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# SYNTHESES OF CYSTINE

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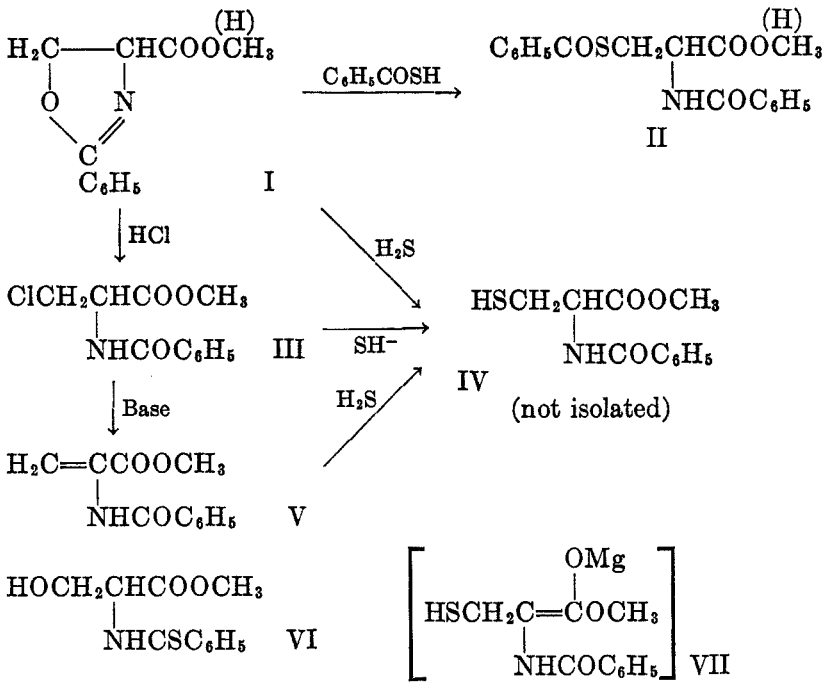
Previous syntheses of cystine designed for the use of isotopic sulfur have been based on the method of Wood and du Vigneaud (1), or on a modification of the procedure of Fischer and Raske (2), in which the benzyl group was used to protect the sulfide function. The routes to cystine described in this paper all depend on the initial transformation of serine into 2-phenyl-4-carboxymethyloxazoline (I). In the first example the hydrochloride salt of the oxazoline was rearranged to methyl $\alpha$-benzamido-$\beta$-chloropropionate (III) and the chlorine replaced directly by the unprotected sulfhydryl group. The other useful method was based on rearrangement of the thiobenzoic acid salt of the oxazoline acid (I) to give N, S-dibenzolycysteine (II). The replacement reaction was useful only for the preparation of inactive cystine in 58% yield, but optically active cystine was made by the oxazoline salt rearrangement in 42% yield. Both yields are based on sulfur and do not take recovered sulfur into account.




Hydrogen sulfide will add to methyl $\alpha$-benzamidoacrylate (V) to yield the corresponding cysteine derivative (IV), but this reaction is of no value in isotopic work because excess hydrogen sulfide was required for a high yield. A similar synthesis utilizing $\alpha$-acetamidoacrylic acid and excess thioacetic acid

has recently been published (3). The addition of hydrogen sulfide to the oxazoline ester (I) also gave the cysteine derivative (IV), but the yield was small and it is probable that the principal product was the thiobenzamide (VI) analogous to that obtained by Goldberg and Kelly (4) from the reaction of hydrogen sulfide with 2-phenyloxazoline.

*The replacement reaction.* Sodium hydrosulfide in methanol reacted with methyl α-benzamido-β-chloropropionate[1] to give dibenzoyllanthionine dimethyl ester as the main product, whereas with magnesium hydrosulfide the cysteine derivative (IV) predominated. This observation led to the use of magnesium hydrosulfide-pyridine in which the formation of the lanthionine derivative was further suppressed. The cysteine derivative was not isolated but was oxidized to a disulfide which proved to be the molecular compound formed from equal amounts of the DL and *meso* forms of inactive N,N′-dibenzoylcystine dimethyl ester. As this compound resulted from the optically active as well as inactive starting material, formulation of the chlorine replacement must include displacement of the hydrogen on the asymmetric carbon. Possible routes to the racemic cysteine derivative are through an enol salt such as VII, or the acrylic ester V, which might subsequently add hydrogen sulfide. Formation of an enol salt is unlikely, in view of the fact that N-benzoyl-L-cysteine methyl ester did not racemize when dissolved in magnesium methoxide solution, nor was any racemization such as that postulated by Elliott (6)[1] observed on alkaline hydrolysis of the oxazoline ester (I). The formation of the acrylate ester V from the chloride III and its bromine analog took place in pyridine solution, and because of the rapidity with which the latter reacted it was used to observe the course of the reaction in the presence of hydrogen sulfide. After an hour in cold pyridine solution, 24% of the bromine had become ionic, but none of the cysteine derivative could be isolated. Its formation on longer standing clearly involved addition to the double bond rather than a direct replacement. In the presence of magnesium ion the mechanism was not as obvious and it is speculative whether replacement took place at the time expulsion of hydrogen and chloride ions became imminent, or whether magnesium catalyzed a subsequent addition to the double bond.

Wood and Gutmann have recently described the resolution of labeled S-benzyl-DL-cysteine by the method of isotopic dilution (7). Following this general procedure, if L-cysteine were added to the N-benzoyl-DL-cysteine methyl ester and the mixture oxidized and hydrolyzed, the resulting L-cystine could easily be freed from the small amount of optically inactive cystine (predominately *meso*) by recrystallization of the hydrochloride salt (12) (If L-cystine were added to labeled DL-cystine that part of the labeled L-form bound as *meso*-cystine would, of course, not be recovered). But if a sample of high activity is desired or if the specific activity of the sulfur is low the following preparation is preferable.

*Oxazoline salt rearrangement.* The rearrangement of the hydrochloride of an oxazoline was first reported by Wislicenus and Körber (8), but apparently has

[1] In the previous paper (10b) two references were omitted. One concerns a preparation of methyl α-benzamido-β-chloro-DL-propionate by Painter (5) and the other is a pertinent oxazoline paper by Elliott (6).

not been studied with respect to organic acids. Although preliminary results indicated general applicability of this scheme, the present report deals with one example only.

By means of the brucine salt, benzoyl-L-serine was conveniently separated from its isomer and converted to 2-phenyl-4-carboxy-L-oxazoline. This acid was added to a pyridine solution of thiobenzoic acid prepared *in situ*, the mixture heated on the steam-bath for two minutes, and the crude N,S-dibenzoyl-L-cysteine isolated. Hydrolysis of the S-benzo group took place easily in sodium hydroxide or sodium methoxide, and the N-benzo group was subsequently removed by acid hydrolysis. Removal of both groups by acid gave somewhat lower yields and took more time.

The hydrogen chloride present in an equivalent amount in the reaction mixture might be expected to compete with the thiobenzoic acid, and it had been observed that heating pyridine hydrochloride with the oxazoline ester in alcohol readily gave III. However, the reactivity of the thio-acid was so much greater that the presence of hydrogen chloride did not noticeably decrease the yield.

## EXPERIMENTAL

*Preparation of* D- *and* L-*benzoylserine.* An equivalent amount of brucine was added to N-benzoyl-DL-serine and the mixture dissolved in a solvent mixture of one part of alcohol and ten parts of acetone. Crystals formed and were filtered after one hour. This fraction was predominantly the brucine-salt of the L-form. Further purification was effected in the same solvent by solution in a little alcohol, and then adding ten times the volume of acetone. Rapidity of crystal formation was taken as an index of purity, but the chief criterion of purity was the absence of the D-form in the material (usually oily) left after removal of the acetone-alcohol mother liquor on the steam-bath under reduced pressure. The brucine salt of N-benzoyl-D-serine was found to be only slightly soluble in cold water, although the presence of the L-salt appeared to increase this solubility and to extend the time necessary for its separation from aqueous solution. To test for the presence of the D-salt, a small portion was dissolved in an equal volume of cold water. If no crystals formed after an hour in the ice-bath the sample was considered to be the L-salt and was recrystallized from alcohol-acetone. Due to the great solubility of the L-salt in water, purification of the D-salt by recrystallization from hot water was easily accomplished.

The brucine salts were dissolved in chloroform and the acid extracted into 2 *N* sodium hydroxide. Addition of hydrochloric acid gave the respective enantiomorphs. In a typical preparation, 39.3 g. of the L-salt was dissolved in 50 ml. of chloroform, extracted with 31 ml. of 2 *N* sodium hydroxide, and both solvents subsequently washed with a fresh portion of the other; then the alkaline solution was acidified and the product precipitated crystalline. The total recoverable *N-benzoyl-*L*-serine* was 11.8 g., m.p. 145–147°. Purified from water it separated in needle clusters and melted at 147–149° (gas). Its dimorphous nature was indicated by the fact that it melted completely when placed in the bath at 130°, resolidified and remelted at 147–148.5°, $[\alpha]_D^{22}$ +43.6° (*c*, 1 in 95% ethanol).

*Anal.* Calc'd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30.
Found: C, 57.59; H, 5.29.

For N-benzoyl-D-serine, $[\alpha]_D^{22}$ −41.8° (*c*, 1 in 95% ethanol).

A commercial sample of L-serine was benzoylated in sodium carbonate solution with benzoyl chloride and although the product was predominately N-benzoyl-DL-serine, the N-benzoyl-L-serine obtained in this manner was used to identify the products of the separation described above. This result corresponds with similar racemizations using acetic anhydride (9).

*N-Benzoyl-*L*-serine methyl ester.* The conversion of the acid to the methyl ester using ethereal diazomethane did not always proceed smoothly, as some unidentified ether-in-

soluble oil was formed, but it is believed the trouble lay in allowing the reaction to take place at the surface of the undissolved acid. N-Benzoyl-L-serine, 6.0 g., was suspended in dry ether and the ethereal diazomethane solution added carefully, to keep the reaction in the solution phase, above and out of contact with the solid. The solution was stirred between additions of diazomethane in order to dissolve more of the acid. The product separated crystalline and no oily residue was apparent. It was filtered and more was obtained from the mother liquor. After recrystallization from benzene it weighed 5.1 g. (80%), m.p. 83–86°. The analytical sample had m.p. 84–86°, $[\alpha]_D^{26}$ +17.7° (*c*, 1 in 95% ethanol).

*Anal.* Calc'd for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87.
Found: C, 59.33; H, 5.87.

*N-Benzyl-D-serine methyl ester* was prepared as above, m.p. 84–86°.

*Anal.* Found: C, 59.15; H, 5.89. $[\alpha]_D^{23}$ −12.1° (*c*, 1 in 95% ethanol).

*2-Phenyl-4-carboxymethyl-L-oxazoline.* The preparation of the racemate has been described (10) but the optically active oxazoline salt was considerably less stable toward rearrangement to the β-chloropropionic acid derivative than the racemate. Its tendency to crystallize was less and this might have been a factor contributing to its instability. For example the DL-oxazoline salt could be kept for thirty minutes at room temperature without appreciable rearrangement, but under these conditions the D-salt (as an oil) completely rearranged to methyl α-benzoylamino-β-chloro-D-propionate. N-Benzoyl-L-serine methyl ester, 3.9 g., was covered with dry ether and 3 ml. of thionyl chloride slowly added while stirring by hand and keeping the temperature at approximately 5°. A heavy gum formed first; this became much lighter-bodied on continued manipulation and began to crystallize in about 20 minutes (the oil did not always crystallize). After approximately two hours, excess ether and thionyl chloride were removed under reduced pressure while keeping cold. Chilled dry pyridine (20 ml.) was slowly added with stirring and chilling. The dark red solution was then poured into a cold solution of 18 g. of sodium carbonate in 200 ml. of water. The product was taken into ether, then recovered, and distilled as a pale yellow oil, b.p. 133–137° at 2 mm. Distilled again at 131–133° (2 mm.), it weighed 2.88 g. (80%) $[\alpha]_D^{23}$ +118° (*c*, 1 in 95% ethanol).

*Anal.* Calc'd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40.
Found: C, 64.18; H, 5.54.

This ester decomposed somewhat on standing several weeks. Hydrolysis in slightly more than the equivalent amount of 2 *N* sodium hydroxide gave a crystalline sodium salt which was recovered by concentrating the aqueous solution under reduced pressure and adding acetone, in which the salt is insoluble. The rotation was unchanged after solution in warm water and reprecipitation with acetone. Probably hydrated, the salt sintered at 85°, resolidified, and was unmelted at 250°. On the basis of four molecules of water of hydration indicated by titration, the yield was 75–80%.

*2-Phenyl-4-carboxy-D-oxazoline sodium salt*, similarly made, showed $[\alpha]_D^{25}$ −66° (*c*, 1 in 95% ethanol).

*2-Phenyl-4-carboxy-L-oxazoline* formed crystalline as an ice-cold suspension of the sodium salt was slowly and with vigorous stirring neutralized with an equivalent amount of 3 *N* hydrochloric acid. Care was necessary because of the ease with which the free acid hydrolyzed, and it was important that the acid separated during the titration. The suspension was seeded if necessary. The sodium salt (1.0 g.) required 1.2 ml. of 3.1 *N* hydrochloric acid to bring the *p*H of the suspension to 3.9 (Accutint 60). Filtered and washed with cold water and dried in a desiccator under reduced pressure, the product weighed 0.62 g., m.p. 125–126°. Yield, 92.5% based on an estimated salt hydration of four molecules of water. Recrystallization from hot, dry benzene lowered the melting point, probably because of unavoidable hydrolysis. $[\alpha]_D^{20}$ +225° (*c*, 1 in dry pyridine).

*Anal.* Calc'd for $C_{10}H_9NO_3$: C, 62.82; H, 4.74.
Found: C, 62.57; H, 4.94.

A pyridine solution showed no significant change in optical rotation on standing 23 hours at 20°.

*Methyl α-benzoylamino-β-chloro-*L*-propionate* was prepared by the method used for the L-oxazoline (see above) with the exception that the temperature was not lowered and the suspension was refluxed for an hour to assure rearrangement. Yield, before purification from alcohol, about 85%; m.p. 114–115.5°, $[\alpha]_D^{23}$ −14° (*c*, 1 in 95% ethanol). Comparable values for the D-isomer have been reported (11).

*Anal.* Calc'd for $C_{11}H_{12}ClNO_3$: C, 54.67; H, 5.01.
Found: C, 55.01; H, 5.23.

In one experiment the rearrangement of the oily oxazoline salt was complete in 30 minutes at room temperature.

*Methyl α-benzoylamino-β-bromo-*DL*-propionate.* 2-Phenyl-4-carboxymethyl-DL-oxazoline, 2.05 g., was dissolved in dry ether and the hydrobromide salt made by passing dry hydrogen bromide through the solution. The oil which separated soon crystallized. The ether was distilled and the salt held for five minutes on the steam-bath. The crude product was washed with water; 2.7 g. (94%), m.p. 115–118°. It was purified by dissolving in hot benzene and adding petroleum ether, m.p. 115–117°.

*Anal.* Calc'd for $C_{11}H_{12}BrNO_3$: Br, 27.93. Found: Br, 27.99.

*N,N′-Dibenzoyl-*DL*-cystine.* DL- and *meso*-cystine were prepared by the method of Loring and du Vigneaud (12). The reaction of benzoyl chloride with DL-cystine (from 5.0 g. of the hydrochloride dissolved in a sodium carbonate solution and chilled in an ice-bath) proceeded slowly over several hours. Acidification gave a solid which was purified by recrystallization from glacial acetic acid; 4.4 g., m.p. 164–167°. The analytical sample had m.p. 165–166.5°. An unidentified inactive form melting at 168° has been reported (13).

*Anal.* Calc'd for $C_{20}H_{20}N_2O_6S_2$: C, 53.55; H, 4.50.
Found: C, 53.46; H, 4.65.

Owing to the fact that this compound was also the principal product obtained from the benzoylation of *meso*-cystine, it was necessary to resolve it in order to prove its structure. Accordingly, N,N′-dibenzoyl-L-cystine was prepared and purified by recrystallization from glacial acetic acid; m.p. 190–192°, $[\alpha]_D^{20}$ −202° (*c*, 1 in 95% ethanol). The melting point 195.5–196.5° and $[\alpha]_D^{20}$ −222° have been reported (14). It was dissolved in alcohol together with an equivalent amount of quinine and the crystalline salt caused to precipitate by the addition of water. The salt had m.p. 161–163°. This same salt was separated from a solution of the quinine salt of N,N′-dibenzoyl-DL-cystine. A mixture melting point of the salts was not lowered, and on decomposition with hydrochloric acid the salt yielded N,N′-dibenzoyl-L-cystine, m.p. 192–194°, $[\alpha]_D^{20}$ −203° (*c*, 1 in 95% ethanol).

*N,N′-Dibenzoyl-meso-cystine.* The benzoylation of *meso*-cystine from 5.0 g. of dihydrochloride in the manner described for DL-cystine yielded a product which on purification from glacial acetic acid proved to be the above-described DL-form; yield 41%. The recrystallization mother liquor yielded a solid which was recrystallized from alcohol to give 0.6 g. (8%) melting at 186–188°. Further purification raised the melting point to 190–191°. It showed no optical activity and a mixture melting point with N,N′-dibenzoyl-L-cystine was lower.

*Anal.* Calc'd for $C_{20}H_{20}N_2O_6S_2$: C, 53.55; H, 4.50.
Found: C, 53.83; H, 4.66.

Its isolation along with the DL-form as one of the oxidation products of N-benzoyl-DL-cysteine further established its identity.

*N-Benzoyl-*DL*-cysteine.* N,N′-dibenzoyl-DL-cystine (0.5 g.) was stirred with zinc in a mixture of equal parts of 6 *N* HCl and dioxane (8 ml.). Most of the solid dissolved in an hour. After 2.5 hours the solvent was taken to a small volume under reduced pressure. The product crystallized, and was recovered. Hot benzene extraction of the product followed by benzene recrystalliation gave 0.14 g., m.p. 136–137°.

*Anal.* Calc'd for $C_{10}H_{11}NO_3S$: C, 53.32; H, 4.92.
Found: C, 53.80; H, 5.05.

*N,N′-Dibenzoyl-*DL*-cystine dimethyl ester.* The acid (0.2 g.) in ethereal suspension was reacted with etheral diazomethane. During the reaction the crystalline product replaced the acid in suspension. The ester was purified from alcohol, m.p. 168–169°, wt. 0.177 g. (83%).

*Anal.* Calc'd for $C_{22}H_{24}N_2O_6S_2$: C, 55.44; H, 5.08.
Found: C, 55.72; H, 5.23.

*N,N'-Dibenzoyl-meso-cystine dimethyl ester.* The ester was prepared as above with diazomethane; m.p. 169.5–171.5°.

*Anal.* Calc'd for $C_{22}H_{24}N_2O_6S_2$: C, 55.44; H, 5.08.
Found: C, 55.21; H, 4.98.

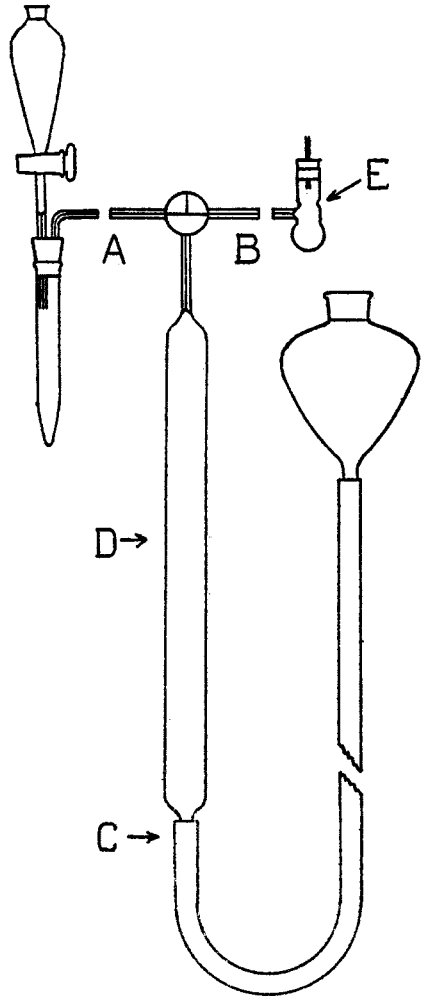



FIGURE 1. HYDROGEN SULFIDE REACTION APPARATUS

*Apparatus for handling hydrogen sulfide.* The apparatus used for all reactions in which hydrogen sulfide was a reactant appears in Figure 1. Stannous sulfide was used as the source of hydrogen sulfide and its properties make it well suited for this purpose. Sulfide in an alkaline solution was easily recovered by first adding stannous chloride solution, then acidifying with acetic acid and centrifuging. Stannous sulfide appeared to be slightly soluble in alkaline solution (lead acetate test), but was not decomposed by acetic acid. The air in the cylinder was displaced by mercury and the leveling bulb lowered below 76 cm. so that an empty space appeared at the top of the cylinder. Then the 15-ml. centrifuge tube containing stannous sulfide and connected at A was evacuated by a water-pump at

B, the stopcock turned to connect it with the cylinder, and the pump shut off. About 3 ml. of 85% phosphoric acid was added through the separatory funnel and the suspension heated with a flame to generate hydrogen sulfide. The leveling bulb was raised to increase the pressure, and hence the temperature to which the phosphoric acid could be heated. The sulfide dissolved to give a yellowish solution which became colorless after brief boiling. After all gas was displaced from the centrifuge tube by hot acid the cylinder was cut off with the stopcock. The reaction flask was attached at B, evacuated by a water-pump at A, and hydrogen sulfide admitted by turning the stopcock.

The cylinder was supported by a clamp at C. The rubber hose provided enough play so that this point was also the pivot for shaking. A cone-drive motor held a short piece of bent rod which was attached to the stopcock through a small spring. The apparatus was shaken at low motor speed and the degree of agitation controlled by a loosely fitting clamp at D.

A constriction in the reaction flask at E supported a circle of filter paper on which the sample of methyl $\alpha$-benzoylamino-$\beta$-chloropropionate rested during absorption of hydrogen sulfide by magnesium methoxide. The tube through the rubber stopper (closed at the upper end) was used to push the sample into the flask, and at the end of the reaction to pass nitrogen through the solution to flush unreacted hydrogen sulfide into a sodium hydroxide absorption tube at A.

*Molecular compound of N,N'-dibenzoyl-*DL*-cystine dimethyl ester and N,N'-dibenzoyl-meso-cystine dimethyl ester. A.* This substance was obtained in 95% yield when 0.5 g. of each of the components were dissolved together in alcohol; m.p. 153–155°, unchanged by recrystallization.

*Anal.* Calc'd for $C_{22}H_{24}N_2O_6S_2$: C, 55.44; H, 5.08.
Found: C, 55.54; H, 5.05.

*B.* Methyl alcohol was removed from 2.1 ml. (2 × theory) of 2.0 *N* magnesium methoxide solution under reduced pressure, the crystalline solid transferred to the reaction flask, and 1.0 ml. of dry pyridine added. The filter paper disk was pressed into the neck of the flask, 0.50 g. of methyl $\alpha$-benzoylamino-$\beta$-chloro-L-propionate placed on the paper, and the flask stoppered. It was attached to the hydrogen sulfide cylinder which contained 65 ml. of hydrogen sulfide generated from 0.390 g. of stannous sulfide (1.25 × theory). The reaction flask was carefully evacuated and the hydrogen sulfide *slowly* admitted (a sudden rush of gas might blow the solid into the nitrogen inlet tube). The flask was gently shaken with absorption of most of the hydrogen sulfide and formation of a yellow solution. After ten minutes it was put in an ice-bath and the pressure dropped in five minutes to a minimum of −27 cm. of mercury, measured by the leveling bulb which was lowered to keep mercury from being drawn into the flask. The solid was then pushed into the pyridine solution with the nitrogen inlet tube (solid which stuck to the sides was freed by tapping). In seven minutes the solid had dissolved to give a yellow-green solution which was shaken for one hour in the ice-bath, and then with a sodium hydroxide absorption tube in place the end of the inlet tube was opened and 0.5 ml. of 6 *N* hydrochloric acid was forced into the flask followed by a slow stream of nitrogen for thirty minutes. The colorless solution was then titrated with 14 ml. of 0.1 *N* iodine solution and the crystalline product recovered and purified from alcohol, wt. 0.363 g., m.p. 152–154.5°. Mixture melting point determinations showed this substance to be identical with that obtained from a similar preparation using inactive starting material and with the molecular compound prepared from N,N'-dibenzoyl-DL-cystine dimethyl ester and its *meso*-isomer.

All remaining organic material was taken into and recovered from chloroform. Five drops of water and 0.2 g. of potassium hydroxide was added and the mixture held on the steam-bath briefly until a solution resulted; then it was heated gradually to 410–420° and held at this temperature for five minutes during which time carbonization took place. The material was taken up in water and added to the sodium hydroxide solution previously used to trap unreacted hydrogen sulfide. Stannous sulfide was precipitated with stannous chloride solution followed by acetic acid. The sulfide was assayed by conversion to hydrogen sulfide which was oxidized by 0.1 *N* iodine solution.

| | | | |
|---|---|---|---|
| S used, mole | 0.00259 | Product, mole | 0.00152 |
| S recovered, mole | 0.00071 | Yield on halide basis, % | 73.5 |
| S reacted, mole | 0.00188 | Yield on S basis, % | 58.7 |
| Halide used, mole | 0.00207 | Yield on recovered S basis, % | 81 |

*C. From methyl α-benzamidoacrylate.* Methyl α-benzoylamino-β-bromo-DL-propionate, 0.15 g., was put in 0.5 ml. of cold pyridine and the suspension held in an ice-bath with occasional shaking for one hour; the solid dissolved. Dilute nitric acid was added in excess and 0.11 g. (72.4%) of starting material was recovered. Silver bromide, 0.024 g. (24%) was recovered from the mother liquor. Under the same conditions and in the presence of an equivalent amount of hydrogen sulfide dissolved in the pyridine, no N,N′-dibenzoyl-aminocystine dimethyl ester was isolated after removal of the hydrogen sulfide and titration with iodine solution (required only 2% of theory). In a similar experiment in which the cold pyridine solution was left in contact with excess hydrogen sulfide for twenty hours the yield of the molecular compound of the N,N′-dibenzoyl-DL- and *meso*-cystine dimethyl esters was 67%. At room temperature the yield was 60% after twenty-three hours with excess hydrogen sulfide. With an equivalent amount of hydrogen sulfide and a reaction time of seventy-two hours at room temperature, (the reaction was ended when the pressure over the reaction mixture stopped dropping) the yield of the isolated product was only 8%.

Although methyl α-benzamidoacrylate was not characterized due to the ease with which it polymerized, its formation was proved in the following way. Methyl α-benzamido-β-bromo-DL-propionate, 0.5 g., was dissolved in 1 ml. of pyridine and the separated pyridine hydrobromide filtered at the end of an hour. From this was obtained silver bromide representing 74% of the original bromine. The filtrate was taken to an oil under reduced pressure and the oil dissolved in 2 *N* sodium hydroxide. Acidification gave α-benzamidoacrylic acid; yield 72%, m.p. 152–155° (gas, turn orange). A mixture melting point with another sample (10b) proved its identity.

The same transformation took place with the chlorine analog but much more slowly. Ionic chlorine amounted to 59% after 24 hours at room temperature.

*D. From 2-phenyl-4-carboxymethyl-*DL*-oxazoline.* A solution of 0.5 g. of the oxazoline in 1 ml. of dry pyridine reacted with hydrogen sulfide containing some oxygen. The only crystalline material recovered weighed 0.033 g., m.p. 151–153°. Its identity as the molecular compound was established by a mixture melting point. The remainder of the material was not identified.

*N,N′-Dibenzoyl-*L*-cystine dimethyl ester* was prepared by the action of diazomethane on N,N′-dibenzoyl-L-cystine. It was purified from alcohol, m.p. 176–178°. This compound was also obtained by the action of hydrogen sulfide on 2-phenyl-4-carboxymethyl-L-oxazoline in the manner described for the optically inactive form and in the same poor yield. A mixture melting point of the two specimens was not depressed. A previously reported (14) melting point is 176–177°.

*Anal.* Calc'd for $C_{22}H_{24}N_2O_6S_2$: C, 55.45; H, 5.08.
Found: C, 55.78; H, 5.32.

*Hydrolysis of* DL*-meso-dibenzoylcystine dimethyl ester.* Three-tenths g. of the molecular compound in one ml. of 48% HBr became oily and evolved gas when the suspension was heated on the steam-bath. The oil shortly dissolved and the pale yellow solution was held on the steam-bath for four hours. During the hydrolysis benzoic acid separated. A little water and excess pyridine were added and the cystine allowed to crystallize over several hours. The yield varied between 0.130 and 0.139 g. (86–92%).

*β,β′-Thiobis-(N-benzoylalanine dimethyl ester) or dibenzoyllanthionine dimethyl ester.* When methyl α-benzamido-β-chloro-DL-propionate, 0.50 g., was added to an equivalent amount of sodium hydrosulfide in absolute methanol, the only product isolated weighed 0.14 g. and was purified from alcohol, wt. 0.11 g., m.p. 153–154°. A mixture melting point with the DL-*meso*-N,N′-dibenzoylcystine dimethyl ester molecular compound was strongly depressed.

*Anal.* Calc'd for $C_{22}H_{24}N_2O_6S$: S, 7.21. Found: S, 7.30.

In a comparable experiment using magnesium hydrosulfide in absolute methanol, only 0.04 g. of the sulfide was isolated; 52% of the product was the cystine derivative.

*N,S-Dibenzoyl-L-cysteine.* Stannous sulfide, 0.301 g., was converted to 50 ml. of hydrogen sulfide and allowed to react with 0.281 g. of benzoyl chloride dissolved in 1 ml. of dry pyridine. The reaction was exothermic and at the end a white salt (probably pyridine hydrochloride) was present in the yellow solution of thiobenzoic acid. A vacuum of 59 cm. of mercury had developed by the end of the reaction which was over in about 15 minutes. Air was admitted, the flask was detached, and 0.40 g. of 2-phenyl-4-carboxy-L-oxazoline was added to the solution. All solid dissolved on shaking and gentle warming, and the solution was then held for two minutes in the steam-bath. Excess 3 *N* HCl was added to the solution and crystallization of the product induced with a drop of benzene. After drying, the product was triturated with petroleum ether which removed a little oil. The crude material weighed 0.52 g. (79% based on SnS); m.p. 171–178°. Other runs gave yields of 78–82%. The compound was recrystallized by dissolving in hot ethyl acetate and concentrating the solution. The analytical sample melted at 179–182° with shrinking from 173°; $[\alpha]_D^{21}$ −76° (*c*, 1 in 95% ethanol).

*Anal.* Calc'd for $C_{17}H_{15}NO_4S$: C, 61.99; H, 4.59.
Found: C, 61.77; H, 4.61.

This compound was hydrolyzed with sodium hydroxide, converted to the methyl ester with diazomethane, and oxidized with iodine to give *N,N'-dibenzoyl-L-cystine dimethyl ester* in 60% yield. A mixture melting point with an authentic sample confirmed its identity.

*N-Benzoyl-S-p-chlorobenzoyl-DL-cysteine* was similarly made in over 80% yield. It was purified from glacial acetic acid, m.p. 203–204°.

*Anal.* Calc'd for $C_{17}H_{14}ClNO_4S$: C, 56.12; H, 3.88.
Found: C, 56.19; H, 4.11.

*N,S-Dibenzoyl-DL-cysteine methyl ester* was similarly made from 2-phenyl-4-carboxymethyl-DL-oxazoline. The crude product was obtained in 82% yield, m.p. 112–117°. It was purified by dissolving in alcohol and adding petroleum ether; m.p. 119–120° (resolidified to melt at 135–136°).

*Anal.* Calc'd for $C_{18}H_{17}NO_4S$: C, 62.96; H, 4.99.
Found: C, 62.67; H, 5.03.

*N-Benzoyl-S-p-nitrobenzoyl-DL-cysteine* was made by heating 2-phenyl-4-carboxy-DL-oxazoline and *p*-nitrothiobenzoic acid together in dry dioxane. The crude product was obtained in 80% yield and was recrystallized from glacial acetic acid, m.p. 178.5–180.5°.

*Anal.* Calc'd for $C_{17}H_{14}N_2O_6S$: C, 54.54; H, 3.77.
Found: C, 54.47; H, 4.03.

*Hydrolysis of N,S-dibenzoyl-L-cysteine.* A number of attempts to hydrolyze this compound with 48% HBr gave crude cystine in not over 48% yield (based on SnS). Both sodium hydroxide and sodium methoxide rapidly removed the S-benzoyl group, and after oxidation to the disulfide the N-benzoyl group was removed by heating in 48% HBr for 4–7 hours at 100°. Whereas the yield from the methoxide hydrolysis was higher (50% based on SnS), the optical activity of the cystine was lower, $[\alpha]_D^{21}$ −172° (*c*, 0.6 in 1% HCl). Since a sample of L-cystine proved relatively stable under these same conditions of heating in acid, partial racemization in sodium methoxide is indicated.

N,S-Dibenzoyl-L-cysteine, 0.27 g., was dissolved in 1.7 ml. of 2 *N* NaOH and after a few minutes the solution was acidified and titrated with 0.2 *N* $KI_3$ solution. The product mixed with benzoic acid came out as a solid which was removed, water-washed, and then heated on the steam-bath with 2 ml. of 48% HBr. The solid dissolved in approximately an hour and benzoic acid separated as the hydrolysis proceeded. After 4 hours the suspension was cooled, water and excess pyridine added and the cystine allowed to form overnight. The cystine was collected, dissolved in a little diluted HCl and the solution filtered. Reprecipitation with pyridine afforded the amino acid. After an hour it was filtered and washed with water and alcohol, wt., 0.051 g. (42.5% based on SnS); $[\alpha]_D^{22}$ −212° (*c*, 0.5

in 1% HCl). A sample of commercial cystine (Merck) showed $[\alpha]_D^{22}$ −230° (*c*, 0.5 in 1% HCl).

*Anal.* Calc'd for $C_6H_{12}N_2O_4S_2$: C, 29.99; H, 5.03.
Found: C, 29.78; H, 5.02.

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SUMMARY

Two syntheses of cystine are presented. One is a modification of an old method and the other depends on the rearrangement of an oxazoline salt. Both are designed for the use of isotopic sulfur.

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