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PROCESS

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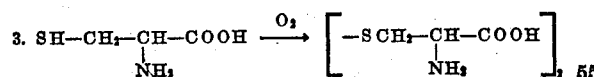
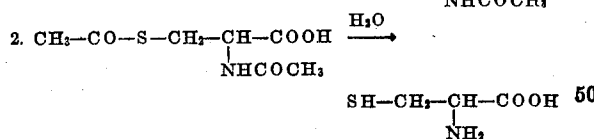
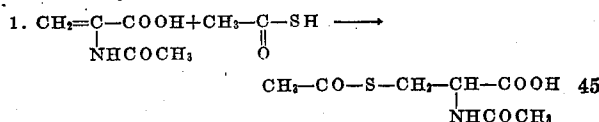
This invention relates to alpha-amino-beta-mercaptoacids. More particularly, it relates to a process for the preparation of these acids and of the corresponding disulfides.

The alpha - amino - beta-mercaptoacids and the corresponding disulfides, of which cysteine and cystine are, respectively, the best known representatives, are extremely important pharmaceutically because of their physiological activity. Methods for preparing these compounds are known, but they involve, in general, a number of complicated steps and the yields are not satisfactory.

An object of this invention is to provide a general method of preparation of alpha-amino-beta-mercaptoacids and the corresponding disulfides. Another object is to provide a two-step process for preparing these compounds in good yields with a minimum of technical difficulties. A particular object of this invention is to provide a satisfactory method of preparing cysteine and cystine. Other objects will appear hereinafter.

These objects are accomplished by the process of this invention, which comprises the steps of adding a thiocarboxylic acid to an alpha-acylamino - alpha, beta-ethylenically unsaturated carboxylic acid or a functional derivative thereof which on hydrolysis reverts to the acid, e. g., a salt, amide, ester, nitrile, acid halide or acid anhydride, and hydrolyzing the resulting alpha - acylamino - beta - (acylthio) carboxylic acid or functional derivative to the corresponding alpha-amino-beta-mercaptocarboxylic acid, or directly to the corresponding β,β' -diamino- $\beta\beta'$ -dicarboxyalkyl disulfide if oxidizing conditions are used during the hydrolytic step.

The process may be illustrated by the following equations, which depict the synthesis of cystine from thiolacetic acid and alpha-acetyl-aminoacrylic acid:



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In practice, steps 2 and 3 may be carried out separately or combined, i.e., the hydrolysis may be carried out under oxidizing conditions, to yield cystine directly.

The more detailed practice of the invention is illustrated by the following example, wherein parts given are by weight. There are, of course, many forms of the invention other than this specific embodiment.

Example

To a mixture of 17.8 parts of alpha-acetyl-aminoacrylic acid (which may be prepared according to the method described by Bergmann and Grafe in Z. physiol. Chem. 187, 191, (1930)) and 100 parts of thiolacetic acid is added approximately 0.1 part of ascaridole. The mixture is refluxed to complete solution, which requires about twenty minutes, then for an additional fifteen minutes. Evaporation of the reaction mixture under reduced pressure gives a crystalline residue which is recrystallized from a mixture of chloroform and petroleum ether. There is obtained 24.1 parts (85% of the theoretical yield) of optically inactive N,S-diacylcysteine (cf. Neuberger, Biochem. J. 32, 1455, (1938)) melting at 118° C. The product has a neutralization equivalent of 203, as compared with the theoretical value of 205.

From N,S-diacylcysteine, cystine is obtained as follows:

To a gently boiling solution of 10.3 parts of N,S-diacylcysteine in 120 parts of concentrated hydrochloric acid is added in small portions, and as fast as it is decolorized, a 0.53 N solution of iodine in methanol, until the yellow iodine color remains for five minutes after the last addition. Somewhat over the calculated quantity of iodine solution is required and the addition takes approximately thirty minutes. The solution is then evaporated nearly to dryness, diluted with water, and sodium acetate added until the solution is alkaline to Congo red. Crystalline cystine separates slowly from the solution. It is removed by filtration and washed with water, alcohol and ether. There is obtained 4.3 parts (72% of the theoretical yield) of dry cystine. Analysis: Calculated for $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2$: C, 30.4%; H, 5.4%; N, 11.0%; S, 26.4%. Found: C, 30.0%; H, 5.0%; N, 11.7; S, 26.6%.

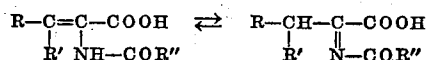
In the above method, it is obviously possible to isolate cysteine as such, if desired. This is done by carrying out the hydrolysis under non-oxidizing conditions, for example by warming

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with dilute hydrochloric acid and evaporating to dryness, giving cysteine hydrochloride from which cysteine is isolated by neutralization in the usual manner.

The invention has been illustrated by reference to cysteine and cystine. It is, however, applicable to the preparation of any desired aliphatic alpha-amino-beta-mercaptocarboxylic acid or functional derivative thereof which reverts to the acid on hydrolysis. For example, in the first step of the method, other carboxylic acids, such as thiolpropionic, thiolbutyric and thiolbenzoic acids may be used. There is, however, little advantage in using rare and expensive thiol acids since the acyl radical is hydrolyzed off during the second step. In place of alpha-acetylaminocacrylic acid, there may be used any desired alpha-acylamino-alpha, beta-ethylenically unsaturated carboxylic acid and functional derivatives thereof such as, for example, alpha-propionylaminocacrylic acid, sodium alpha-acetylaminocacrylate, alpha-acetylaminomaleic acid, ethyl alpha-benzoylaminoacrylate, alpha-stearoyl-aminoacrylamide, alpha-lauroylaminocrotonic acid, alpha-butyrylamino-cinnamic acid, alpha-acetylaminocacrylonitrile, alpha-acetylaminocacrylic anhydride, alpha-caprylamino-beta, beta-dimethylacrylic acid, alpha-acetylaminoundecylenic acid, alpha-acetylaminotetrahydrofurylacrylic acid etc. Here again, the most economical acylamino group is preferably used since the acyl radical is hydrolyzed off during the second step. In the case of certain functional derivatives of the alpha-acylamino-alpha, beta-unsaturated acids, e. g., the chloride, an excess of the thiolcarboxylic acid may be needed. This reacts with the functional groups but is hydrolyzed off later.

The alpha-acylamino-alpha, beta-ethylenic acids are sometimes assigned alternative formulas as shown below:



where R, R' and R'' are hydrogen or substituents. The two types are tautomeric, hence compounds represented by either formula are suitable starting materials in the process of this invention.

A catalyst for the addition of the thiol acid to the alpha-acylamino-alpha, beta-ethylenic acid is not necessary, though it facilitates the reaction. Addition-promoting catalysts such as benzoyl peroxide, ascaridole, etc. are satisfactory for this purpose.

The reaction may be carried out in an inert solvent such as benzene or ether, in which case an excess of thiol acid, or of functional derivative thereof, is unnecessary, or, as in the example, an excess of thiol acid may be used as solvent. When either the reaction mixture or the reaction product is liquid, neither a solvent nor an excess of thiol acid is required. The reaction sometimes take place at room temperature, but mild heating accelerates the addition and is therefore advantageous. The choice of the proper conditions for any given set of reactants offers no difficulties to those skilled in the art.

In the second step of the process, any convenient hydrolyzing agent may be used, such as mild alkalis, e. g., alkali carbonates or alkaline earth oxides, acids, etc., but neutral or acidic conditions are much preferred because of the relative instability of the products toward alkalis. The hydrolysis may be carried out either under non-oxidizing conditions, if it is desired to isolate the alpha-amino-beta-mercaptoacid, or

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under oxidizing conditions if it is desired to obtain the corresponding disulfide directly. The hydrolysis may be carried out in aqueous media or in partially-aqueous media, e. g., aqueous methyl alcohol. Suitable oxidizing agents are oxygen itself, iodine, bromine, hydrogen peroxide, ferric sulfate or any agent which is mild enough not to decompose the reaction product.

The above description and example are intended to be illustrative only. Any modification of or variation therefrom which conforms to the spirit of the invention is intended to be included within the scope of the claims.

What is claimed is:

1. Process which comprises reacting a thiolcarboxylic acid with a member of the class consisting of alpha-acylamino-alpha, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product to the corresponding alpha-amino-beta-mercapto carboxylic acid.

2. Process which comprises reacting a thiolcarboxylic acid with a member of the class consisting of alpha-acylamino-alpha, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product to the corresponding alpha-amino-beta mercapto carboxylic acid in an acidic aqueous medium.

3. Process which comprises reacting a thiolcarboxylic acid with a member of the class consisting of alpha-acylamino-alpha, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product in an acidic aqueous medium under oxidizing conditions to the corresponding β,β' -diamino- β,β' -dicarboxydialkyl disulfide.

4. Process for the preparation of alpha-acylamino-beta-acylthio monocarboxylic compounds which comprises reacting a thiolcarboxylic acid with a member of the class consisting of alpha-acylamino-alpha, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis.

5. Process which comprises reacting thiolacetic acid with a member of the class consisting of alpha-acetylaminocacrylic acid, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product to the corresponding alpha-amino-beta-mercapto carboxylic acid.

6. Process which comprises reacting thiolacetic acid with a member of the class consisting of alpha-acetylaminocacrylic acid, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product to the corresponding alpha-amino-beta-mercapto carboxylic acid in an acidic aqueous medium.

7. Process which comprises reacting thiolacetic acid with a member of the class consisting of alpha-acetylaminocacrylic acid, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis and hydrolyzing the addition product in an acidic aqueous medium under oxidizing conditions to the corresponding β,β' -diamino- β,β' -dicarboxydialkyl disulfide.

8. Process for the preparation of alpha-acetylaminocacrylic acid, beta-acylthio monocarboxylic compounds which comprises reacting thiolacetic acid with a member of the class consisting of alpha-acetyl-

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amino-alpha, beta-ethylenically unsaturated carboxylic acids and functional derivatives thereof which revert to the acid on hydrolysis.

9. Process which comprises reacting thiolacetic acid with alpha-acetylaminoacrylic acid and hydrolyzing the alpha-acetylamino-beta-acetylthiopropionic acid to alpha-amino-beta-mercapto-propionic acid.

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10. Process which comprises reacting thiolacetic acid with alpha-acetylaminoacrylic acid and hydrolyzing the alpha-acetylamino-beta-acetylthiopropionic acid under oxidizing conditions in an aqueous acid medium to cystine.

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