angiotensin II. Rupture of the aliphatic ring of proline causes a marked decrease in the pressor activity of the peptide. Since the removal of this ring would be expected to cause a marked change in the conformation of the C-terminus of the peptide, this evidence further suggests the importance of conformation in angiotensin structure for biological activity.

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**On Cysteine and Cystine Peptides. I. New S-Protecting Groups for Cysteine**

By Leonidas Zervas and Iphigenia Photaki

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The problem of the synthesis of unsymmetrical cystine peptides with two or more cystine -S-S- bridges is discussed. For a solution of this problem, the following requirements must be fulfilled: (a) cysteines bearing different S-protecting groups selectively removable must be available and (b) procedures must be worked out for preventing the rearrangement of cystine chains during synthesis until their final incorporation in a multimered ring system. Concerning the first of the above requirements, S-di phenylmethyI-L-cystine (I) and S-trityl-L-cystine (II) are proposed as the most suitable S-protected cysteines for the incorporation of cystine residues in a peptide chain. The S-trityl group can be easily split off with heavy metal salts at room temperature, whereas the removal of the S-di phenylmethyl group is also easily effected by the action of trifluoroacetic acid. The SH-groups thus liberated can be oxidized to the corresponding -S-S- derivatives. Several peptides of cysteine and cystine have been synthesized in this way.

**Introduction**

An attempt by Fischer and Gerngross to prepare monoglycyl- and monoleucyl-L-cystine through aminolysis of their respective monohaloacyl-L-cystine precursors resulted in the formation, in each instance, of a dipeptide which was not pure and appreciable amounts of cystine. This unusual aminolysis of an α-haloacylaminoc acid can be easily explained by the well-known fact that unsymmetrical open chain derivatives of cystine (Fig. 1) are not stable but rearrange very easily to the symmetrical ones. The existence of unsymmetrical cystine peptides, as in oxytocin and in vasopressin, may apparently be attributed to the fact that these compounds are of cyclic structure, the only cystine -S-S- bridge being implicated in the ring system. Most of the proteins can be considered, in principle, as unsymmetrical polypeptides of cystine. These proteins, i.e., insulin, whose structure has been elucidated by Sanger, et al., are more or less stable, because in this case more than one cystine -S-S- bridge holds the polypeptide chains together, forcing them to participate in a multimered ring system.

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**Contributions from the Laboratory of Organic Chemistry, University of Athens, Greece**

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For the synthesis of unsymmetrical cystine peptides with two or more cystine -S-S- bridges is discussed. For a solution of this problem, the following requirements must be fulfilled: (a) cysteines bearing different S-protecting groups selectively removable must be available and (b) procedures must be worked out for preventing the rearrangement of cystine chains during synthesis until their final incorporation in a multimered ring system. Concerning the first of the above requirements, S-di phenylmethyL-L-cystine (I) and S-trityl-L-cystine (II) are proposed as the most suitable S-protected cysteines for the incorporation of cystine residues in a peptide chain. The S-trityl group can be easily split off with heavy metal salts at room temperature, whereas the removal of the S-di phenylmethyl group is also easily effected by the action of trifluoroacetic acid. The SH-groups thus liberated can be oxidized to the corresponding -S-S- derivatives. Several peptides of cysteine and cystine have been synthesized in this way.

Owing to the carbobenzyloxy method as well as to various other methods the synthesis of common polypeptide chains (i.e., peptides of different amino acids including cysteine, symmetrical cystine peptides or peptides of the oxytocin type) is no longer a problem, especially since these methods have already been adapted to the peculiarities of some amino acids, as in the case of lysine and arginine and—what was more requisite—of cysteine-cystine. However, an inspection of the insulin -S-S- bridge system (Fig. 2) shows that an approach to the synthesis of unsymmetrical cystine peptides with two or more cystine -S-S- bridges would be facilitated if, in addition to the methods mentioned above, the following requirements could be met: (a) the availability at cysteines bearing different S-protecting groups (R,R') Fig. 3) which could be incorporated into a peptide chain;

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(1) A summary of this paper was presented at the 4th European Peptide Symposium, Moscow, August, 1961; L. Zervas, Collection Caschion, Commun., in press.

(2) This investigation was supported by the Royal Hellenic Research Foundation, to which we are greatly indebted.

(3) E. Fischer and O. Gerngross, Ber., 48, 1485 (1919).


these S-protecting groups must be selectively removable in such a way that the peptide bond and an already existing -S-S- bridge in the molecule would be removed by catalytic hydrogenation, the simultaneous hydrogenolytic cleavage of the -S-S- bridge cannot be excluded, since cystine, its ester and some cystine peptides have been catalytically reduced to the corresponding -SH derivatives. In recent years, many other S-protecting groups (i.e., the tetrahydrourylyl-, benzylthiomethyl, butyrophosphoryl- and p-chlorophenoxyethyl groups) have been used and the SH group has been incorporated, by interaction with acetone, in a 2,2-dimethylthiazolidine; all these groups and the thiazolidine ring as well can be split off not by hydrogenation but by other more or less mild methods. However, up to the present it is not always certain whether the removal of these protecting groups is not accompanied by side reactions.

For our purposes S-protecting groups are needed which can be removed by chemical means under mild conditions without affecting sensitive parts of the molecule. S-Diphenylmethyl(DPM)-L-cysteine (I) and S-trityl(Tr)-L-cysteine (II) appear to be such protecting groups. Their cysteine derivatives can be prepared easily by interaction of cysteine hydrochloride, dissolved in dimethylformamide, and diphenylmethyl chloride or trityl chloride without addition of any acid-binding agent. Under these conditions the amino group is not affected. On the other hand, N-tritylation by known methods of S-tritylcysteine (II) in the presence of diethylamine and of L-cysteine methyl ester in the presence of triethylamine afforded S,N-ditryl-L-cysteine (IIIA) and its methyl ester VII, respectively. Direct pertitylation of L-cysteine in the presence of diethylamine leads also to S,N-ditryl-L-cysteine (IIIA). From the mother liquors of this last substance, after treatment with acetic acid, an S-trityl-L-cysteine was isolated which melted at 202-205° and possessed [a]D \(-16^\circ\) in 0.1 N sodium hydroxide. Our S-trityl-L-cysteine crystallizes nicely but its melting point and optical rotation (m.p. 182°, [a]D \(-16^\circ\) in 0.1 N sodium hydroxide), even after many recrystallizations, were different from those reported. For comparison, pure S,N-ditryl-L-cysteine was N-detritylated with dilute acetic acid and an S-trityl-L-cysteine was obtained which exhibited the same specific rotation and had the same m.p. Our S-trityl-L-cysteine (II) is beyond any doubt analytically, chromatographically and optically pure.

In order to make the S-DPM- and S-Tr-cysteine suitable for peptide syntheses we prepared their esters (V, VI), as well as their N-trityl- (IIIA, IVa), N-carbobenzoxy- (IIIb, IVb) and N-
Formyl- (IIIc, IVc) derivatives. The esters were obtained either by the action of diphenylmethyl chloride or trityl chloride on L-cysteine methyl ester hydrochloride in dimethylformamide solution, or, as in the case of S-tritylcysteine methyl ester (VI), by the selective N-detritylation of the corresponding S,N-ditrityl ester17 VII with one equivalent of hydrogen chloride in methanolic solution.17,25 On the other hand, the N-carbobenzoxy derivatives IIIb and IVb can be obtained in good yields by reductive cleavage of dicarbobenzoxy-L-cystine (IIId) with zinc dust in hydrochloric acid, and by subsequent treatment of the reaction product with diphenylmethyl or trityl chloride. However, the usefulness of all the above-mentioned S-DPM- and S-Tr-cysteine derivatives for our purposes depends upon the possibility of splitting off the S-protecting groups under experimental conditions such that the lengthening of the peptide chain would not be hindered, in addition to the requirement that the peptide bond and an already existing -S-S- bridge remain intact.

Like S-benzylcysteine,11 S-tritylcysteine,14 and S-DPM-cysteine are cleaved with sodium in liquid ammonia to free cysteine. However, in contrast to the S-benzyl group,25,26 the S-Tr- and S-DPM-groups are split off by hydrogen bromide in acetic acid and by trifluoroacetic acid. Both of these reagents are known to be N-decarboxybenzoylating agents without effect on peptide bonds.25,26 More sensitive to HBr is the S-trityl group; dilute HBr in acetic acid (4 equivalents of 1 N HBr) detritylates S-tritylcysteine almost quantitatively within 3 minutes and at 8-10°; even 0.2 N HBr converts II to cysteine to an extent of approx. 75% within 5 minutes. The rate of cleavage of the S-trityl group is slightly slowed by acylation of the α-amino group. Thus, N-carbobenzoxy-S-tritylcysteine (IIIb) is detritylated with 0.2 N HBr to an extent of 65-75% within 5-15 minutes; because of the low concentration of HBr, N-decarboxybenzoylation is retarded and, after oxidation, N,N'-bis-carboxybenzoxycystine can be isolated in good yield. The cleavage of the S-DPM- group requires a higher concentration of hydrogen bromide (2 N HBr), higher temperature (about 55°) and longer reaction time (90 minutes). Even under these conditions the cleavage is seldom more than 50%. If the temperature is kept at 20° and the reaction time is reduced to about 20 minutes, the removal of the S-DPM group from S-DPM-N-carbobenzoxy-L-cysteine (IVb) amounts to an extent of only 8-10%, and it is easy to isolate S-DPM-cysteine (I) in high yield (85%).

Boiling trifluoroacetic acid very rapidly converts S-DPM-cysteine (I) and S-Tr-cysteine (II) quantitatively to cysteine, but in contrast to hydrogen bromide it splits off the S-protecting group from II a little more slowly than from I (1 in 15 minutes and II in 30 minutes); under the same conditions N-carbobenzoxy-S-DPM-cysteine (IVb) is transformed to cysteine only to an extent of 75% and this cannot be substantially increased even if the
boiling time is prolonged for 30–40 minutes. On the other hand, N-decarbonylirenecycloxylation of I by with 2 N hydrogen chloride in acetic acid followed by warming with trifluoroacetic acid leads to a quantitative conversion of I by to cysteine (cf. Experimental part). This “inhibitory” effect is not observed in the case of N-decarbonylirenecycloxy peptides in which I is not the N-terminal amino acid. 27

According to the literature, 28 S-tritylcysteine derivatives can be S-detritylated in the cold by hydrogen chloride in chloroform solution within a few minutes. In our opinion, this statement must be modified. It has been found that S-detritylation of S-tritylcysteine and of numerous derivatives of it (i.e., S-trityl-N-carbonycloxy-L-cysteine, S-trityl-N-benzoyl-L-cysteine methyl ester, S-trityl-dipeptide ester, etc.) by this method never exceeds 40–50% of cleavage more than 30 minutes treatment with HCl. Furthermore, one must always keep in mind the possible formation of a thiazoline ring during the action of strong acids 28 under anhydrous conditions. S-DPM-derivatives are not cleaved by hydrogen chloride in chloroform solution.

Tritylthiocarbinol and its S-derivatives are known to be sensitive to heavy metal salts. 29 In the case of these S-Tr-cysteine derivatives the splitting off of the S-trityl group can be accomplished almost instantly even at 0°C with an equivalent amount of silver nitrate-pyridine in alcohol solution. The silver mercaptide and trityl ether are formed almost quantitatively; finally, the free sulfhydryl derivative can be liberated from the mercaptide with one equivalent of hydrogen halogenide in chloroform, silver chloride forming the thiocarbonyl-

\[ \text{AgNO}_3 + 3 \text{H}_2 \text{N}_2 \text{H}_2 \text{N}_2 \text{NO}_2 \text{H} \rightarrow \text{AgCl} + 3 \text{NO}_2 \text{H} \text{H}_2 \text{N}_2 \text{H}_2 \text{N}_2 \text{NO}_2 \text{H} \]

silver mercaptide and trityl ether are formed almost quantitatively; finally, the free sulfhydryl derivative can be liberated from the mercaptide with one equivalent of hydrogen halogenide in chloroform, dimethylformamide etc. 30 An illustrative example is the selective detritylation of S-N-detrityl-cysteine methyl ester (VII). S-Detritylation takes place with one equivalent of hydrogen chloride in methanol with formation of VI, whereas the S-trityl group is selectively removed by silver nitrate-pyridine. The mercaptide VIII thus formed is converted to the sulfhydryl derivative IX which can be oxidized to N,N'-bistrityl-L-cystine dimethyl ester (X). S-Trityl-N-benzoyl-L-cysteine methyl ester is also quantitatively S-detritylated with silver nitrate-pyridine forming silver mercaptide of N-benzoyl-L-cystine methyl ester. S-Detritylation can also be achieved by mercury chloride, but using it is of no advantage. The S-DPM-group is not affected at all by heavy metal salts as of silver or mercury.

Mercury and silver salts form insoluble mercaptides with cystine, 31, 32 by dismutation, the over-all reaction being:

\[ 2 \text{R}-\text{S}-\text{S}-\text{R} + 3 \text{Ag}^+ + 2 \text{H}_2 \text{O} \rightarrow 3 \text{Ag}_2 \text{S} + \text{R} \text{SO} + 3 \text{H}^+ \]

N,N'-bisacetyl cystines as well as oxidized gluthione also react with these metal salts in the same manner, 31 although slowly even at 37°C, whereas N,N'-bisacetyl- 32 and N,N'-bisbenzoyl-cystine diester, N,N'-bis-carbonycloxy cysteinyldiglycine diester and other similar derivatives are resistant to these reagents. Apparently, the blocking of the amino and the carboxyl groups either strongly suppresses or fully inhibits the cleavage of the S-S-cystine bond by silver or mercury salts.

Since the removal of the S-trityl group is almost instantaneously affected even at 0°C with the equivalent amount of silver salt, there is no danger of breaking or-S-S- bridges already present in the same molecule. Therefore, this procedure constitutes the method of choice for the S-detritylation. On the other hand, for the removal of the S-DPM group, boiling with trifluoroacetic acid is recommended, especially since during this treatment no formation of a thiazoline ring takes place.

It is evident that S-trityl and S-DPM-L-cysteine are useful intermediates for the synthesis of symmetrical or oxytocin like peptides. Furthermore, their introduction fulfills the first requirement (a) for the synthesis of unsymmetrical cysteine peptides with at least two -S-S- bridges.

The following examples of the synthesis of some peptides are presented simply to illustrate the possibilities of the incorporation of cysteine into a peptide chain using S-trityl- and S-DPM-cysteine.

Depending on the type and the size of peptide to be synthesized one must use N-formyl, N-carbonyl, N-trityl, N-dibenzyloxophosphoryl or N-trifluoroacetyl derivatives 3 of the above S-protected cysteines. By coupling of such N-protected S-trityl- or S-DPM-cysteines with glycine ester by known methods, S-trityl-N-formyl-L-cysteinylglycine ethyl ester (XI), S-trityl-N-carbonyloxy-L-cysteinylglycine ethyl ester (XII), N,S-ditrityl-L-cysteinylglycine p-nitrophenyl ester (XIII), S-diphenylmethyl-N-carbonyloxy-L-cysteinylglycine ethyl ester (XIV) and S-diphenylmethyl-N-formyl-L-cysteinylglycine ethyl ester (XV) were obtained. After treatment of compound XI with two equivalents of hydrogen chloride in alcohol the formyl group was cleaved and the hydrochloride of the corresponding dipeptide derivative was obtained and this was coupled with carbobenzyloxy-L-phenylalanine forming the tripeptide derivative XVI. S-Detritylation of compound XII with silver nitrate-pyridine, removal of the silver with hydrogen chloride and oxidation of the compound formed (XVII), afforded N,N'-bis-carbonyloxy-L-cysteinylglycine ethyl ester (XVIII). Similarly, S-trityl-tripeptide ester XVI is transformed in good yield first to XIX and after oxidation to the cysteine peptide ester XX. Furthermore, the ditryptidylpeptide ester was selectively S-detritylated with one equivalent of hydrochloric acid affording a dipeptide derivative XXI which, as an “active” ester, offers, in principle, two alternatives for the lengthening of the peptide chain, i.e., either at the amino or at the carboxyl end. Saponification of XIV to XIVa and N-decarbonylirenecycloxylation of the acid formed yields XIVb which upon
treatment with trifluoroacetic acid afforded cysteinylglycine and after oxidation L-cystinylglycine (XXII). On the other hand, N-deformylation of the S-DPM-dipeptide ester XV followed by coupling with N-trifluoroacetyl-L-valine yields the N,S-protected tripeptide ester XXIII; the S-DPM group is selectively removed from this substance by trifluoroacetic acid yielding almost quantitatively the cysteinyl peptide XXIV and, upon oxidation, the corresponding cystinyl peptide XXV.

Other S-protecting groups for cysteine suitable for our purposes are the S-acyl groups. After the incorporation of S-acetyl-L-cysteines (i.e., S-acetyl-, S-benzoyl-, S-carbobenzoxy-L-cysteine) in a peptide chain by means of their corresponding N-formyl or N-carbobenzoxy derivatives, or the S-acyl groups as ester groups can be removed, in this particular case, rapidly with very dilute alkali or with dilute ammonia; on the other hand, they are resistant, with the exception of the S-acetyl group, to hydrogen bromide in acetic acid. Examples of such syntheses will be described in one of our next communications.

Commercially available cystine and cysteine are usually not optically pure; moreover their purification, especially that of cystine, is quite difficult. The same applies to their esters. The preparation of all these compounds in a state of high purity, both chemical and optical, was greatly facilitated by the observation that cysteine, but not cystine, forms a quite insoluble salt with hydrogen sulfide and the cysteine hydrobromide. L-Cysteine obtained this way possesses a higher specific rotation than that reported in the literature. We prefer to store L-cysteine in the form of its tosylate, because recrystallization from water is sufficient to remove any L-cysteine tosylate formed by air oxidation during storage. On the other hand, oxidation of L-cysteine tosylate in aqueous solution affords very pure L-cysteine possessing a specific rotation of at least $-212^\circ$ (in $N\ HCl$). This sequence of reactions constitutes a simple way of purifying L-cysteine.

From purified L-cysteine it is possible easily to obtain the corresponding methyl and benzyl esters. Pure L-cysteine can also be converted easily to its crystalline methyl ester hydrochloride, but the product must be recrystallized in a special way (cf. Experimental part). The best way to prepare L-cysteine benzyl ester is the reductive cleavage of L-cystine benzyl ester with zinc--hydrochloric acid, followed by the isolation of the cysteine benzyl ester formed as its mercury mercaptide; the removal of mercury with hydrogen sulfide affords pure benzyl ester hydrochloride.

### Experimental

For the coupling reactions anhydrous reactants and dry solvents were used; the ether used was free of peroxides. Freshly prepared solutions of pure hydrogen bromide, free of bromine, were always used. Evaporations were carried out in vacuo at 35–40°C. The melting points are not corrected.

Prior to analysis the compounds were dried at 58°C in high vacuum over phosphorus pentoxide. Cysteine and its derivatives were determined by titration with 0.1 $N$ iodine at pH 5–6; the method was sufficiently accurate for the purposes of this work. Prior to titration, strong acidic solutions were adjusted to the above pH by addition of sodium acetate.

The $R_f$ values were determined by thin-layer chromatography in 1-butanol–acetic acid–water–pyridine. The high vacuum over phosphorus pentoxide. Cysteine and its derivatives were determined by titration with 0.1 $N$ iodine at pH 5–6; the method was sufficiently accurate for the purposes of this work. Prior to titration, strong acidic solutions were adjusted to the above pH by addition of sodium acetate.

The $R_f$ values were determined by thin-layer chromatography in 1-butanol–acetic acid–water–pyridine. L-Cystine Tosylate.—To a suspension of 24 g. (0.1 mole) of L-cystine in 80 ml. of concd. hydrochloric acid and 2 ml. of 85% sulfuric acid, 30 ml. of saturated sodium bisulfite solution was added and the mixture was boiled for 30 minutes. The solution was then evaporated to dryness, and the residue was recrystallized several times from methanol.

### References

3. N-Carbobenzoxy-S-acetyl-L-cysteine, m.p. 110–117°C, [α]D = $-52.4^\circ$ (c 2, ethanol); N-carbobenzoxy-S-benzyl-L-cysteine, m.p. 139°C, [α]D = $-26.4^\circ$ (c 0.5, ethanol); N-formyl-S-benzyl-L-cysteine, m.p. 165°C, [α]D = $-40^\circ$ (c 1, ethanol); N-formyl-S-carbobenzoxy-L-cysteine, m.p. 139–140°C, reported $141–142^\circ$ (unpublished data of work conducted in our laboratory by Mr. N. Ghelis).
4. Microanalyses were carried out by Mr. H. Mantzos in the Analytical Laboratory of the Royal Hellenic Research Foundation.
40 ml. of water there was added 8 g. of zinc dust in many portions over a period of 10 minutes with vigorous stirring and cooling at 5°-10°. The mixture was stirred for 5 minutes more, filtered, and the filtrate was added to a solution of 44 g. of p-toluenesulfonic acid hydrate in 20 ml. of water. To the resulting L-cystine tosylate separated out; after standing for many hours in the ice-box it was collected, washed with a small amount of cold 10% p-toluenesulfonic acid solution, recrystallized, when still wet, from the necessary amount of hot 10% p-toluenesulfonic acid solution and dried over calcium chloride. The yield was 48-58 g. (75-90%) depending on the quality of tosylate used. Whereafter, for L-cystine [α]D = -210° (c 1, N HCl)4 was used the yield was at least 90%; with L-cystine [α]D = -190° the yield dropped to 75%. The substance, melted at 223-225°, [α]D +4.2° (c 10, dimethylformamide). Iodine titration revealed that the substance was composed entirely of cystine tosylate.

Purification of L-Cystine.—Commercial preparations of cystine having specific rotations lower than the generally accepted value of [α]D = -210° (in N HCl), i.e., values between -195° and -205°, were reduced with zinc dust-hydrochloric acid and converted to L-cystine tosylate as described above. The pH of the 20% water solution of the tosylate thus obtained was adjusted at 8.5. Upon addition of several hundred cubic centimeters as followed by the pH adjustment at 5.5 optically pure L-cystine separated out (recovery 68-75%); [α]D = -210° to -214° (c 1, N HCl). L-Cystine Dimethyl Ester Dihydrochloride.—Pure L-cystine was esterified by the thionyl chloride method.41 The crude ester hydrochloride was recrystallized by dissolving in methanol and precipitating again by addition of ether. The yield was 95%, m.p. 181-182°, [α]D = -16.6° (c 1, N HCl).42 L-Cystine Benzyl Ester Dihydrochloride.—The suspension of the tosylate dissolved in 6 ml. of methanol and precipitating again by addition of ether. The yield was 93%, m.p. 73-75° (reported46 76-78°). The substance consisted entirely of L-cysteine ester.

Anal. Calcd. for C20H24N2O2S2: C, 40.9; H, 5.15; N, 5.68; S, 14.33.

Hydrogen sulfide was bubbled through a solution of 7.2 g. (0.015 mole) of the above mercaptide in 15 ml of dimethylformamide until all the mercury was converted to mercury sulfide. After adding 10 ml. of ethyl alcohol the last mercury sulfide was removed by centrifugation and was washed with a few ml of ethyl alcohol. Upon addition, the mercuric salt was suspended in a solution of 1.2 g. (0.01 mole) of selebic acid in 4 ml. of benzene. The mixture was cooled to 0° and then 10 ml. of ethyl alcohol was added. The solution was filtered off and washed with cold isopropyl alcohol. The residue was dried in vacuo.

Purification of L-Cystine Methyl Ester Hydrochloride.—The esterification of L-cystine methyl ester hydrochloride by the thionyl chloride method could be successfully accomplished as follows: L-Cysteine methyl ester hydrochloride (45 g.) of m.p. around 100° was dissolved in 90 ml of hot methanol. After adding 225 ml of isopropyl alcohol most of the mercury was removed by distillation into vacuo at 90°. During the distillation, and especially upon cooling in the ice box, pure ester hydrochloride separated; it was collected, washed with ether saturated with hydrogen chloride the dinitrohydrochloride suspension of the tosylate disappeared. The ethyl acetate was removed by distillation in vacuo at 90°. The residue was dissolved in 40 ml of hot methanol. On cooling, 8 g. of crude mercapptide of L-cystine benzyl ester hydrochloride separated; it was recrystallized from a mixture of dimethylformamide-methanol-water (1-4-5) and washed with a small amount of cold methanol. The yield on pure mercapptide was 5.4 g. (80%), m.p. 157-158°.


L,N'-Bis(carbzenoxy)-L-cystine Dimethyl Ester.—Into the mixture of 30 ml of saturated solution of potassium hydroxide carbonate in water and 30 ml of chloroform, 3.4 g. (0.1 mole) of L-cystine methyl ester dihydrochloride and 4 ml of carbonzolylbenzoxycarbonyl chloride were added. After shaking the mixture for about 30 minutes at 4° the water layer was discarded; to the chloroform solution 1 ml of pyridine was added and then it was washed successively with dilute sulfuric acid, water and dilute potassium hydrogen carbonate, dried over sodium sulfate and evaporated to dryness. A residual syrup was obtained which crystallized after the addition of petroleum ether; it was recrystallized from hot methyl alcohol. The yield was 5.1 g. (95%), m.p. 73-75° (reported47 74-75°). L-L-Cysteine Benzyl Ester Dihydrochloride.—The suspension of 4.9 g. (0.01 mole) of L-cystine dibenzyl ester dihydrochloride in 25 ml of dioxane and 3.5 ml of conc. hydrochloric acid there was added 1 g. of zinc dust in many portions over a period of 15 minutes with vigorous stirring. Undissolved zinc was filtered off and washed with 5 ml of dioxane; to the combined filtrates 5.5 g. of mercury chloride was added and the solution was concentrated to dryness (in methanol).

L-Cystine Benzyl Ester Dihydrochloride.—To the suspension of 4.9 g. (0.01 mole) of L-cystine dibenzyl ester dihydrochloride in 25 ml of dioxane and 3.5 ml of conc. hydrochloric acid there was added 1 g. of zinc dust in many portions over a period of 15 minutes with vigorous stirring. Undissolved zinc was filtered off and washed with 5 ml of dioxane; to the combined filtrates 5.5 g. of mercury chloride was added and the solution was concentrated to dryness (in methanol).

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N. N'-Bis-carcobenzoxy-L-cystine dibenzyl ester was obtained as described for the preparation of the corresponding dimethyl ester. After recrystallization of the product from ethanol the yield was 75%, m.p. 78-80° reported,46 m.p. 79°, [α]D +45.8° (c 1.5, chloroform).

Found: N, 6.05; S, 10.43.

N. N'-Bisentzenoyl-L-cystine dibenzyl ester was prepared as described for the preparation of the dimethyl ester. After recrystallization of the product from ether the yield was 80%, m.p. 90-92°, [α]D +54.1° (c 3, chloroform).

Found: N, 4.51; S, 9.25.

S-Diphenylmethyl-L-cystine (I).—The solution of 29.3 g. of L-cysteine tosylate or 15.7 g. (0.1 mole) of anhydrous diphenylmethyl chloride and 20.5 g. (0.1 mole) of diphenylmethyl chloroform in 30 ml. of dimethylformamide was heated under nitrogen for 2 hours and then allowed to cool. Pyridine (15 ml.), water (25 ml.) and ethanol (200 ml.) were then added, and the mixture was heated for 10 minutes under nitrogen. After cooling in the ice-box for 2 hours, the crystalline precipitate of I was collected and washed with ethanol; yield 15 g. (55%), needles of m.p. 186-190°.

The substance was purified by dissolving it in a mixture of 75 ml. of hot ethanol and 10 ml. of 5 N HCl, and adding 8 ml. of pyridine (recovery 90%); m.p. 202-205°, [α]D +16.9° (c 2, ethanol).

Anal. Calcd. for C₂₃H₂₀N₂O₂S: N, 4.64; S, 5.19.
Found: N, 4.78; S, 5.56.

Removal of the S-Diphenylmethy1 Group. (a).—The reduction of I (2.87 g., 0.01 mole) with sodium in liquid ammonia and the isolation of the reaction products was effected in the same manner as in the case of S-benzylcysteine.44 Diphenylmethane (1.5 g., 90%, m.p. 24-26°) and, after air oxidation, L-cystine (0.9 g., 80%) was isolated. On the other hand, iodine titration of an aliquot of the solution, prior to the air oxidation, indicated a 97% formation of cysteine during the reductive cleavage.

(b).—The solution of 0.57 g. (0.002 mole) of I and 0.4 g. of 3.5 ml. of trichloroacetic acid was refluxed for 20 minutes and then it was evaporated to dryness. Water was added to the residue and the mixture was extracted with ether. Oxidation with iodine solution revealed that the water-soluble fraction consisted of cystine. The water solution was washed with ether, reduced and the ether solution was washed with water until the water layer became neutral to Congo red; the chloroform layer was dried, evaporated to dryness in vacuo and the residue was dissolved in ethyl acetate-ether (1:1). Some undissolved material was removed by filtration; upon adding 2 ml. of diethylene the corresponding salt of IVA crystallized out (recovery 90%), m.p. 187-188°, [α]D +42.2° (c 2, chloroform).

Anal. Calcd. for C₂₃H₂₀N₂O₂S: N, 6.43; S, 11.32.
Found: N, 6.32; S, 11.15.

Removal of the S-Diphenylmethyl Group. (a).—To the solution of 5.7 g. of II in 20 ml. of 1 N sodium hydroxide, 4 ml. of carboxybenzylchloride and 30 ml. of 1 N sodium hydroxide were added in 4 portions over a period of 20 minutes with stirring and cooling at approx. 5° then by stirring at room temperature for 15 minutes. During the reaction the sodium salt of IVB precipitated out; it was redissolved by addition of water. After standing for 15 minutes at room temperature the solution was diluted with water, extracted twice with ether and then added with sulfuric acid. The mixture was extracted with ether, the ether solution was washed first with just the necessary amount of 1 N sodium acetate solution until the water layer became neutral to Congo red and then with water and finally was dried over sodium sulfate. Upon adding cyclohexylamine to the ether solution the corresponding salt of IVA crystallized out; yield 8.1 g. (90%), m.p. 139° and 145-146° after recrystallization from ethanol, [α]D +3.8° (c 2, ethanol).

Found: N, 5.56; S, 6.05.

Upon adding dilute sulfuric acid to the hot methanol solution of the above salt, 1VB precipitated as a sirup which crystallized after standing in the ice-box for several hours; the yield was 90%, m.p. 102-103°, after recrystallization from diisopropyl ether, [α]D +2.0°. (c 0.1, ethanol).

Found: N, 3.31; S, 7.50.

(b).—Three grams of zinc dust was added to the solution of 5.1 g. (0.01 mole) of bis-carbobenzoxy-L-cystine in 20 ml. of methanol and 6.5 ml. of conc. hydrochloric acid with continuous vigorous shaking. The addition was effected in ten equal portions, within a period of 10-15 minutes at 5-10°. Undissolved zinc was then filtered off and washed with methanol. The combined filtrates were concentrated in vacuo.

N. N'-Decarbobenzoxylation.—The above salt of IVB (3.5 g. 30%) separated out, m.p. 140° and 142-145°, after recrystallization from ethanol.

N. N'-Decarbobenzoxylation. — The above salt of IVB (1.6 g., 0.003 mole) was dissolved in 12 ml. of 2 N sodium hydroxide in acetic acid. After standing for 20 minutes at room temperature, the solution was evaporated to dryness in vacuo. Almost complete removal of the acetic acid was achieved by the addition of dioxane and recrystallization in vacuo. The residue together with 0.4 g. of phenol was dissolved in 3.5 ml. of triethylacetic acid; after refluxing for 20 minutes, the solution was worked up as described above. Iodine

acetone; the yield of the addition of 500 ml. of 10% sodium acetate solution, 111 p. 159-160°. The yield of the corresponding hydrochloride was 14 g. (91%), m.p. 145-147°, [α]D +18.1° (c 2, ethanol).

Anal. Caled. for C11H20N02S: C, 54.5%; H, 5.3%; S, 10.16. Found: C, 54.0; H, 5.2; S, 10.2.

Upon adding 1.3 ml. of cyclohexylamine to a solution of 1.8 g. of I in ethyl acetate, C (35 g.), 95% of crystalline cyclohexylammonium salt of I was separated, m.p. 170-172° after recrystallization from alcohol, [α]D +29.9° (c 1, ethanol).

Anal. Caled. for C11H20N02S: C, 54.5%; H, 5.3%; S, 10.16. Found: C, 54.0; H, 5.2; S, 10.2.

From this salt the corresponding free acid I was recrystallized; it melted at 147-148° and possessed [α]D +18.1° (c 2, ethanol).

S-Diphenylmethyl-L-cysteine Methyl Ester Hydrochloride (V).—(a).—The solution of 1.7 g. (0.01 mole) of pure L-cysteine methyl ester hydrochloride and of 2.1 g. (0.01 mole) of diphenylmethyl chloride in 7 ml. of dimethylformamide was heated at 90° for 2 hours under an atmosphere of nitrogen. Upon adding ether an oil precipitated; it was treated with saturated potassium hydrogen carbonate and immediately thereafter extracted twice with ether. The ether layer was washed with a small amount of water and dried over sodium sulfate; upon adding ether saturated with hydrogen chloride, compound I precipitated as needles. The yield was 1.1 g. (33%), m.p. 150-160° and 182° after recrystallization from methanol-acetone, [α]D +5.5° (c 3, methanol).

Anal. Caled. for C11H20N02S: C, 54.5%; H, 5.3%; S, 10.16. Found: C, 54.0; H, 5.2; S, 10.2.

(b).—N,N′-Biscarbobenzoxy-L-cystine dimethyl ester (3.4 g., 0.01 mole) was reduced with zinc dust as described for carboxbenzoxyl-L-cystine. To the solution of the reduction product in anhydrous benzene, 2.8 ml. of triethylamine and 5 g. of diphenylmethyl bromide were added. After standing for 4 days at room temperature the benzene was removed by distillation in vacuo, and ether was added. The mixture of bromonium chloride was removed, the ether was evaporated, then the filtrate was successively washed with water, 20 ml. of 4% sodium hydroxide, and again with water. After drying over sodium sulfate, it was evaporated to dryness, and the residue was dissolved in 500 ml. of anhydrous ether containing approx. 14 g. of hydrogen bromide. Upon standing for 2 days in the ice-box the hydrobromide of S-diphenylmethyl-L-cysteine methyl ester separated out (2.7 g.); it was converted to the corresponding hydrochloride by dissolving in a small amount of water, adding potassium hydrogen carbonate, extracting the free ester with ether, and adding ether saturated with hydrochloric acid to the ether extract. The yield of V was 1.7 g. (28%), m.p. 159-160°.

(c).—Compound I (2.9 g., 0.01 mole) was esterified in the same manner as I as the diethylammonium salt; the yields were 2.8 ml. of triethylamine and 5 g. of diphenylmethyl bromide were added. The solution of 1.7 g. (0.01 mole) of pure L-cysteine tosylate or 15.7 g. (0.1 mole) of anhydrous L-cysteine hydrochloride in 60 ml. of dimethylformamide was heated at 90° for 2 hours under an atmosphere of nitrogen. Upon adding 1.5 ml. of cyclohexylamine to a solution of 1.8 g. of IVa in ethyl acetate, 2 g. (95%) of crystalline N,N′-bis-carbobenzoxy-L-cystine was isolated as the diethylammonium salt; the yield was 5.1 g. m.p. 191-192° and 192-193° after recrystallization from ethyl acetate, [α]D +68.8° (c chloroform), reported3 71.7° (c 2).

Anal. Caled. for C31H32N02S: C, 72.85; H, 5.84; S, 9.31. Found: C, 72.8; H, 5.9; S, 9.4.

N-Detritylation. —A solution of 3.4 g. (0.005 mole) of the diethyllammonium salt of IVa in 10 ml. of acetic acid and 2 ml. of water was heated for 2 minutes on the steam-bath. On cooling, triphenylcarbinol separated out; upon adding ether the carbinol was dissolved and I was precipitated as needles. The yield was 2.3 g. (70%), m.p. 191-192°, [α]D +68° (c 2, chloroform).

Anal. Caled. for C11H20N02S: X, 4.18; S, 4.71. Found: X, 4.29; S, 4.95.

(b).—L-Cysteine hydrochloride hydrate (8.75 g., 0.05 mole) was pertritylated as described.8 The ether was free of peroxides. The isolation of the diethyllammonium salt of IVa was carried out as described in the case of IVa. The yield was 24° (70%), m.p. 191-192°, [α]D +68° (c 2, chloroform).

N-Detritylation of IIIa. —A solution of 3.4 g. (0.005 mole) of the diethyllammonium salt of IIIa in 10 ml. of acetic acid and 2 ml. of water was heated for 2 minutes on the steam-bath. On cooling, triphenylcarbinol separated out; upon adding ether the carbinol was dissolved and II precipitated. The yield was 3.3 g. (92%), m.p. 181-182° unchanged even after repeated recrystallizations; [α]D +108° (c 1.45, 0.04 N ethanolic hydrogen chloride).

N-Carboxbenzoxyl-S-trityl-L-cysteine (IIIb) was prepared (a) by the carboxbenzoxylation of II dissolved in a mixture of dioxane-1 N sodium hydroxide (1:1) and (b) by refractionation of biscarboxbenzoxyl-L-cystine with zinc dust-hydrochloric acid followed by S-tritylation, in both cases in the same manner as IVb from I. Compound IIIb was isolated as the diethyllammonium salt of IIIb was (27%) and (b) 55%; m.p. 168° and 169° after recrystallization from acetone; [α]D +21.4° (c 5, methanol).


N-Detritylation. —The diethyllammonium salt of IIb (1.14 g., 0.002 mole) was dissolved in 30 ml. of 0.2 N HBr in acetic acid and the solution was kept for 15 minutes at room temperature. Then, sodium acetate solution was added until the solution became neutral to congo red paper and the mixture was oxidized with 0.1 N iodine; 15 ml. of this iodine solution was consumed corresponding to 75% S-detritylation. The oxidized solution was concentrated to dryness and the residue was treated with chloroform and dilute sulfuric acid. The chloroform solution was washed repeatedly with water until the water extract was neutral to congo red paper, dried and evaporated to dryness. Upon dissolving the residue in ethyl acetate and adding cyclohexylamine, 0.310 g. (30%) of N,N′-bis-carboxbenzoxyl-L-cystine cyclohexylammonium salt was obtained, m.p. 178° and 181° after recrystallization from ethanol, [α]D +182° calculated as bis-carboxbenzoxyl-L-cystine (c 3.5, 0.1 N hydrochloric acid in ethanol).


For comparison purposes pure N,N′-bis-carboxbenzoxyl-L-cystine repeatedly recrystallized from ethanol was prepared by mixing ethyl acetate solutions of pure bis-carboxbenzoxyl-L-cystine and cyclohexylamine; the yield was 90%, m.p. 184° after recrystallization from ethanol. The mixed m.p. of this
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material with that of the product from the S-detritylation and reaction described above was 184°.

N-Formyl-S-trityl-L-cysteine (IIIc) was prepared by formylation of II in the same manner as IVc from I. The yield was 91%, m.p. 103° unchanged by recrystallization from anhydrous methanol. [α]D +59° (c 2.6, ethanol).

Anal. Calcd. for C46H34NO4S: N, 6.05; S, 6.05. Found: N, 6.00; S, 6.09.

Upon adding diethylamine to the solution of IIIc in ethyl acetate—ether the diethylanmonium salt precipitated; the yield was 89%; m.p. 163° [α]D +56° (c 2, ethanol).

Anal. Calcd. for C46H34NO4S: N, 6.05; S, 6.05. Found: N, 6.10; S, 6.15.

Upon condensation of II with that of the product from the S-detritylation described above was 140°.

A solution of 0.481 g. (0.001 mole) of the N-benzoyl derivative in 5 ml. of chloroform, was treated with pure anhydrous hydrogen chloride (bubbled in) for 30 minutes. Iodine titration revealed a 90% conversion to N-benzoyl-L-cysteine methyl ester.

S-Detritylation of N-S-Ditryptyl-L-cysteine Methyl Ester.

-1-Cysteine methyl ester hydrochloride (1.7 g., 0.01 mole) was perbenzoylated in quantitative yield. To the solution of the sirupy N,S-ditryptyl derivative VII thus obtained in 55 ml. of acetone, there was added a solution of 1.7 g. (0.01 mole) of silver nitrate and 0.95 ml. of pyridine in 75 ml. of methanol. The silver mercaptide of N,S-ditryptyl cysteine methyl ester (VIII) precipitated immediately. After standing for 30 minutes at room temperature and adding 30 ml. of methanol, the mixture was concentrated to a small volume and placed on a steam bath. The residue was filtered and washed with methanol; yield 4.55 g. (95%).

Anal. Calcd. for C29H27N03S: N, 7.50; S, 8.38. Found: N, 7.45; S, 8.35.

N-Benzoyl-S-trityl-L-cysteine Methyl Ester. (a).—A solution of L-cysteine methyl ester hydrochloride (0.85 g., 0.005 mole) and 1.4 g. of trityl chloride in 5 ml. of dimethylformamide was kept for 48 hours at room temperature. Upon adding ether, oily S-trityl-L-cysteine methyl ester hydrochloride VI precipitated; it was purified in the same manner as the corresponding S-diphenylmethyl derivative V and was obtained also as sirup. The yield was 0.6 g. (30%); this product was benzoylated with benzoyl chloride in pyridine solution yielding 0.6 g. (85%) of the above N-benzoyl derivative, m.p. 132–133° after recrystallization from methanol, [α]D +11.4° (c 3, chloroform).

Anal. Calcd. for C30H27N03S: N, 7.50; S, 8.38. Found: N, 7.45; S, 8.35.

To the solution of 2.4 g. (0.005 mole) of the mercaptide VIII in 20 ml. of anhydrous chloroform, 1 ml. of anhydrous dimethylformamide was kept for 48 hours at room temperature and adding 10 ml. of methanol and cooling, 2.4 g. (90%) of triphenylmethyl ether (m.p. 81–82°) was obtained. The filtrate was evaporated to dryness. The residue was triturated with anhydrous ether; the sirupy residue was triturated with anhydrous ether; the sirupy N,S-ditrityl-L-cysteine methyl ester hydrochloride (VI) thus obtained in 25 ml. of chloroform, 1 ml. of anhydrous dimethylformamide was kept for 48 hours at room temperature and adding 50 ml. of methanol, the mixture was concentrated to a small volume of approx. 30 ml. The mercaptide was collected and washed with methanol; yield 4.55 g. (95%).

Anal. Calcd. for C29H27N03S: N, 7.50; S, 8.38. Found: N, 7.45; S, 8.35.

N-Formyl-S-trityl-L-cysteinylglycine Ethyl Ester (XI).—To a solution of 0.481 g. (0.001 mole) of the N-benzoyl derivative prepared above, 0.7 g. of silver nitrate and 0.95 ml. of pyridine in 75 ml. of anhydrous dichloroethane, m.p. 132–133° after recrystallization from ethyl-methanol (reported yield 73%).

N-Formyl-S-trityl-L-cysteinylglycine Ethyl Ester (XII) was prepared in the same manner as the corresponding N-formyl derivative XI by coupling of IIIb with glycine ethyl ester by the carbodiimide method. After the evaporation of the chloroform solution to dryness, the residue was twice recrystallized from ethanol; yield 3.9 g. (86% calculated on glycine ethyl ester used), m.p. 78–77°. After repeated recrystallizations from a small amount of ethyl acetate the m.p. was raised to 78–79°, [α]D +30.5° (c 4, ethanol).

Anal. Calcd. for C40H34NO7S: N, 5.50; S, 5.72. Found: N, 5.45; S, 5.61.

N-Carbobenzoxy-S-trityl-L-cysteinylglycine ethyl ester (XIII) was prepared in the same manner as the corresponding N-formyl derivative XI by coupling of IIIb with glycine ethyl ester by the carbodiimide method. The free and the residue was then heated for 2 minutes on the steam bath. The silver chloride was removed by centrifugation and the clear solution was concentrated in vacuo to a volume of 4–5 ml. Upon benzoylation with benzoyl chloride in the presence of potassium hydroxide carbonate, followed by addition of water, 1.3 g. (78%) of N-S-trityl-L-cysteine methyl ester was obtained; m.p. 198° and 141° after recrystallization from ethanol, [α]D +55.7° (c 2, chloroform).

N-S-Ditriethyl-L-cysteinylglycine p-Nitrophenyl Ester (XIII).—To the suspension of 7.5 g. (0.01 mole) of diethylammonium salt of IIIa in water, there was added 14 ml. of 1 N sulfuric acid and the mixture was immediately thereafter extracted with 150 ml. of ether. The ether layer was repeatedly washed with water until the water extracts were neutral to congo red paper, dried over sodium sulfate and evaporated in vacuo to dryness. The residue consisting of the free IIIa was dissolved in chloroform and this solution was mixed with a chloroform solution of 2.8 g. of glycine p-nitrophenylester hydrobromide and 1.4 ml. of triethylamine; N,N'-dicyclohexylcarbodiimide (2.2 g.) was added to the mixture. The peptide formed (XIII) was isolated as described for the isolation of XI and XII, with the exception that the chloroform solution was washed only with water. The crude crystalline product XIII was twice recrystallized by slow evaporation of a solution in acetone-ethanol (1:1); yield 2 g. (25%), m.p. 184-185°, [α]D +62.4° (c 0, chloroform).

Anal. Calcd. for C₂₀H₂₄N₂O₁₁S: N, 5.49; S, 4.08. Found: N, 5.49; S, 4.08.

N-Carbobenzyoxys-diphenylmethyl-L-cysteinylglycine Ethyl Ester (XIV).—To a solution of 2.1 g. (0.004 mole) of XIV in ethyl acetate. The ethyl acetate extract was repeatedly evaporated with water, acidified with sulfuric acid and extracted with a chloroform solution of 0.46 g. of glycine ethyl ester in 20 ml. of tetrahydrofuran and 5 ml. of 1 N hydrochloric acid; N,N'-dicyclohexylcarbodiimide (2.2 g.) was added to the mixture. The peptide formed (XIVa) was isolated as described for the isolation of XI and XII, with the exception that the chloroform solution was washed only with water. The crude crystalline product XIV was twice recrystallized from ethanol, 1.7 g. (75%) of XIV, m.p. 117-118°, [α]D -30°.

Anal. Calcd. for C₂₀H₂₄N₂O₁₁S: N, 5.84; S, 0.62. Found: N, 5.57; S, 6.62.

N-Carbobenzyoxys-diphenylmethyl-L-cysteinylglycine Ethyl Ester (XIVb).—The solution of 2.1 g. (0.004 mole) of XIVb in ethyl acetate was treated with 0.4 ml. of ethyl chlorofosmate in chloroform. After standing for several hours at room temperature the solution was successively washed with hydrochloric acid and potassium hydrogen carbonate and water; 1 ml. solution was evaporated to dryness and the crystalline residue (XIVi) was recrystallized from ethanol. The yield was 1.6 g. (70%), m.p. 153°, 156-157° after recrystallization from ethanol (recovery 95%).[α]D +14° (c 0.5, dimethylformamide).

Anal. Calcd. for C₂₀H₂₄N₂O₁₁S: N, 5.49; S, 4.08. Found: C, 70.85; H, 6.02; N, 5.84; S, 4.20.

N-Carbobenzyoxys-L-cysteinylglycine Ethyl Ester (XVII).—Upon adding a solution of 0.51 g. (0.003 mole) of silver nitrate and 0.24 ml. (0.003 mole) of pyridine in 15 ml. of ethanol to a saturated solution of XII in methanol, the silver mercaptide of XVII was separated; after standing for a few hours at room temperature under nitrogen it was filtered off and washed with methanol. The yield was 1.25 g. (94%).

Anal. Calcd. for C₂₀H₂₄N₂O₁₁S: N, 6.26; Ag, 24.12. Found: N, 6.45; Ag, 24.35.

To the suspension of 0.9 g. of the above silver mercaptide in 10 ml. of dimethylformamide, 0.25 ml. of conc. hydrochloric acid and 0.4 ml. of sodium hydroxide was added. The mixture was heated at room temperature, and then it was heated on the water-bath for 1 minute. The precipitate, consisting of silver chloride, was removed and washed with a little dimethylformamide. To the filtrate the chloroform was added, the mixture was repeated washed with water. Traces of silver chloride were removed by filtration and the filtrate was concentrated in vacuo. Upon adding water to the residue (73%) of XII in methanol, the silver mercaptide of XVII separated; after standing for a few hours at room temperature under nitrogen it was filtered off and washed with methanol. The yield was 1.25 g. (94%).

N,N'-Bis-carbobenzyoxys-L-cysteinylglycine Diethyl Ester (XIX).—Compound XVII (0.34 g., 0.001 mole), dissolved in 20 ml. of 50% acetic acid, was oxidized with 0.1 ml. of silver nitrate and 0.16 ml. of pyridine in 10 ml. of ethanol to a saturated hot alcoholic solution of 1.47 g. (0.002 mole) of XVI, the silver mercaptide of XIX precipitated. After standing for 2 hours at room temperature, it was filtered off and washed with ethanol. The yield was 1.15 g. (98%); it was purified by trituration with a little chloroform (recovery 90%).


N-Carbobenzyoxys-2-phenylalanyl-L-cysteinylglycine Ethyl Ester (XIII).—Upon adding a solution of 0.530 g. of silver nitrate and 0.16 ml. of pyridine in 10 ml. of ethanol to a saturated hot alcoholic solution of 1.47 g. (0.002 mole) of XVI, the silver mercaptide of XIX precipitated. After standing for 2 hours at room temperature, it was filtered off and washed with ethanol. The yield was 1.15 g. (98%); it was purified by trituration with a little chloroform (recovery 90%).


To the suspension of 1.32 g. of the dicyclohexylcarbodiimide and 2.8 g. of glycine ethyl ester in 20 ml. of 1 N sodium hydroxide was added. Shaking was continued for 30 minutes at room temperature and the mixture was evaporated to dryness. Upon dissolving the residue in water and adjusting the pH to 4-6 with sodium acetate, XIVb gave a turbid solution. The yield was 0.480 g. (70%); needles, m.p. 224°, [α]D +53.3° (c 1.7 in 65% ethanol containing 1 equivalent of HCI).


N-Formyl-diphenylmethyl-L-cysteinylglycine ethyl ester (XV) was prepared by coupling 1Vc with glycine ethyl ester by the carbodiimide method (cf. the similar procedure for the preparation of XI). The yield was 2.2 g. (83%), m.p. 89-90°. After recrystallization from ethyl acetate the m.p. was 92-93°.


N-Carbobenzyoxys-2-phenylalanyl-S-trityl-L-cysteinylglycine Ethyl Ester (XVI).—A solution of 2.4 g. (0.005 mole) of XI in 12.5 ml. of 1 N ethanolic hydrogen chloride was warmed at 40-45° for 15 minutes and then kept at room temperature for 2 days. Upon evaporation to dryness and trituration with anhydrous ether, 1.7 g. (70%) of pure, amor phous S-trityl-L-cysteinylglycine ethyl ester hydrochloride[51] was obtained, m.p. 109-115°. To a chloroform solution of 1.9 g. (0.004 mole) of this hydrochloride and of 0.5 ml. of triethylamine, was added the mixed anhydride prepared in the usual way from 1.2 g. of carbobenzyoxys-2-phenylalanine (0.004 mole) and 0.4 ml. of ethyl chloroformate in chloroform. After standing for several hours at room temperature the solution was successively washed with hydrochloric acid and potassium hydrogen carbonate and water; 1 ml. solution was evaporated to dryness and the crystalline residue (XVI) was recrystallized from ethanol (recovery 95%).[α]D +14° (c 0.5, dimethylformamide).

Anal. Calcd. for C₂₀H₂₄N₂O₁₁S: N, 5.49; S, 4.08. Found: C, 70.85; H, 6.02; N, 5.84; S, 4.20.

N,N'-Bis-carbobenzyoxys-2-phenylalaninyl-L-cysteinylglycine Diethyl Ester (XX).—The solution of 0.974 g. (0.002 mole) of XIX in 50% acetic acid was titrated with 0.1 N iodine; the theoretical amount of iodine was consumed (20.2 ml. instead of 10 ml.) was consumed, whereas XVIII separated out. Upon adding water to the residue (0.003 mole) of XII in methanol, the silver mercaptide of XVII separated; after standing for a few hours at room temperature under nitrogen it was filtered off and washed with methanol. The yield was 1.25 g. (94%).


(52) Carbobenzyoxys of this product yielded 75% of N-carbobenzyoxys-S-trityl-L-cysteinylglycine ethyl ester (XII), m.p. 110-111°.
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### Organic Sulfur Compounds. VIII. Addition of Thiols to Conjugated Dienes

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**Abstract.** Radical addition of simple aliphatic and aromatic thiols and of thiolacetic acid to 1,3-butadiene, 2,3-dimethyl-1,3-butadiene, isoprene and chloroprene yields predominantly the 1,4-trans-monoadducts. In the case of unsymmetrically substituted butadienes (isoprene, chloroprene and piperylene), the addition of aromatic thyl radicals is highly selective to the first carbon atom. Aliphatic thyl radicals are less selective; their addition to carbon four leads to significant amounts of the “reverse adducts” as by-products. The adducts are derived from the intermediate allene radical through subsequent hydrogen abstraction from the thyl by the more reactive primary carbon atom. Thiols add to piperylene to yield both 1,2- and 1,4-adducts. This is expected since both reactive carbons of the intermediate allene radical are of the secondary type.

Addition of ionic and free radical reagents to conjugated dienes usually gives rise to both normal 1,2-addition and 1,4-conjugate addition. Kharasch and co-workers reported about a quarter of a century ago that in the presence of peroxides the addition of hydrogen bromide and hydrogen chloride to butadiene resulted predominantly in 1,4-adducts while in the absence of peroxides mainly the 1,2-adducts were formed. Carbon tetrachloride and butadiene also formed a 1,4-addition product under free radical conditions, along with larger amounts of telomer products. The first addition of a thiol to a conjugated diene was reported by Posner in 1905. On treating benzylethynyl with 1-phenyl-1,3-butadiene, he obtained an unidentified liquid adduct. On oxidation with potassium permanganate, this yielded a crystalline sulfone corresponding to the 1,4-adduct in an unreported yield.

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