The Dehydrating Cyclization of S-Carboxymethyl-cysteine and N-Acetyl-S-carboxymethyl-cysteine

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It is well known that acidic amino acids and their peptides form ring compounds by dehydration between their β- or γ-carboxyl group and the α-amino group or adjacent imino group of peptide linkage. For instance, glutamic acid and glutamine can be converted to pyrrolidonecarboxylic acid[1]. Aspartyl-ε-lysine can form ε-(amino succinyl)-lysine when treated with concentrated hydrochloric acid. It is to be expected, therefore, that *S*-carboxymethyl-cysteine (SCMC), a reaction product of cysteine with iodoacetic acid, can cyclize in a similar way by dehydration between its amino group and the carboxyl group of the carboxymethyl moiety. This paper describes the formation of 3-oxo-5-carboxyperhydro-1, 4-thiazine (SCMC-lactam) from SCMC and *N*-Ac-SCMC.

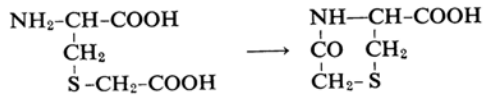
Experimental and Results

The Synthesis of SCMC.—A solution of 1.6 g. of cysteine in 30 ml. of water was neutralized with 1 N ammonium hydroxide. A solution of 2.9 g. of iodoacetic acid in 5.0 ml. of water was adjusted to pH 7.6 with 1 N ammonium hydroxide. Both solutions were mixed and left to stand for one hour. The pH value was kept at 7.6 by adding 1 N ammonium hydroxide occasionally. A small amount of precipitate produced during the reaction was filtered off, and this filtrate was passed through a column (3×15 cm.) of Amberlite IR-120 (hydrogen form) resin which was then washed thoroughly with water. The column was then treated with 1 N ammonium hydroxide. The acidic fraction showing a positive ninhydrin reaction was evaporated to dryness under reduced pressure. About 2 g. of SCMC (free acid) was obtained and recrystallized from water-ethanol. From the last fraction with a positive ninhydrin reaction, about 0.4 g. of the ammonium salt of SCMC was obtained. It was converted to the free acid by absorbing it on a column (4×20 cm.) of Amberlite IR-45 (acetate form) resin and eluting it with 2 N acetic acid. The preparation melted at 196~198°C. The overall yield was 95% of the theoretical.

The Synthesis and Properties of SCMC-lactam.—SCMC (400 mg.) was dissolved in water (5.0 ml.), and the pH brought to 4.8 by the addition of 1 N sodium hydroxide. The solution was then heated in a sealed glass tube at 110°C for 48 hr. and poured onto a column (1.5×15 cm.) of Amberlite IR-120 (hydrogen form) resin which was then washed with water (30 ml.). The filtrate and washings were combined and concentrated under reduced pressure. To a solution of an oily residue in a small amount of glacial acetic acid, ether was added drop by drop. Hexagonal crystals with a melting point of 182~183°C were obtained (about 260 mg., 74% of the theoretical).

Found: C, 37.68; H, 4.65. Calcd. for $C_5H_7O_3NS$: C, 37.26; H, 4.38%.

The compound was acidic and gave a negative ninhydrin reaction. When it was hydrolyzed with 1 N hydrochloric acid at 110°C for 1 hr., SCMC was recovered. The electrophoretic mobility was equal to that of pyrrolidonecarboxylic acid, as is shown in Fig. 3. The infrared absorption spectrum of the preparation is shown in Fig. 1, together with that of SCMC. From these results, it was concluded that SCMC-lactam was obtained as follows:



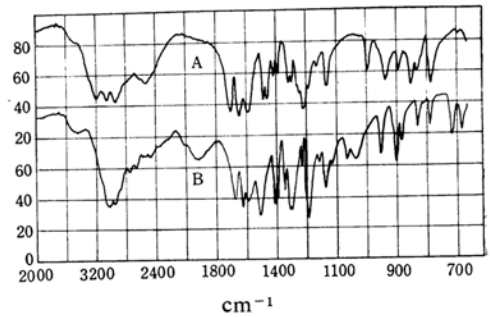


Fig. 1. Infrared absorption spectra of
A *S*-carboxymethyl-cysteine lactam
B *S*-carboxymethyl-cysteine

The Cyclization of SCMC under Various Conditions.—An aqueous solution of SCMC or of its mono sodium salt (1.0 mM) was heated at various temperatures for 1 to 48 hr., and the amount of amino acid remaining was estimated by the ninhydrin method of Yemm and Cocking[3]. The

* The following abbreviations are used: SCMC, *S*-carboxymethyl-cysteine; *N*-Ac-SCMC, *N*-acetyl-*S*-carboxymethyl-cysteine.

1) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. 3, John Wiley and Sons, Inc., New York (1961), p. 1937.

2) D. L. Swallow and E. P. Abraham, *Biochem. J.*, **70**, 364 (1958).

3) E. W. Yemm and E. C. Cocking, *Analyst*, **80**, 209 (1955).

results are summarized in Fig. 2. When SCMC was heated at 130°C, about 70% of the amino group disappeared in 1 hr., while no measurable reaction occurred at this temperature in the case of glutamic acid, in agreement with the report[4] that the rate of dehydration is very slow under these conditions. The disappearance of the ninhydrin positive substance, however, did not proceed to its completion. The paper chromatogram of the reaction mixture (*n*-butanol : acetic acid : water, 4 : 1 : 2, v/v) gave several ninhydrin positive spots, but no spot of SCMC, indicating that some side-reactions occurred in addition to the main cyclization reaction. When the reaction was carried out at 180°C, only 30% of the amino group disappeared, showing that the side reactions occurred to a larger extent. With SCMC mono sodium salt, the reaction rate was smaller, but the reaction proceeded to its completion. The paper chromatogram of the reaction mixture showed no by-products.

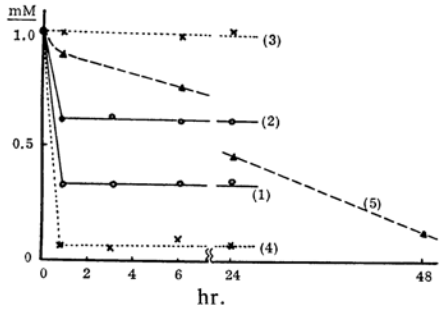


Fig. 2. Disappearance of amino group of *S*-carboxymethyl-cysteine (SCMC) and glutamic acid. Aqueous solution of SCMC (1.0 mM) was heated at (1) 130°C and (2) 180C° for 0 to 24 hr. and tested for ninhydrin reaction. (5) Solution of SCMC-mono sodium salt (1.0 mM) was heated at 110C° for 0 to 48 hr. Solution of glutamic acid (1.0 mM) was treated at (3) 130°C and (4) 180C°.

The Synthesis of *N*-Ac-SCMC.—SCMC (200 mg.) was suspended in water (1 ml.) and dissolved by neutralizing it with sodium hydroxide (4.0 M). Acetic anhydride (0.4 ml.) was added drop by drop to the solution, which was kept about pH 7.0 by adding sodium hydroxide (4.0 M). The reaction mixture, which showed a negative ninhydrin reaction on a filter paper, was diluted with water (20 ml.), passed through a column (1.5×15 cm.) of Amberlite IR-120 (hydrogen form) resin to remove the sodium ion, and evaporated to dryness under reduced pressure. *N*-Ac-SCMC was obtained as an oily residue. The electrophoretic mobility of the compound was similar to that of *N*-acetyl-aspartic acid (Fig. 3). The oily preparation was used as such for further experiments.

The Cyclization Reaction of *N*-Ac-SCMC.—*N*, *N'*-Dicyclohexylcarbodiimide (DCCD) (380 mg.) was added to a suspension of *N*-Ac-SCMC (120 mg.) in ethyl acetate (8.0 ml.) A massy precipitate was produced in ten minutes. After the reaction mixture had been kept at room temperature for two hr., glacial acetic acid (two drops) was added to decompose excess DCCD. Paper chromatography and paper electrophoresis of the reaction mixture showed acidic compounds upon its being sprayed with a 0.05% solution of bromcresol green in ethanol. The spot of the starting material, *N*-Ac-SCMC, was not detected. A large new spot with a somewhat smaller electrophoretic mobility (pyridine : acetic acid : water, 27 : 1 : 142, pH 6.0) and a larger R_f value (0.85 with *n*-butanol : acetic acid : water, 4 : 1 : 2) than SCMC-lactam was detected. Two minor spots with lower electrophoretic mobilities and R_f values were also observed (Fig. 3). The reaction mixture was evaporated to dryness under reduced pressure. The residue was mixed with 2.0 ml. of water, and the pH was adjusted to 9.0 with ammonium hydroxide (1.0 N). After the mixture had been left standing at 37°C for 30 min., the precipitate was centrifuged off and extracted twice with water; then the combined extract was evaporated to dryness in vacuo. The residue gave two spots, together with two other minor spots, by paper chromatography and paper electrophoresis, as is shown in Fig. 3. The main spot had the same electrophoretic mobility and R_f value as SCMC-lactam. The second spot coincided with *N*-Ac-SCMC. The water extract was poured onto a column (1.5×15 cm.) of Amberlite IR-45 (acetate form) resin. After washing with 1.0 N acetic acid (100 ml.), the column was eluted with 0.1 N hydrochloric acid. The crystals of SCMC-lactam were obtained by evaporating the eluate under reduced pressure and were identified by means of the infrared absorption spectrum. The cyclization reaction was also carried out under the following conditions: one mole of *N*-Ac-SCMC was treated (a) with 10 mol. of DCCD at 77°C for 2 hr., (b) with 3 mol. of DCCD at 77°C for 2 hr., or (c) with 10 mol. of DCCD at room temperature for 24 hr. There was no difference in the results.

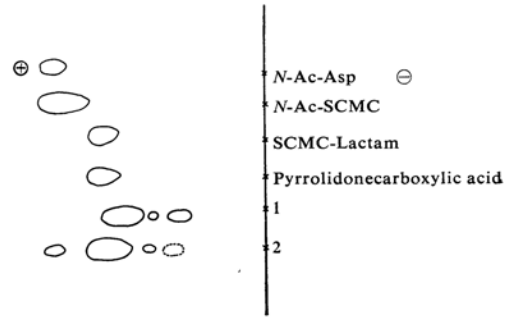


Fig. 3. Drawing of paper electrophoresis of *S*-carboxymethyl-cysteine lactam (SCMC-lactam), *N*-acetyl-SCMC and reaction products of *N*-acetyl-SCMC with *N*, *N'*-dicyclohexylcarbodiimide. 1: Direct dehydration products. 2: After treatment at pH 9.0. Electrophoresis was carried out at pH 6.0, 18 v/cm. for two hr. on Toyo-Roshi filter paper No. 51.

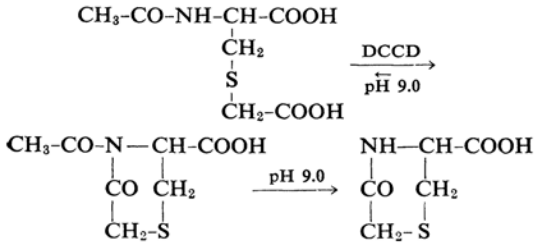
4) H. Wilson and K. Cannan, *J. Biol. Chem.*, **119**, 309 (1937).

SCMC-lactam was not formed when the aqueous solution of *N*-Ac-SCMC was heated at various temperatures (100~180°C) for 10 to 48 hr. Under these conditions, *N*-Ac-SCMC was completely decomposed. No SCMC-lactam was formed either when diethyl ester of *N*-Ac-SCMC was suspended in a 5% sodium hydrogen carbonate solution and heated at 80°C for two hours. The product obtained in this reaction was *N*-Ac-SCMC.

Discussion

SCMC cyclizes easily by dehydration to form 3-oxo-5-carboxy-perhydro-1, 4-thiazine (SCMC-lactam).

The same ring compound was also obtained from *N*-Ac-SCMC when the latter was treated with DCCD at room temperature for two hours and hydrolyzed at pH 9.0. The direct dehydration product of *N*-Ac-SCMC had a larger R_f value and a smaller electrophoretic mobility than SCMC-lactam. The course of the reaction may be explained as follows:



A minor part of the intermediate may be converted to the starting material under hydrolysis at pH 9.0.

An attempt to apply the cyclization reaction to the splitting of cysteine-containing peptides is now in progress.

Summary

S-Carboxymethyl-cysteine (SCMC) cyclizes by dehydration to form 3-oxo-5-carboxy-perhydro-1, 4-thiazine (SCMC-lactam) when the aqueous solution was heated at 110~180°C. SCMC-lactam was also obtained from *N*-acetyl-SCMC when the latter was treated with *N*, *N'*-dicyclohexylcarbodiimide and hydrolyzed subsequently at pH 9.0.

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