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On Cysteine and Cystine Peptides. Part V.[1] *S*-Trityl- and *S*-Diphenylmethyl-cysteine and -cysteine Peptides [2]

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A new method for the preparation of *S*-trityl- (*i.e.* triphenylmethyl) and *S*-diphenylmethyl-cysteines or -cysteine peptides involves treatment of cysteine or cysteine peptides with aralkylcarbinols in the presence of trifluoroacetic acid or hydrogen halides in acetic acid solution. The optimal conditions for the removal of these *S*-protecting aralkyl groups by trifluoroacetic acid or hydrogen halides in acetic acid solution under various conditions have been determined.

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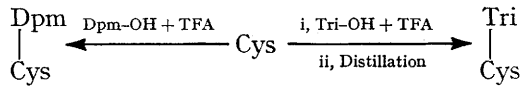
For the synthesis of complicated cysteine–cystine peptides, especially those containing S–S bridges and free SH groups, one requires cysteine derivatives bearing different *S*-protecting groups which may be removed selectively, especially without affecting any already existing S–S bridges.[3] In our experience, for these purposes, the most effective *S*-protecting groups for cysteine have proved to be the *S*-trityl (Tri),[3] *S*-diphenylmethyl (Dpm),[3] and *S*-acyl groups.[4] The intermediate *S*-trityl- and *S*-diphenylmethyl-L-cysteines were originally prepared in our laboratory under acidic conditions from L-cysteine or its hydrochloride and the corresponding aralkyl chloride without the addition of any acid binding agent.[3] Later, other procedures for the preparation of *S*-diphenylmethyl- and *S*-trityl-cysteines, under acidic conditions were developed.[5]

It has been reported that the *S*-trityl group in both cysteine and cysteine peptides is split off to some degree at room temperature by trifluoroacetic acid (TFA).[1a,2,3,6] The situation is confused by the varying results obtained by different methods of work-up of the reaction solution. For example, after a solution of *S*-tritylcysteine in anhydrous trifluoroacetic acid has been kept for 15 min. at room temperature, *S*-tritylcysteine may be recovered largely unchanged by evaporation, but the addition of water causes separation of cysteine (70—75%). Such results might suggest that the presence of water is necessary for the cleavage. However, determination of the SH content of the reaction solutions indicated rapid cleavage of *S*-tritylcysteine in anhydrous trifluoroacetic acid, and the rate of cleavage was significantly slower in 90% aqueous trifluoroacetic acid (see Table). Apparently the removal of the *S*-trityl group by trifluoroacetic acid is an equilibrium reaction, and the equilibrium is displaced during the removal of trifluoroacetic acid by distillation so that retritylation of the SH group is favoured. We have thus elaborated a simple method for preparing *S*-trityl-L-cysteine. Interaction of triphenylmethanol and cysteine in trifluoroacetic acid for 15 min., followed by removal of the excess of trifluoroacetic acid under vacuum and addition of ether and sodium acetate solution, gives *S*-tritylcysteine in about 90% yield.



L-Cysteine also reacts with diphenylmethanol in the presence of trifluoroacetic acid, giving *S*-diphenylmethyl-L-cysteine in over 90% yield. In this case removal of trifluoroacetic acid by distillation is not necessary, since apparently the equilibrium favours the formation of *S*-diphenylmethylcysteine.

Furthermore experiments in this laboratory have shown that trifluoroacetic acid alone does not in fact remove the *S*-diphenylmethyl group. Only in the presence of aromatic compounds, such as phenol or anisole, *etc.*, capable of undergoing electrophilic substitution reactions, does trifluoroacetic acid remove the *S*-diphenylmethyl group to any great extent. In the presence of these compounds the yield of *S*-diphenylmethylcysteine is reduced to *ca.* 15%.

The method can be applied to the preparation of other aralkylcysteines, *e.g.* bis-*p*-methoxyphenylmethanol and cysteine give *S*-bis-*p*-methoxyphenylmethyl-L-cysteine. The use of cysteine peptides, *e.g.* (III), instead of cysteine gives the corresponding *S*-protected peptides, *e.g.* (Ia—c).

Diaryl- and triaryl-methanols and cysteine can also be induced to react by hydrogen halides in acetic acid solution instead of trifluoroacetic acid. In this case the reaction must be facilitated by warming at 40—50°. It is known that hydrogen bromide and, to a lesser extent, hydrogen chloride in acetic acid can remove the *S*-trityl group, by an equilibrium reaction.[2,3,5] Therefore in the preparation of *S*-tritylcysteine from cysteine

[1] (*a*) Part IV, L. Zervas, I. Photaki, and I. Phocas, *Chem. Ber.*, 1968, **101**, 3332; (*b*) Part III, L. Zervas, I. Photaki, A. Cosmatos, and D. Borovas, *J. Amer. Chem. Soc.*, 1965, **87**, 4922.

[2] Presented in part at the Ninth European Peptide Symposium: I. Photaki, I. Phocas, J. Taylor-Papadimitriou, and L. Zervas, 'Peptides: Proceedings of the Ninth European Symposium,' Orsay, France, 1968, ed. E. Bricas, North Holland Publishing Co., Amsterdam, 1968, p. 201.

[3] (*a*) L. Zervas and I. Photaki, *Chimia* (*Switz.*), 1960, **14**, 375; (*b*) L. Zervas, *Coll. Czech. Chem. Comm.*, 1962, **27**, 2229; (*c*) L. Zervas and I. Photaki; *J. Amer. Chem. Soc.*, 1962, **84**, 3887.

[4] L. Zervas, I. Photaki, and N. Ghelis, *J. Amer. Chem. Soc.*, 1963, **85**, 1337.

[5] (*a*) H. D. Law and R. W. Hanson, *J. Chem. Soc.*, 1965, 7285; (*b*) R. G. Hiskey and J. B. Adams, Jun., *J. Org. Chem.* 1965, **30**, 1340.

[6] L. Zervas, I. Photaki, A. Cosmatos, and N. Ghelis, 'Peptides: Proceedings of the Fifth European Symposium,' Oxford, 1962, ed. G. T. Young, Pergamon, Oxford, 1963, p. 27; L. Zervas, I. Photaki, C. Yovanidis, J. Taylor, I. Phocas, and V. Bardakos, 'Peptides: Proceedings of the Eighth European Symposium,' Noordwijk, The Netherlands, 1966, ed. H. C. Beyerman, North-Holland Publishing Co., Amsterdam, 1967, p. 28; I. Photaki, 'Pharmacology of Hormonal Polypeptides and Proteins,' Plenum Press, London, 1968, p. 1.

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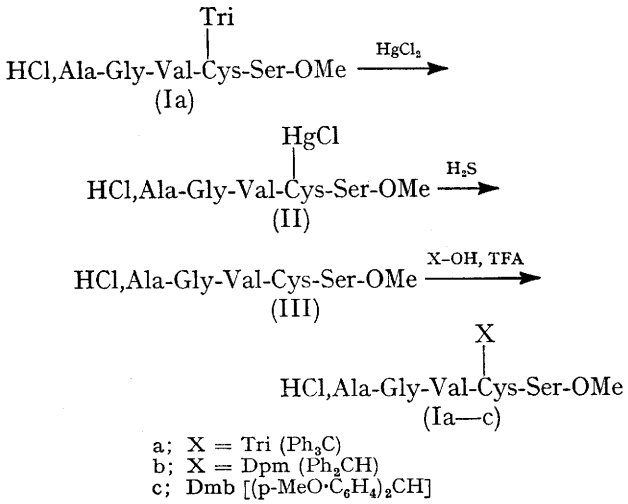

a; X = Tri (Ph_3C)
b; X = Dpm (Ph_2CH)
c; Dmb [$(p\text{-}MeO\cdot C_6H_4)_2CH$]

and triphenylmethanol in hydrogen bromide–acetic acid, the acid must be removed by distillation prior to isolation of *S*-protected cysteine. A similar effect is observed in treatment of *N*-benzyloxycarbonyl-*S*-trityl-L-cysteinyl-L-leucine ethyl ester with hydrogen bromide–acetic acid, followed by distillation of the reagents. The trityl bromide initially formed attacks the liberated SH group during the distillation to give *S*-trityl-L-cysteinyl-L-leucine ethyl ester hydrobromide in good yield.[7]

In the preparation of *S*-diphenylmethylcysteine from L-cysteine and diphenylmethanol in the presence of hydrogen bromide in acetic acid, removal of the acid by distillation prior to the isolation of the *S*-protected cysteine has again no significant influence on the yield.

Our results show that in order to use trifluoroacetic acid or hydrogen bromide in acetic acid as cleavage reagents, the reaction conditions must be carefully controlled. This point is important because of the wide use of *S*-trityl- and *S*-diphenylmethyl-cysteines in peptide synthesis. The results in the Table illustrate the variation in the removal of *S*-trityl, *S*-diphenylmethyl, and *S*-bismethoxyphenylmethyl groups by various reagents under different conditions. The following points are of practical importance. The extent of removal of the *S*-protecting groups was measured by determination of SH by the original[8] or a modified Ellman procedure (see Experimental section), or by titration with iodine at a defined concentration.[4] The Ellman method could only be applied to cleavage by trifluoroacetic acid; hydrogen bromide in acetic acid interfered with the estimation. In the case of the titration with iodine, the titration must be done quickly in aqueous solutions in order to avoid the interaction of iodine with the *S*-trityl group.[9]

In order to avoid retritylation, the reagents trifluoroacetic acid and hydrogen halide–acetic acid should not be removed by distillation. Furthermore, prior to titration (or isolation of the product), water must be added to the mixture. The acetic acid must be pure and absolutely dry otherwise the cleavage rate is greatly diminished. Finally, the extent of cleavage by tri-

Compound	Reagent	Cleavage (%)	Time [a] (Temp. [b])
Tri-Cys [c,d,e]	TFA	70–75 [f,g]	15 min.
Tri-Cys [i]	TFA	85 [g]	30 min.
Tri-Cys [c]	TFA + 10% H_2O	45 [f]	30 min.
Tri-Cys [h,e]	N-HBr–AcOH	90–95 [f]	5 min. (10°)
Tri-Cys [h]	N-HBr–AcOH + 2·5% H_2O	20–25 [f]	5 min. (10°)
Tri-Cys [h,j]	N-HBr–AcOH + 5 equiv. Tri-Cl	80 [f]	5 min.
Tri-Cys [h]	N-HBr–AcOH + 5 equiv. Tri-OH	5 [f]	5 min.
Tri-Cys [h,e]	0·2N-HBr–AcOH	70–75 [f]	10 min.
Tri-Cys [h]	N-HCl–AcOH	50 [f]	1—3 hr.
Tri-Cys [h]	0·5N-HCl–AcOH	40–45	1—3 hr.
Tri-Cys [h]	0·5N-HCl–AcOH + 5 equiv. Tri-OH	1 [f]	3 hr.
(IV) [c]	TFA	19 [f]	30 min.
(IV) [k]	TFA	37 [f]	30 min.
(IV) [i]	TFA	67 [f]	30 min.
(IV) [h]	N-HBr–AcOH	83 [f]	5 min.
(IV) [h]	0·2N-HBr–AcOH	75 [f]	10 min.
(IV) [l]	HBr–TFA	45 [f]	45 min.
(Ia) [c]	TFA	18 [f,g]	30 min.
(Ia) [k]	TFA	33 [g]	30 min.
(Ia) [i,m]	TFA	60 [g,f]	30 min.
(Ia) [c]	TFA + 5 equiv. Tri-OH	1 [g]	30 min.
(Ia) [k]	TFA + 5 equiv. Tri-OH	1 [g]	30 min.
(Ia) [i,m]	TFA + 10 equiv. Tri-OH	5 [g]	30 min.
(Ia) [i,m]	TFA + 10 equiv. Tri-Cl	6 [g]	30 min.
(Ia) [h]	N-HBr–AcOH	82 [f]	5 min.
(Ia) [h]	0·2N-HBr–AcOH	65 [f]	15 min.
(Ia) [l]	HBr/TFA	50 [f]	20 min.
Dpm-Cys [c,d,e]	TFA + 15% phenol [n]	98 [f]	15 min. (70°)
Dpm-Cys [c,e]	TFA	1 [f]	30 min. (70°)
Dpm-Cys [c,e]	TFA + 15% phenol [n]	75 [f]	18 hr.
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	25 [f,g]	30 min.
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	50 [f,g]	2 hr.
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	88 [f]	16 hr.
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	35 [f]	30 min. (30°)
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	65 [f]	2 hr. (30°)
Dpm-Cys [o]	TFA + 2·5% phenol [n,p]	95 [f]	16 hr. (30°)
Dmb-Cys [c]	TFA + 15% phenol [n]	45 [f]	30 min.
Dmb-Cys [c]	TFA + 15% phenol [n]	80 [f]	2 hr.
Dmb-Cys [c]	TFA	5 [f]	2 hr.
Dmb-Cys [h,j]	2N-HBr–AcOH	10 [f]	1 hr.
Dmb-Cys [q,j]	6N-HBr–AcOH	30 [f]	1 hr. [r]
(Ib) [o]	TFA + 2·5% phenol [n]	40 [g]	30 min. (30°)
(Ib) [o]	TFA + 2·5% phenol [n]	65 [g]	2 hr. (30°)
(Ib) [o]	TFA + 2·5% phenol [n] + 10% H_2O	11 [g]	30 min. (30°)
(Ic) [o]	TFA + 2·5% phenol [n]	71 [g]	30 min. (30°)
(Ic) [o]	TFA + 2·5% phenol [n]	84 [g]	1 hr. (30°)
(Ic) [o]	TFA + 2·5% phenol [n] + 10% H_2O	58 [g]	30 min. (30°)
(VI) [s]	TFA	20 [g]	15 min.
(VI) [s]	TFA	1 [f]	15 min.

[a] Measured after the *S*-protected compound had been dissolved; [b] room temperature, *i.e.* 20—25° except where otherwise noted; [c] *c* 0·6M; [d] *cf.* ref. 1; [e] *cf.* refs. 3 and 6; [f] titration of SH group by iodine; [g] titration of SH group by the Ellman method; [h] treated with 4 equiv. of hydrogen halide in acetic acid; [i] *c* 0·07M; [j] *cf.* ref. 5; [k] *c* 0·3M; [l] hydrogen bromide was passed through a solution (0·05M) of the compound in trifluoroacetic acid containing 2% phenol (w/v) for 10 min. at 0° and then at 10°; [m] unchanged in the presence of 20% phenol (w/v); [n] w/v; [o] *c* 0·08M; [p] before titration with iodine, trifluoroacetic acid was removed *in vacuo* at room temperature; [q] treated with 12 equiv. of hydrogen bromide in acetic acid; [r] a solution obtained after warming the mixture for 1—2 min. on a water-bath; [s] 0·09M.

[7] Y. Hirotsu, T. Shiba, and T. Kaneko, *Bull. Chem. Soc. Japan*, 1967, **40**, 2950.
[8] G. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.
[9] D. S. Tarbell and D. P. Harnisch, *J. Amer. Chem. Soc.*, 1952, **74**, 1862; B. Kamber and W. Rittel, *Helv. Chim. Acta*, 1968, **51**, 2061.

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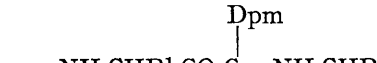
fluoroacetic acid depends upon the concentration of the substrate. Diminution of the concentration can result in a higher percentage cleavage.

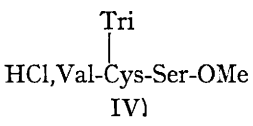
We wish to make the following suggestions regarding the removal of *S*-trityl and *S*-diphenylmethyl groups from *S*-protected peptides.

(i) Trifluoroacetic acid in the presence of phenol is an excellent cleavage reagent for the *S*-diphenylmethyl group both from *S*-diphenylmethyl-cysteine and -cysteinylpeptides, and from peptides bearing an *S*-diphenylmethylcysteine residue at positions other than the *N*-terminal.[3c,10] The *S*-diphenylmethyl group can



be removed preferentially at 20—30°, especially in dilute solutions, in which case up to 90% cleavage has been observed. The *S*-bismethoxyphenylmethyl group is cleaved appreciably faster.

(ii) In the case of the *S*-trityl group, trifluoroacetic acid is a good cleavage reagent only for *S*-tritylcysteine and (probably) for peptides bearing an *S*-tritylcysteine residue at the amino-end. For all other peptides, *e.g.* (Ia) or (IV), the optimal conditions for cleavage need to be determined. In the cases so far studied, the per-



(IV)

centage cleavage did not exceed *ca.* 60%. For preparative purposes the percentage cleavage can be increased by isolation of the product and repetition of the reaction with trifluoroacetic acid.

(iii) The removal of *S*-trityl groups by trifluoroacetic acid is almost fully suppressed in the presence of triphenylmethanol or triphenylmethyl chloride; this fact can be used to great advantage in cases where a polypeptide bears other side-chain protecting groups (*e.g.* *N*-t-butoxycarbonyl, t-butyl ester, diphenylmethyl ester, *etc.*) quantitatively removable by trifluoroacetic acid.*

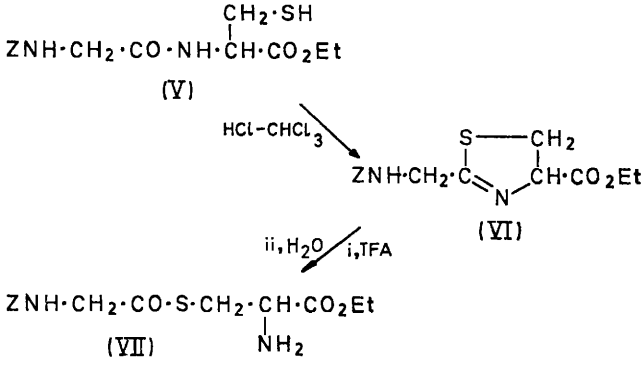
(iv) Hydrogen bromide in acetic acid is the reagent of choice for removal of the *S*-trityl group in all peptides. Triphenylmethanol inhibits removal of the *S*-trityl group (see Table), whereas triphenylmethyl chloride has no appreciable influence.[5]

(v) The *S*-trityl group is removed less efficiently by hydrogen bromide in trifluoroacetic acid and phenol than by hydrogen bromide in acetic acid; in the case of the pentapeptide (Ia) the cleavages are 50 and 82%, respectively. For preparative purposes this procedure must be repeated.†

(vi) Hydrogen chloride in acetic acid removes the *S*-trityl group to a lesser extent; cleavage is also suppressed in the presence of triphenylmethanol.

(vii) In the light of past results and of a new case described in the Experimental section, the removal of the *S*-trityl group by Hg^{2+} (or Ag^{+}), followed by the action of hydrogen sulphide on the thiolate thus formed, is still to be recommended.

In the removal of the *S*-trityl group by trifluoroacetic acid, one cannot exclude *a priori* the possibility that a thiazoline derivative is formed, since cysteine peptides can be converted to thiazoline derivatives in the presence of high concentrations of acids, in non-aqueous solutions (*e.g.* hydrogen chloride in chloroform [7,12] or methanol [13]). Bearing in mind that the action of aqueous strong acids on thiazolines gives *S*-acyl derivatives, treatment of the thiazoline (VI) with trifluoroacetic acid followed by addition of water was examined. Trifluoroacetic acid has no effect on the SH-peptide (V) but cleaves the



(V) (VI) (VII)

thiazoline derivative (VI) to some extent, giving, presumably, an *S*-peptide (VII). The cleavage is estimated to be *ca.* 20% ‡ by the Ellman method which, because of the alkaline pH, hydrolyses the *S*-peptide bond and detects the SH-groups thus formed. Iodine titration is done under acidic conditions and therefore does not detect *S*-acyl or *S*-peptide bonds. Since the Ellman method and the iodine titration gave the same figure for the percentage of free SH formed in the cleavage of *S*-trityl peptides, a thiazoline derivative is probably not formed during splitting of the *S*-trityl bond by trifluoroacetic acid. Further evidence is provided by the u.v.

* Peptide (IV) was recovered unchanged in 90% yield after treatment with trifluoroacetic acid (0·3M-solution containing 5 equiv. of triphenylmethanol; 30 min. at room temperature), concentration *in vacuo*, and addition of ethyl acetate followed by a tenfold quantity of ether and then some drops of ether saturated with hydrogen chloride {m.p. 100° (decomp.), $[\alpha]_D^{20}$ + 26·1° (*c* 2 in MeOH), R_F 0·69; lit.,[1b] m.p. 100° (decomp.), $[\alpha]_D^{18}$ + 26·6° (*c* 3 in MeOH), R_F 0·69}.

† In *S*-detritylation of another polypeptide with hydrogen bromide in trifluoroacetic acid Zahn *et al.*[11] obtained only 8% cleavage. This low value could be understood if water was not added prior to the isolation in order to avoid retritylation (see before). In spite of the small yield, these workers repeated the reaction for preparative purposes.

‡ There is also a simultaneous decrease (to *ca.* 20%) in the thiazoline absorption at 267 nm.

[10] I. Photaki and V. Bardakos, *Chem. Comm.*, 1967, 275; I. Photaki, V. Bardakos, A. W. Lake, and G. Lowe, *J. Chem. Soc.* (*C*), 1968, 1860.

[11] H. Zahn, W. Danho, H. Klostermeyer, H. C. Gattner, and J. Repin, *Z. Naturforsch.*, 1969, **24***b*, 1127.

[12] Y. Hirotsu, T. Shiba, and T. Kaneko, *Bull. Chem. Soc. Japan*, 1967, **40**, 2945.

[13] W. Stoffel and L. C. Graig, *J. Amer. Chem. Soc.*, 1961, **83**, 145.

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absorption curves obtained before and after treatment with trifluoroacetic acid of the *S*-tritylpentapeptide (Ia); in the region 260—270 nm. no increase in the absorption characteristic of thiazoline formation was observed.

EXPERIMENTAL

For *S*-protection and *S*-deblocking of cysteine and cysteine peptides, anhydrous reactants and dry solvents were used unless otherwise stated. Peroxide-free ether, dry acetic acid, freshly distilled trifluoroacetic acid, and freshly prepared solutions of pure hydrogen bromide free of bromine in acetic acid were always used. Evaporations were carried out *in vacuo* at 35—40°. M.p.s quoted are mostly points of decomposition. Purity of compounds was confirmed by t.l.c. on Kieselgel G, in the solvent system n-butanol–acetic acid–water (10 : 1 : 3); the chromatograms were developed with ninhydrin.

Prior to analysis the compounds were dried at 56° under high vacuum over phosphoric oxide; microanalyses were performed by Dr. H. Mantzos, Analytical Laboratory, Royal Hellenic Research Foundation.

S-*Trityl*-L-*cysteine*.—(*a*) L-Cysteine or its hydrochloride (0·005 mole) and triphenylmethanol (0·005 mole) were dissolved by gentle shaking in trifluoroacetic acid (9 ml.). The clear solution was set aside for 15 min. at room temperature and the trifluoroacetic acid was removed *in vacuo*. Ether was added to the residue and then 10% aqueous sodium acetate until the mixture was neutral to Congo Red. After cooling, the precipitate was filtered off and washed with water. The material thus obtained was dried on a porous plate and triturated several times with hot ether, yielding S-*trityl*-L-*cysteine* (85—90%), m.p. 177—179°, $[\alpha]_D^{28}$ +15·6° (*c* 2 in 0·1N-NaOH) {lit.,[3c] m.p. 178—179°, $[\alpha]_D^{24}$ +16·2° (*c* 2 in 0·1N-NaOH, for the recrystallized compound)}.

The yield was unchanged (87%) when phenol (1 g.) was added to the reaction mixture; the product had m.p. 180—181°, $[\alpha]_D^{25}$ +16·1° (*c* 2 in 0·1N-NaOH). On the other hand, when trifluoroacetic acid was not removed by distillation the yield was reduced to *ca.* 15%.

(*b*) A mixture of L-cysteine (0·005 mole) and triphenylmethanol (0·005 mole) in N-hydrogen bromide in acetic acid (15 ml.) was warmed with occasional stirring so that the temperature of the mixture was *ca.* 40°. After 1 hr. the mixture was distilled and treated as described in (*a*). The yield of *S*-trityl-L-cysteine was 58%, m.p. 179°. When the acids were not removed by distillation no tritylcysteine was obtained.

(*c*) A mixture of L-cysteine (0·005 mole) and triphenylmethanol (0·005 mole) in 1·6N-hydrogen chloride in acetic acid (20 ml.) was stirred for 4 hr. at *ca.* 40° before work-up as described in (*a*). The yield of crude *S*-trityl-L-cysteine was 94%, m.p. 180—181°, $[\alpha]_D^{28}$ +15·6° (*c* 2 in 0·1N-NaOH).

S-*Diphenylmethyl*-L-*cysteine*.—(*a*) This was prepared from L-cysteine and diphenylmethanol in the presence of trifluoroacetic acid as described in (*a*) for *S*-trityl-L-cysteine; the yield of crude S-*diphenylmethylcysteine* was 91%, m.p. 194—195°, $[\alpha]_D^{25}$ +17·1° (*c* 2·9 in ethanolic 0·1N-HCl) {lit.,[3c] m.p. 198—199°, $[\alpha]_D^{25}$ +16·9° (*c* 2·9 in ethanolic 0·1N-HCl)}.

When trifluoroacetic acid was not removed by distillation the yield of recrystallized *S*-diphenylmethyl-L-cysteine was 80%, m.p. 196—198°, $[\alpha]_D^{23}$ +16·75° (*c* 2·9 in ethanolic 0·1N-HCl). Addition of phenol (1 g.) to the reaction mixture reduced the yield to 15%.

(*b*) A mixture of L-cysteine (0·005 mole) and diphenylmethanol (0·005 mole) in N-hydrogen bromide in acetic acid (15 ml.) was warmed with stirring on a water-bath at 50° for 2 hr. The solution was distilled and the residue treated as described in (*a*) for *S*-trityl-L-cysteine. The yield of crude *S*-diphenylmethyl-L-cysteine was 80—95%, m.p. 190—193°, $[\alpha]_D^{25}$ +16·5° (*c* 2·9 in 0·2N-HCl in ethanol).

When the hydrogen bromide–acetic acid was not removed by distillation the yield of crude *S*-diphenylmethyl-L-cysteine was 75%, m.p. 192—195°, $[\alpha]_D^{23}$ +16·6° (*c* 2·9 in 0·2N-HCl in ethanol).

S-*Bis*-p-*methoxyphenylmethyl*-L-*cysteine*.—(*a*) This was prepared from L-cysteine and bis-*p*-methoxyphenylmethanol in the presence of trifluoroacetic acid as described in (*a*) for *S*-trityl-L-cysteine. The yield of crude S-*bismethoxyphenylmethyl*-L-*cysteine* was 94%, m.p. 201°. A sample was recrystallized by dissolving it in N-hydrochloric acid, extracting the solution twice with ethyl acetate, and reprecipitating the substance from the aqueous layer with sodium acetate. The m.p. did not change; $[\alpha]_D^{28}$ +9·5° (*c* 2 in 0·1N-NaOH) {lit.,[5] m.p. 211°, $[\alpha]_D^{20}$ +10·2° (*c* 2 in 0·1N-NaOH)}.

(*b*) L-Cysteine (0·005 mole) and bis-*p*-methoxyphenylmethanol (0·005 mole) were dissolved with stirring in 0·7N-hydrogen bromide in acetic acid (14 ml.) at room temperature. After 15 min. the solution was distilled and the residue was treated as described in (*a*) for *S*-trityl-L-cysteine; it was also washed with acetone, yielding 93% of crude *S*-bismethoxyphenylmethyl-L-cysteine, m.p. 196—197°, $[\alpha]_D^{30}$ +9·2° (*c* 2 in 0·1N-NaOH after the solution had been filtered through a sintered glass funnel to remove a slight turbidity).

L-*Alanylglycyl*-L-*valyl*-S-*chloromercuri*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (II).—To a solution of L-alanylglycyl-L-valyl-*S*-trityl-L-cysteinyl-L-serine methyl ester hydrochloride (Ia) [1b] (0·73 g., 0·001 mole) in dry methanol (5 ml.), mercury(II) chloride (0·54 g., 0·002 mole) was added. The mixture was shaken to dissolve the ingredients and refluxed on a water-bath for 20 min.; a white precipitate was formed. Ether was added and the precipitate was filtered off and washed with ether. Trituration with hot methanol–ether yielded the S-*chloromercuripentapeptide hydrochloride* (0·63 g., 88%), m.p. 190° (sinters at 145°) (Found: Cl, 10·1; N, 9·8. $C_{17}H_{31}Cl_2HgN_5O_7S$ requires Cl, 9·8; N, 9·7%).

L-*Alanylglycyl*-L-*valyl*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (III).—Compound (II) (1·1 g., 0·0015 mole) was suspended in dry methanol (30 ml.) and hydrogen sulphide was bubbled in for 1 hr. Mercury(II) sulphide was removed by centrifugation and extracted twice with methanol. The extracts were combined with the supernatant liquid and concentrated *in vacuo*. Ether was added to obtain complete precipitation of the *hydrochloride* (III) (65%), m.p. 175—178°, free SH (Ellman's method) 96% of theoretical (Found: C, 41·7; H, 6·6; Cl, 7·5; N, 14·4; S, 6·35. $C_{17}H_{32}ClN_5O_7S$ requires C, 42·0; H, 6·6; Cl, 7·3; N, 14·4; S, 6·6%).

L-*Alanylglycyl*-L-*valyl*-S-*trityl*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (Ia).—A solution of the pentapeptide ester hydrochloride (III) (0·24 g., 0·0005 mole) and triphenylmethanol (0·145 g., 0·00055 mole) in trifluoroacetic acid (1 ml.) was set aside at room temperature for 15 min. then concentrated *in vacuo*. Addition of ether to the residue gave the S-*protected ester hydrochloride* (0·340 g., 93%), m.p. 197—199°, $[\alpha]_D$ −31° (*c* 2·5 in MeOH) {lit.,[1b] m.p. 204—205°, $[\alpha]_D^{18}$ −32·2° (*c* 2·5 in MeOH)}.

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L-*Alanylglycyl*-L-*valyl*-S-*diphenylmethyl*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (Ib).—A mixture of the pentapeptide ester hydrochloride (III) (0·24 g., 0·0005 mole) and diphenylmethanol (0·110 g., 0·0006 mole) were treated with trifluoroacetic acid as described for the preparation of the *S*-tritylpeptide ester hydrochloride (Ia). The yield of crude S-*diphenylmethylpeptide ester hydrochloride* was 0·32 g. (95%). This material was dissolved in hot methanol–ethyl acetate and the solution was filtered. Addition of hydrogen chloride in ethyl acetate precipitated the pure compound (recovery 80%). The chromatographically pure substance (R_F 0·45) has no definite m.p. but starts decomposing at *ca.* 157—160°. For analysis a sample was suspended in ethyl acetate and shaken with 5% aqueous sodium carbonate. The ethyl acetate layer was washed twice with water, dried (K_2CO_3), and filtered. Addition of hydrogen chloride in ethyl acetate to the filtrate gave the *pentapeptide hydrochloride*, which was washed with ethyl acetate and then with ether (recovery 60%) (Found: C, 54·1; H, 6·5; N, 9·8. $C_{30}H_{42}ClN_5O_7S,H_2O$ requires C, 53·8; H, 6·6; N, 10·4%).

L-*Alanylglycyl*-L-*valyl*-S-*bis*-p-*methoxyphenylmethyl*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (Ic).—A mixture of the pentapeptide ester hydrochloride (III) (0·24 g., 0·0005 mole) and bis-*p*-methoxyphenylmethanol (0·13 g., 0·00055 mole) was treated with trifluoroacetic acid as described for the preparation of the *S*-tritylpeptide (Ia). The yield of crude S-*bismethoxyphenylmethylpeptide ester hydrochloride* was 0·34 g. (96%); it was purified by conversion into the free ester and reprecipitation as the hydrochloride as described for the corresponding *S*-diphenylmethyl derivative (recovery 70%). The substance has no definite m.p.; it starts decomposing at *ca.* 135° (Found: C, 53·9; H, 6·64; Cl, 5·0; N, 9·6; S, 4·4. $C_{32}H_{46}ClN_5O_9S$ requires C, 54·0; H, 6·6; Cl, 5·0; N, 9·8; S, 4·5).

Determination of the Amounts of Cleavage of S-*Protected Cysteines and Cysteine Peptides.*—(*a*) In all cases concerning de-aralkylation of cysteine and cysteine peptides by means of trifluoroacetic acid the free SH groups thus formed were estimated by iodine titration or by the Ellman method, as described earlier for the case of *S*-trityl-L-cysteine.[1a] For estimation of SH groups in peptides insoluble in water the Ellman procedure was modified as follows.

(i) *Reagents.* An aqueous 0·5M-tris(hydroxymethyl)-aminomethane solution is adjusted to pH 8·0 with glacial acetic acid. This buffer is used in a mixture (1 : 4 v/v) with dimethylformamide (DMF). Ellman stock solution is prepared by dissolving 5,5′-dithiobis-(2-nitrobenzoic acid) (0·396 g.) in 0·1M-phosphate buffer (pH 7; 100 ml.). The solution is diluted 1 : 100 in DMF–tris buffer to give the diluted Ellman reagent.

(ii) *Solutions of thiols.* A fresh cysteine solution (0·5 mM in water) was always made up for comparison with the sample to be estimated. For making the sample solution the material treated with trifluoroacetic acid is diluted with DMF–tris buffer to a final concentration of 0·25—1 mM.

(iii) *Procedure.* Duplicate samples of the solution to be estimated (0·05—0·2 ml. containing *ca.* 0·05 μM of SH group) and of the cysteine solution (0·1 ml.) are made up with DMF–tris buffer to a final volume of 0·2 ml. For the blank 0·2 ml. of DMF–tris buffer is used. To each sample diluted Ellman reagent (3 ml.) is added and the absorption developed is measured after 10 min. in the spectrophotometer, at 450 * nm.

(*b*) In all cases concerning *S*-de-aralkylation by means of hydrogen bromide (or chloride) in acetic acid, cold water followed by sodium acetate † must be added before proceeding to iodine titration. The final concentration of the solution should not be less than 0·045—0·05M; 0·1N-iodine solution is used.[4]

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* When the DMF–tris buffer is used the extinction is maximal at 450 nm. and ε_M of cysteine is *ca.* 15,000. For water soluble thiols the same SH values were obtained by use of either the original or the modified Ellman procedure.

† A weighed amount of solid sodium acetate approximately equivalent to the hydrogen bromide present is added.