmann's "Biochemisches Praktikum." The titer of the acid solution remained unaffected. Methylamine hydrochloride distilled under the same conditions gave values corresponding to 50–60% of the theoretical. After hydrolysis in 20% hydrochloric acid, the material was heated with excess calcium hydroxide at 35–40° using the same apparatus. The distillate in the hydrochloric acid solution was concentrated by evaporating over a water-bath, and the crystalline residue dried over sulfuric acid and soda lime and finally over phosphorus pentoxide in a vacuum desiccator. *Anal.* Calcd. for methylamine hydrochloride: N, 20.75; Cl, 52.51. Found: N, 20.44; Cl, 52.01.

γ-Ethylamide of Glutamic Acid.—A 2.5-g. portion of pyrrolidonecarboxylic acid which had been recrystallized from water was treated with 25 cc. of 33% aqueous solution of ethylamine for twenty days at 37° in a sealed glass tube. The further treatment was as described for the methyl derivative; yield 0.25 g.; m. p. 200° (uncor.); $[\alpha]^{24}D$ +6.25°. *Anal.* Calcd.: N, 16.09. Found: N (Kjeld.), 16.15; NH_2-N (Van Slyke), 14.15. The ninhydrin reaction was strongly positive. The material was tested for amine salt as described for the methyl derivative and with the same result. (Ethylamine hydrochloride distilled under the same conditions gave values corresponding to about 50%.) The markedly hygroscopic crystalline product obtained by distillation of the hydrolyzate with excess of calcium hydroxide into hydrochloric acid and subsequent evaporation to dryness contained 43.33% Cl; calcd. for ethylamine hydrochloride, 43.50%.

Summary

γ-Methylamide and γ-ethylamide of glutamic acid have been obtained by treating pyrrolidonecarboxylic acid with aqueous solutions of methylamine and ethylamine, respectively. Glutamine was not obtained by the treatment of pyrrolidonecarboxylic acid with aqueous ammonia.

The two alkyl derivatives of glutamine gave abnormally high Van Slyke amino nitrogen values such as are characteristic of glutamine itself. A possible explanation of the peculiar behavior of glutamine and its derivatives in this respect is suggested.

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