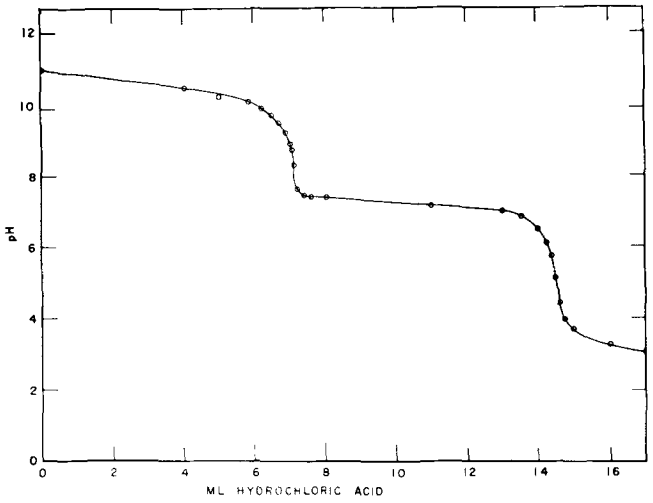

FIG. 2. Electrometric titration curve of 0.1013 g. of gossypol, prepared from dianilinogossypol, dissolved in an excess of NaOH (15 ml., 0.0503 N) with 0.0516 N HCl.

from gossypol-acetic acid by an earlier method (11). The nitrogen content of representative samples Nos. 2, 4, and 5 were respectively 0.093, 0.008, and 0.040% by the Macro-Kjeldahl method.

Discussion

Considerably more gossypol can be precipitated from an ether extract of cottonseed as dianilinogossypol than as gossypol-acetic acid. For example, 16 kg. of flaked cottonseed meats were extracted with ether, and the resulting oil was divided into two equal parts. The gossypol isolated as dianilinogossypol from one-half of the oil was 1.2%, based on the weight of the seed, while the gossypol isolated as gossypol-acetic acid from the other half of the oil was only 0.51%. An additional 0.56% gossypol precipitated from the filtrate of the latter by adding aniline.

The isolation of gossypol as the aniline derivative eliminates the necessity for removing most of the oil in order to precipitate gossypol-acetic acid more completely. The reddish color associated with gossypol appears to be more readily removed by the hydrolysis of the dianilinogossypol than it is when the gossypol is isolated from the extract as gossypol-acetic acid.

The conditions of hydrolysis described apparently circumvent the decomposition of gossypol as observed by Murty *et al.* (9) when large amounts of dianilinogossypol were hydrolyzed with hot concentrated H_2SO_4. Moreover the recombination of the hydrolyzed gossypol and aniline is not a problem, as was noted by King and Thurber (8), when the hydrolysis was carried out with ethanolic KOH. This recombination was apparently suppressed by the presence of the strong H_2SO_4 with which the aniline was probably combined as the aniline salt.

In purification, the yield is improved because of the low solubility of gossypol in the ether-ethanol-water mixture. The yield of pure gossypol was approximately 75%. Additional gossypol could be recovered by treating the filtrates obtained in the purification process with aniline.

Summary

A simple method has been developed for the acid hydrolysis of dianilinogossypol, resulting in gossypol yields of about 86%. The gossypol may be readily purified by recrystallization as gossypol-acetic acid from a mixture of ethyl ether and acetic acid. After removal of the acetic acid the gossypol may be crystallized either from a mixture of ether and light petroleum ether or from a mixture of ether, ethanol, and water. Crystallization from the latter mixture results in a yield of about 75% of gossypol, based on the amount of dianilinogossypol hydrolyzed, with a high degree of purity as is indicated by spectrophotometric and titrimetric methods.

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Synthesis of Amino Acid Derivatives of Ethanolamine[1,2]

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UNTIL QUITE RECENTLY little interest has been shown in the amino acid derivatives of ethanolamine. Although Fränkel and Cornelius (1) synthesized N,O-diglycylaminoethanol in 1918, it remained for James and Synge (2) in 1951 to detect an amino acid-ethanolamine linkage in a naturally-occurring material, the cyclic peptide gramicidin. Cheftel and co-workers (3, 4) have since been puzzled by the existence of what appear to be phosphatidyl ethanolamine-amino acid combinations of either a physical or chemical nature in blood. These materials, called "peptidic cephalins," behave in a peculiar manner during the process of purification. Their behavior pattern resembles the instability of the simple fatty acid esters and amides of ethanolamine under certain conditions of acidity and basicity (5).

This paper describes several attempts to find suit-

[1] Portion of a thesis presented by Elizabeth C. Smith as partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2] Authorized for publication as Paper No. 2399 on Sept. 1, 1959, in the Journal Series of the Pennsylvania Agricultural Experiment Station.

able methods for the synthesis, purification, and characterization of simple amino acid and peptide derivatives of ethanolamine.

Experimental

A simple amino acid derivative of ethanolamine, N-phthaloylglycylaminoethanol, was prepared by a modification of the Sheehan synthesis of peptide derivatives of hydroxy amino acids (6).

To a solution of 0.738 g. (0.0036 mole) of phthaloylglycine in 10 ml. of freshly distilled acetonitrile was added 0.147 g. (0.00241 mole) of ethanolamine and 0.495 g. (0.00241 mole) of N,N′-dicyclohexylcarbodiimide. The reaction was allowed to proceed for 5 hrs. at room temperature. The acetonitrile was removed under reduced pressure, and the residue was extracted with approximately 200 ml. of boiling acetone. Upon cooling, crude phthaloylglycylaminoethanol was crystallized from the acetone. This was recrystallized from hot water, yielding a white statically-charged material with a melting point of 216–217°C.[3] The yield of N-phthaloylglycylaminoethanol from this synthesis was 24% of theoretical.

N-Phthaloylglycylaminoethanol was soluble in water and hot acetone but relatively insoluble in cold 95% ethanol. Acid hydrolysis of the monosubstituted amino alcohol yielded only glycine and ethanolamine when checked electrophoretically for ninhydrin-positive compounds. No free amino group was present in N-phthaloylglycylaminoethanol as evidenced by a lack of reaction when it was treated with nitrous acid. The percentage of nitrogen as determined by the micro-Kjeldahl method was 11.23 compared to a theoretical value of N-phthaloylglycylaminoethanol of 11.28%.

N-Phthaloylglycylaminoethanol reacted with acetic anhydride to yield a white crystalline solid, melting at 145°C., which was assumed to be N-phthaloylglycylaminoethyl acetate. This compound was shown to contain 9.48% nitrogen; theoretical percentage of nitrogen for N-phthaloylglycylaminoethyl acetate is 9.65.

A comparison of the infrared spectra of N-phthaloylglycylaminoethanol and N-phthaloylglycylaminoethyl acetate showed the presence of a peak at 9.58 microns in the case of the former compound and two small peaks, one at 8.04 microns and the other at 8.12 microns, in the case of the latter compound. Since the peak at 9.58 microns is often associated with the free alcohol and the peaks at 8.04 and 8.12 microns with the ester bond, additional evidence is offered for the preparation of both the acetate of N-phthaloylglycylaminoethanol and the free alcohol.

An investigation of the reaction of acid with N-phthaloylglycylaminoethanol was undertaken to determine whether or not this compound would undergo an aminoacyl shift (5). A small quantity of this compound was dissolved in absolute ethanol saturated with hydrogen chloride gas and allowed to remain one to four days at room temperature. Removal of the solvent under reduced pressure yielded a solid melting over a range of 100–180°C. Recrystallization of this residue from 1 *N* HCl gave two fractions, one acid-insoluble portion melting at 115–120°C. The infrared spectra of these compounds was almost identical, each showing only a faint indication of peaks at 3.06 and 6.34 microns. In neither case was a peak observed at 9.58 microns. The disappearance of these peaks indicates that the products isolated from ethanol probably do not contain an amide (peaks at 3.06 and 6.34 microns) or a free hydroxyl group (peaks at 9.58 microns) as does N-phthaloylglycylaminoethanol. Further purification of this material failed to yield a product with a sharp melting-point.

An attempt was made to prepare N-glycylaminoethanol from N-phthaloylglycylaminoethanol by a modification of the method of Sheehan and Frank (7). A mixture of 0.0044 mole of N-phthaloylglycylaminoethanol, 0.0044 mole of hydrazine hydrate (in the form of a 1 molar alcoholic solution), and 50 ml. of alcohol were heated under reflux for one hour. The solvent was removed under reduced pressure. The residue was extracted with 25 ml. of 1-propanol to remove the N-glycylaminoethanol. Although an electrophoretic analysis of the propanol solution showed the presence of a ninhydrin-positive compound other than glycine or ethanolamine, N-glycylaminoethanol hydrochloride could not be isolated by passing dry hydrogen chloride through the alcohol solution. Several very hygroscopic compounds were isolated, but in each instance they contained only 1% nitrogen whereas theoretically they should contain 18.12% nitrogen.

The procedure described for the synthesis of N-phthaloylglycylaminoethanol was modified to prepare N-phthaloylglycylglycylaminoethanol. In this case the phthaloylglycylglycine was used instead of phthaloylglycine, tetrahydrofuran was substituted for acetonitrile, and the time of reaction was increased from 5 hrs. to 6 days. The crude N-phthaloylglycylglycylaminoethanol was recrystallized from dioxane to yield a product melting at 210–212°C. Although an electrophoretic analysis of the mixture obtained on hydrolysis of this compound showed the presence of glycine and ethanolamine, the N-phthaloylglycylglycylaminoethanol was impure as shown by the amount of nitrogen in the sample. The product of this synthesis contained 11.87% nitrogen; theoretically the N-phthaloylglycylglycylaminoethanol has 13.77% nitrogen.

N-Phthaloylalanylaminoethanol was synthesized by the procedure used for the preparation of N-phthaloylglycylaminoethanol; phthaloylalanine was substituted for phthaloylglycine. In spite of the fact that electrophoretograms of the acid hydrolysis of a fraction of the reaction mixture melting at 218–219°C. indicated the presence of N-phthaloylalanylaminoethanol, this compound was not actually isolated.

During the investigation of methods for synthesizing amino acid derivatives of ethanolamine a number of synthetic methods were shown electrophoretically to be satisfactory for the production of N-glycylaminoethanol. The Fränkel and Cornelius synthesis of N,O-diglycylaminoethanol (1) resulted in a reaction mixture which contained five ninhydrin-positive compounds: glycine, ethanolamine, N-glycylaminoethanol, and two other compounds suspected of being O-glycylaminoethanol and N,O-diglycylaminoethanol.

The Desnuelle synthesis of fatty acid amides of ethanolamine (5) was modified in the following manner to yield N-glycylaminoethanol. Pure monochloracetic acid (0.1 mole) was heated for 3 hrs. with an excess of ethanolamine (0.2 mole) at a temperature not exceeding 180°C. The reaction mixture was allowed to cool and then diluted with 100 ml. of distilled water. The aqueous solution was extracted with five 50-ml.

[3] All melting points recorded in this paper are uncorrected.

portions of ether to remove unreacted monochloracetic acid and some ethanolamine. Prior to the ammoniation of this solution by the addition of an excess (three times the volume) of ammonium hydroxide, the water was removed under reduced pressure. The flask was stoppered and allowed to stand several days, after which time the ammonium hydroxide was removed under reduced pressure and the residue in the flask was concentrated to its smallest volume. An electrophoretic analysis of the reaction mixture indicated the presence of three ninhydrin-positive compounds: glycine, ethanolamine, and a compound occupying a position intermediate between these two, N-glycylaminoethanol.

A modification of the carbodiimide synthesis (6) in which the trifluoroacetyl group was used as the blocking agent instead of the phthaloyl group resulted in the synthesis of N-glycylaminoethanol. To a solution of 0.412 g. (0.00241 mole) of trifluoroacetyl glycine in 10 ml. of 80% aqueous dioxane was added 0.495 g. (0.00241 mole) of N,N'-dicyclohexylcarbodiimide. To this was added (dropwise, with swirling) 0.159 g. (0.00260 mole) of ethanolamine. The reaction mixture was allowed to stand for seven days, after which time the solvent was removed under reduced pressure. The residue was extracted with two 25-ml. portions of boiling benzene to remove the unreacted trifluoroacetyl glycine. The material insoluble in benzene showed the presence of trifluoroacetyl glycine, glycine, N-glycylaminoethanol, and ethanolamine when subjected to electrophoretic analysis, followed by basic hydrolysis and color development with acetic-ninhydrin reagent (8).

Discussion

Synthesis of the lower amino acid derivatives of ethanolamine has been avoided because of the difficulties encountered in preparation and in purification. Kipriyanov (9) found that glycyl and alanyl aleamino acids, formed by the interaction of α-hydroxides and amino acid esters, were impossible to purify. The problem of purification was also encountered by Crowhall and Elliott (10) in the isolation of amino alcohols prepared by the reduction of peptides.

Despite the fact that N-glycylaminoethanol was produced in each of the syntheses mentioned, this compound could not be isolated. Ion exchange chromatography, according to the method of Moore and Stein (11), resulted in some decomposition of the desired compound when the pH of the buffer was raised above 6.3. Although starch block electrophoresis gave a separation of the components of the various reaction methods, the N-glycylaminoethanol fraction was not entirely free of glycine and ethanolamine. Not enough is known about stability of N-glycylaminoethanol at pH 6 under the influence of an electric current to determine whether the contamination of the amide occurred because of decomposition or a poor separation. In any event the separation obtained was not such that it can be recommended as a method of purification for glycine derivatives of ethanolamine. Two other methods of purification, solvent extraction and distillation, proved valueless when applied to this problem.

Preparation of N-phthaloylglycylaminoethanol by means of the carbodiimide method appeared to be the most satisfactory means for the synthesis of this derivative of ethanolamine. Although the yield of amide was only 24% of theoretical by this method, little difficulty was encountered in obtaining a pure product. The solubilities of phthaloylglycine, N-phthaloylglycylaminoethanol, ethanolamine, carbodiimide, and dicyclohexyl urea were sufficiently different to permit isolation and purification by recrystallization alone, eliminating the necessity for the acid and bicarbonate washes used by Sheehan (6).

Summary

N-Phthaloylglycylaminoethanol may be prepared in 24% yield by a modification of the Sheehan carbodiimide synthesis of peptides of hydroxy amino acids (6). The amino alcohol is characterized as to melting point, solubility in various solvents, the ability to form a derivative with acetic anhydride, percentage of nitrogen, and infrared spectra. In acid solution this compound appears to undergo the aminoacyl shift characteristic of compounds containing an amide linkage and a free hydroxyl group. Attempts to isolate N-glycylaminoethanol by hydrolysis of the phthaloyl derivative with hydrazine hydrate have been unsuccessful. Impure N-phthaloylalanylaminoethanol and N-phthaloylglycylglycylaminoethanol were also synthesized by means of this procedure. Several other methods for synthesizing N-glycylaminoethanol are discussed.

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