A NEW SYNTHESIS OF CYSTINE

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The lack of synthetic methods for the preparation of cystine has become more acute through the need of cystine containing isotopes for various metabolic investigations. In previous syntheses of cystine reported by Erlenmeyer (1) and by Fischer and Raske (2) serine was used as the starting material. We have therefore felt it would be of value to attempt to work out a synthesis of this compound from simple starting materials which would be suitable for the introduction into the molecule of isotopic atoms.

The success which attended the use of benzyl mercaptan as a means of introducing sulfur into the molecule in the synthesis of homocystine encouraged us to explore the possibilities of this method for the preparation of cystine. The benzyl derivative as an intermediate in such syntheses has the advantage of affording protection of the sulfur during the various intermediate reactions and the benzyl group can be readily removed at the end of the series of reactions by reduction.

The desired halide containing the benzylthiol radical for this method of approach was obtained by condensing benzyl mercaptan, polyoxymethylene, and HCl by the process used by Böhme (3) in the synthesis of the ethyl analogue. An excellent yield of the benzylthiolmethyl chloride was obtained. Attempted condensation of this chloride with sodium ethyl malonate failed, but condensation with sodium phthalimidomalonic ester was found to proceed smoothly. Upon hydrolysis of the condensation product, dl-benzylcysteine was obtained. The benzyl group was cleaved by sodium in liquid ammonia. Subsequent oxidation of the dl-cysteine yielded a mixture of meso- and dl-cystine, which may be
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separated by procedures already presented (4). The racemic cystine may then be resolved to give the d and l isomers (5). On the other hand, the latter may be prepared from the optically active benzylcysteines readily obtainable through the resolution of dl-benzylcysteine recently reported (6).

An attractive feature of the synthesis presented is its versatility for the introduction of isotopic atoms, depending on the particular isotopic cystine desired. It is obvious that deuterium or isotopic carbon can be introduced into the molecule if formaldehyde containing the appropriate isotope is used for the preparation of the benzylthiomethyl chloride which is the starting point of the present synthesis. The preparation of benzyl mercaptan can be accomplished by means of the reaction of benzyl magnesium chloride with elementary sulfur (7) and can thus serve as the starting point for the introduction of the sulfur isotopes, either stable or radioactive. The poor yield of 10 to 15 per cent reported in the preparation of cystine from serine makes the method suggested appear preferable. The preparation of potassium phthalimide as described by Schoenheimer and Ratner (8) is very economical from the standpoint of nitrogen, and thus the phthalimidomalonic ester benzylthiomethyl chloride condensation in the present paper affords a practical approach for the preparation of cystine containing the N\textsuperscript{15} isotope.

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**Benzylthiomethyl Chloride**—73 gm. of benzyl mercaptan were placed in a flask with 25 gm. of polyoxymethylene. The mixture was cooled in an ice bath and was saturated with dry HCl. 30 gm. of CaCl\textsubscript{2} were then added and the mixture was allowed to stand at room temperature for 24 hours. The solid material was removed by filtration and was washed with dry ether. The solution and washings were then distilled at reduced pressure and the fraction boiling at 102\textdegree C at 2 mm. pressure was collected. The yield was 69.7 gm. This product was redistilled and 64.5 gm. of benzylthiomethyl chloride (b.p. 102\textdegree C at 2 mm. pressure) were obtained. This represented 64 per cent of the theoretical amount.

C\textsubscript{6}H\textsubscript{5}Cl\textsubscript{2}S. Calculated, S 18.58; found, S 18.9

**S-Benzylthiomethylphthalimidomalonic Ester**—92.4 gm. of sodium phthalimidomalonic ester, 50.4 gm. of benzylthiomethyl
chloride, and 200 cc. of dry toluene were heated together under a reflux for 2½ hours. The precipitated sodium chloride was filtered from the solution and was washed with toluene. After the toluene had been distilled at reduced pressure, the residue was dissolved in hot absolute ethanol. 81.5 gm. of crystalline material separated when the solution cooled. The product after recrystallization from absolute ethanol melted at 81–82°. The amount obtained represented a yield of 70 per cent of the theoretical amount.

C_{23}H_{33}O_{4}NS. Calculated, S 7.48; found, S 7.6, 7.4

*S-Benzyl-dl-Cysteine*—81.5 gm. of benzylthiolmethylphthalimidomalonic ester were suspended in 640 cc. of a 1:1 mixture of 95 per cent ethanol and water, and 50 cc. of dioxane were added. 2 drops of phenolphthalein were added and the mixture was heated to 50°. 73 cc. of 5 N NaOH were added dropwise with stirring at a rate to maintain the temperature at 55–60°. When all of the alkali had been added, the temperature of the solution was brought to 70°. The solution was then stirred for 15 minutes, while the temperature was allowed to fall spontaneously. Enough HCl was added to make the mixture acid to phenolphthalein. The solution was then distilled to 0.5 volume *in vacuo*. Water was added to make the volume 1 liter, and 120 cc. of concentrated HCl were added. Upon acidification, an evolution of carbon dioxide took place. The solution was heated for 1½ hours and then 600 cc. of HCl were added and heating was continued for 2 hours. The solution was distilled to dryness, the residue was taken up with water, and the solution was distilled again. It was then taken up in 400 cc. of water and ammonium hydroxide was added until the solution gave a reaction neutral to Congo red. After the precipitate had been filtered and washed with water, it was suspended in boiling 95 per cent ethanol, heated for a moment, and was filtered while hot. The extraction was repeated several times until the phthalic acid was washed out, leaving benzylcysteine as a crystalline residue. The total weight of the combined fractions was 24.7 gm. of benzyl-dl-cysteine. This represented a yield of 64 per cent of the theoretical amount. 21.4 gm. of the material were recrystallized from warm HCl solution by the addition of

1 The melting points are corrected.
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NH₄OH. 20 gm. of halogen-free benzyl-dl-cysteine were recovered. The melting point was 215–216°.

\[ \text{C}_{12}\text{H}_{16}\text{O}_3\text{NS. Calculated, N 6.63; found, N 6.6} \]

**N-Acetyl-S-Benzyl-dl-Cysteine** 0.5 gm. of benzyl-dl-cysteine was dissolved in 4 cc. of n NaOH and the solution was cooled in an ice bath. 1 cc. of acetic anhydride was added dropwise with shaking. After the solution had been allowed to stand at room temperature for 2 hours, 0.5 cc. of concentrated HCl was added and the solution was cooled. The precipitated acetyl derivative was removed by filtration, washed with water, and crystallized from dilute ethanol. The compound melted at 158°. The melting point of N-acetyl-S-benzyl-dl-cysteine prepared from naturally occurring l-cystine by reduction, benzylation, acetylation, and racemization was likewise 158°. A mixture of the two samples possessed the same melting point.

\[ \text{C}_{12}\text{H}_{16}\text{O}_3\text{NS. Calculated, N 5.46; found, N 5.4} \]

**Optically Inactive Cystine**—15 gm. of synthetic benzyl-dl-cysteine were added portionwise to 250 cc. of liquid ammonia. Strips of sodium were added as fast as the metal reacted. When all of the compound had been added and a permanent blue color of sodium had remained for 15 minutes, ammonium chloride was added until the excess sodium was destroyed. The ammonia was allowed to evaporate spontaneously and the residue was taken up in 100 cc. of ice and water. The solution was extracted with ether and then concentrated HCl was added to the highly alkaline solution until it was just alkaline to phenolphthalein. 2 drops of ferric chloride solution were added and air was bubbled through the solution until the nitroprusside test for the sulfhydryl group was negative. The solution was neutralized to litmus with HCl and allowed to stand overnight. The precipitate was filtered, washed with water, and then dissolved in hot 1 N HCl. The solution was boiled for a few minutes with a small quantity of norit. The cystine was precipitated from the clear solution with NH₄OH. It was filtered and was washed with water, alcohol, and ether. The weight of the cystine was 7.0 gm., which represented 80 per cent of the theoretical amount. The synthetic cystine was compared with an analytically pure sample of naturally occurring
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l-cystine by the Sullivan reaction. They agreed quantitatively in color response.

\[ \text{C}_4\text{H}_6\text{O}_4\text{N}_2\text{S}_2. \text{ Calculated, N 11.66; found, N 11.5} \]

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**SUMMARY**

A new synthesis of cystine from simple starting materials has been presented. Benzyl mercaptan was condensed with formaldehyde in the presence of hydrogen chloride. The benzylthiolmethyl chloride was then condensed with phthalimidomalonic ester and the condensation product was hydrolyzed to produce benzyl-dl-cysteine. The benzyl group was removed by sodium in liquid ammonia and optically inactive cystine was formed from the dl-cysteine by oxidation.

The adaptability of the synthesis for the introduction of isotopes into the cystine molecule has been discussed.

*Addendum*—After the completion of our synthesis of cystine we learned through a private communication from Dr. Tarver that he had likewise accomplished a synthesis of cystine from trioxymethylene. Dr. C. L. A. Schmidt with whom this work of Dr. Tarver's was carried out has just informed us that Dr. Tarver had utilized Böhme's reaction but that methyl mercaptan was used, giving S-methylcysteine as the intermediate. We would like to call attention to the fact that this synthesis of Tarver's has been presented in a thesis and has been deposited in the library of the University of California.

**BIBLIOGRAPHY**

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