The Conversion of L-β-Chloroalanine Peptides to L-Cysteine Peptides

M. WILCHEK, C. ZIOUDROU, AND A. PATCHORNIK

The Department of Biophysics, Weizmann Institute of Science, Rehovoth, Israel, and the Nuclear Research Center, Democritas, Athens, Greece

Received March 14, 1966

The reaction of thio reagents such as thioacetate, thiobenzoate, and benzyl mercaptide, with L-β-chloroalanine peptides in N,N-dimethylformamide or ethyl acetate solution yields the corresponding optically active L-cysteine peptides, in high yield. The method has been applied for the synthesis of several di-, tri-, and penta-L-cysteine peptides.

In previous communications[1,2] the reaction of tosyl-L-serine derivatives with thio acids and thiols to yield S-substituted L-cysteine peptides was described. However, these methods are of limited value for the synthesis of cysteine peptides for several reasons. Firstly, the O-tosylserine peptides are rather unstable derivatives. In particular, O-tosylserine residues which are not in the N-terminal position cyclize easily to yield Δ^2-oxazoline derivatives under mild conditions.[3,4]

Secondly, there is no good method yet available to tosylate quantitatively serine residues inside a long peptide chain. The formation of O-*p*-toluenesulfonylserine in a protein may only be applied at the present time for specific chemical modification of proteins at an "active serine" residue.[5]

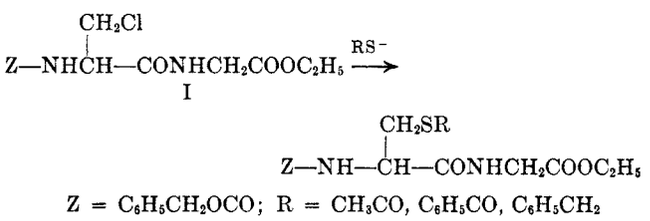
Although significant progress has been made in the development of the selective and reversible protection of the sulfhydryl group of cysteine,[6] the introduction of stable alanine derivatives which could be converted to cysteine residues in a complicated peptide molecule still seems to be desirable.

In searching for groups which could be displaced from β-substituted alanine derivatives by thiol reagents without affecting the stereochemistry of the α-carbon, it was found that L-β-chloroalanine derivatives could be converted to the corresponding L-cysteine derivatives. In earlier studies by other workers, β-chloroalanine has been transformed to cystine in order to ascertain the stereochemistry of some amino acids[7] and optically active derivatives of lanthionine[8] and cystathionine[9] have been prepared by treating L-β-chloroalanine esters with cysteine and homocysteine, respectively, under strongly alkaline conditions.[8,9]

The present study describes the reaction of the anions of thioacetic acid thiobenzoic acid and benzylmercaptan with L-β-chloroalanine (L-α-amino-β-chloropropionic acid) peptides to yield L-cysteine peptides.

N-Carbobenzoxy-L-β-chloroalanylglycine ethyl ester was allowed to react with various salts of thioacetic acid, thiobenzoic acid, and benzylmercaptan in solvents such as ethyl acetate, dimethylformamide, or 50% dimethylformamide–phosphate buffer, pH 7.0. The resultant N-carbobenzoxy-S-acetyl-L-cysteinylglycine ethyl ester (II), N-carbobenzoxy-S-benzoyl-L-cysteinylglycine ethyl ester (III), and N-carbobenzoxy-S-benzyl-L-cysteinylglycine ethyl ester (IV) were isolated in excellent yields (85–90%).

The various cysteine peptides thus formed were identical with authentic samples prepared by independent methods.[1b,10]



$Z = C_6H_5CH_2OCO$; $R = CH_3CO$, C_6H_5CO, $C_6H_5CH_2$

Similarly, N-carbobenzoxy-L-β-chloroalanyl-L-phenylalanine benzyl ester (V) and N-carbobenzoxy-L-β-chloroalanylglycylglycine ethyl ester (VI) were converted in high yields to the corresponding N-carbobenzoxy-S-acetyl-L-cysteinyl-L-phenylalanine benzyl ester (VII) and N-carbobenzoxy-S-acetyl-L-cysteinylglycylglycine ethyl ester (VIII) by treatment with sodium thioacetate in dimethylformamide or with the triethylammonium salt of thioacetic acid in ethyl acetate solution. The products VII and VIII are optically active. Compound VII has the same specific rotation as reported previously.[1b] The retention of optical configuration supports the hypothesis that the reaction proceeds by nucleophillic attack of the thiol anion on the β-carbon of the chloroalanine residue with displacement of chloride anion to yield the S-acetyl-L-cysteine peptide.

The displacement of β-chloro groups by thioacetic acid has been extended to peptides bearing the chloroalanine moiety between two other amino acid residues in a tripeptide, N-carbobenzoxyglycyl-L-β-chloroalanylglycine ethyl ester (IX), and in a pentapeptide, N-carbobenzoxyglycylglycyl-L-β-chloroalanylglycylglycine ethyl ester (X). These peptides have been converted to the corresponding N-carbobenzoxyglycyl-S-acetyl-L-cysteinglycine ethyl ester (XI) and N-carbobenzoxyglycylglycyl-S-acetyl-L-cysteinylglycylglycine ethyl ester (XII) in 80–90% yields of optically and analytically pure products. All reactions were followed iodimetically by measuring the disappearance of the thioreagent.[1b] The purity of the chloroalanine peptides was determined either by nonaqueous titration of the hydrochloric acid released after treatment with stand-

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(8) V. du Vigneaud and G. B. Brown, *J. Biol. Chem.*, **138**, 151 (1941).

(9) G. B. Brown and V. du Vigneaud, *ibid.*, **137**, 611 (1941).

(10) L. Zervas, I. Photaki, and N. Ghelis, *J. Am. Chem. Soc.*, **85**, 1337 (1963).

ard sodium alkoxide solution or by argentometric titrations of the chloride ion released after treatment with 1 *N* sodium hydroxide solution. The alkali treatment promoted the β-elimination reaction resulting in the release of the chloride ion and formation of dehydroalanine. For the determination of the sulfur-containing compounds oxidative methods with iodine and bromine were used.[1b]

Experimental Section

All melting points are uncorrected. Prior to analysis the compounds were dried *in vacuo* over phosphorus pentoxide at 60°. Microanalysis was performed by the Microanalytical Laboratory of the Weizmann Institute of Science, Rehovoth, Israel.

N-Carbobenzoxy-L-β-chloroalanine.—A solution of 15.9 g of L-β-chloroalanine hydrochloride (0.1 mole)[11] was adjusted to pH 9–9.5 with 1 *N* sodium hydroxide and was treated with stirring and cooling in an ice bath with benzyl chloroformate (0.11 mole) over a 30-min period. During this time the pH was maintained between 8.5 and 9.5 by addition of 1 *N* sodium hydroxide. After further stirring for 20 min at room temperature, the reaction mixture was extracted with ether (250 ml in three portions); the aqueous layer was acidified with 5 *N* hydrochloric acid. The resulting crystalline product was filtered, washed with water, and dried *in vacuo*. The product was recrystallized from ethyl acetate–petroleum ether; yield 20.4 g (80%); mp 88°; $[\alpha]^{25}D$ +27° (*c* 1, methanol).

Anal. Calcd for $C_{11}H_{12}NO_4Cl$: C, 51.35; H, 4.66; N, 5.44; Cl, 13.81. Found: C, 51.49; H, 4.73; N, 5.11; Cl, 13.70.

A neutral equivalent of 130 was found by nonaqueous titration with 0.1 *N* sodium methoxide. A molecular weight of 250 was found by titration with 0.01 *N* silver nitrate.

N-Carbobenzoxy-L-β-chloroalanylglycine Ethyl Ester (I).—To a solution of 1.4 g of glycine ethyl ester hydrochloride (10 mmoles) and 1.4 ml of triethylamine (10 mmoles) in 50 ml of chloroform, 2.6 g of N-carbobenzoxy-L-β-chloroalanine (10 mmoles) in 30 ml of chloroform and 2.1 g of dicyclohexylcarbodiimide (10 mmoles) were added at 0°. The solution was then stirred for 6 hr at room temperature. Dicyclohexylurea was removed by filtration and the filtrate was washed with 0.5 *N* hydrochloric acid, water, and 5% sodium bicarbonate solution, and finally dried over sodium sulfate. Removal of the solvent yielded a crystalline residue which was recrystallized from ethyl acetate or methanol; yield 2.77 g (81%); mp 138°; $[\alpha]^{23}D$ − 3.9° (*c* 5, dimethylformamide).

Anal. Calcd for $C_{15}H_{19}N_2O_5Cl$: C, 52.56; H, 5.59; N, 8.17. Found: C, 52.77; H, 5.72; N, 8.27.

A molecular weight of 350 was found by titration with silver nitrate and a neutral equivalent of 344 by nonaqueous titration with 0.1 *N* sodium methoxide.

The procedure described for the synthesis of compound I was used for the preparation of the following compounds:

N-Carbobenzoxy-L-β-chloroalanyl-L-phenylalanine Benzyl Ester (V).—Compound V was obtained in 82% yield; mp 145°; $[\alpha]^{25}D$ −13.8° (*c* 5, dimethylformamide) recrystallized from ethanol.

Anal. Calcd for $C_{27}H_{27}N_2O_5Cl$: C, 65.51; H, 5.50; N, 5.66. Found: C, 65.39; H, 5.60; N, 5.60.

The molecular weight estimated from chloride titration was 500.

N-Carbobenzoxy-L-β-chloroalanylglycylglycine Ethyl Ester (VI).—Compound VI was obtained in 74% yield; mp 159–160°; $[\alpha]^{25}D$ −5.4° (*c* 2, dimethylformamide) recrystallized from ethyl acetate.

Anal. Calcd for $C_{17}H_{22}N_3O_6Cl$; C, 51.06; H, 5.55; N, 10.51; Cl, 8.87. Found: C, 51.31; H, 5.65; N, 10.71; Cl, 9.20.

The molecular weight estimated from chloride titration was 400.

N-Carbobenzoxy-glycyl-L-β-chloroalanylglycine Ethyl Ester (IX).—N-Carbobenzoxy-L-β-chloroalanylglycine ethyl ester (1.7 g) was treated with 7 ml of 33% HBr in acetic acid. Carbon dioxide evolution ceased after 15 min and anhydrous ether was added after another 20 min to precipitate the dipeptide ester hydrobromide which was triturated with ether until entirely crystalline. The compound was washed with ether and dried *in vacuo;* yield 1.4 g. This compound was coupled with 1.1 g of N-carbobenzoxyglycine by the carbodiimide method as described above; yield 1.8 g (80%); mp 132°; $[\alpha]^{25}D$ −9.4° (*c* 5, dimethylformamide) recrystallized from ethyl acetate.

Anal. Calcd for $C_{17}H_{22}N_3O_6Cl$: C, 51.06; H, 5.55; N, 10.51; Cl, 8.87. Found: C, 51.28; H, 5.60; N, 10.69; Cl, 8.75.

The molecular weight estimated from chloride titration was 395.

N-Carbobenzoxyglycylglycyl-L-β-chloroalanylglycylglycine Ethyl Ester (X).—A solution of 2 g of compound VI (5 mmoles) in 4 ml acetic acid was treated with 6 ml of 33% HBr in acetic acid as described above. The crystalline residue which was precipitated by the addition of ether was filtered and dried *in vacuo;* yield 1.7 g (96%); mp 165°. This compound was reacted with 1.3 g (5 mmoles) of N-carbobenzoxyglycylglycine by the carbodiimide method in a mixture of dimethylformamide and chloroform; yield 1.75 g (70%); mp 166°; $[\alpha]^{25}D$ −5.6° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{21}H_{28}N_5O_8C$: C, 49.12; H, 5.45; N, 13.64; Cl, 6.82. Found: C, 48.91; H, 5.35; N, 13.22; Cl, 6.55.

The molecular weight estimated from chloride titration was 520.

N-Carbobenzoxy-S-acetyl-L-cysteinylglycine Ethyl Ester (II). Method A.—To a solution of 0.3 ml of thioacetic acid in 5 ml of ethyl acetate, 0.5 ml of triethylamine and 0.34 g of N-carbobenzoxy-L-β-chloroalanylglycine ethyl ester (I) (1 mmole) were added. After allowing the mixture to stand for 2.5 hr at 50° (or 16 hr at room temperature) ethyl acetate was added and the triethylamine hydrochloride (136 mg 100%) was filtered off. The solution was washed with water several times and with 5% sodium hydrogen carbonate. It was dried over sodium sulfate and evaporated until dry. The crystalline residue was triturated with ether and filtered; yield 0.33 g (86%); mp 135–136°; $[\alpha]^{25}D$ −48.7° (*c* 1, dimethylformamide) [lit.[2] mp 135–136°; $[\alpha]^{18}D$ −48.4° (*c* 1, dimethylformamide)].

Anal. Calcd for $C_{17}H_{22}N_2O_6S$; C, 53.50; H, 5.80; N, 7.33; S, 8.38. Found: 53.81; H, 5.65; N, 7.20; S, 8.35.

Method B.—Thioacetic acid (0.5 ml) was treated with 2.1 ml of 3.25 *M* sodium methoxide and the solution was diluted to 4 ml with dimethylformamide. Compound I (0.34 g, 1 mmole) was dissolved in 4 ml of dimethylformamide and treated with 1 ml (1.5 mmoles) of the thioacetate solution. The reaction mixture was kept at 50° and the reaction was followed by iodine titration. After 90 min when the theoretical amount of thioacetate had been consumed, it was diluted with ethyl acetate and treated as described in method A above; yield 0.31 g (81%); mp 134°; $[\alpha]^{23}D$ −48.4° (*c* 1, dimethylformamide).

N-Carbobenzoxy-S-benzoyl-L-cysteinylglycine Ethyl Ester (III).—To a solution of 0.6 g of thiobenzoic acid and 0.6 ml of triethylamine in 5 ml of ethyl acetate 0.34 g of I was added. The reaction was allowed to stand for 2.5 hr at 50°, then diluted with ethyl acetate, and the triethylamine hydrochloride (132 mg 97%) was filtered off. It was then processed as described in method A above; yield 0.33 g (84%); mp 152°; $[\alpha]^{23}D$ −58.3° (*c* 1, dimethylformamide) [lit.[2] mp 153°; $[\alpha]^{18}D$ −58.5° (*c* 1, dimethylformamide)].

N-Carbobenzoxy-S-benzyl-L-cysteinylglycine Ethyl Ester (IV).—Benzyl mercaptan (0.74 g) was treated with 1.7 ml of 3.25 *M* sodium methoxide solution and the solvent was removed *in vacuo*. The residue was dissolved in 5 ml of dimethylformamide. Compound I (0.34 g, 1 mmole) was dissolved in 4 ml of dimethylformamide and treated with 2 ml (2.4 mmoles) of the sodium benzyl mercaptide solution. The reaction was followed iodometrically. After 30 min at room temperature, the solution was diluted with ethyl acetate and treated as described under method A above. The compound was recrystallized from ether–petroleum ether, or ethyl acetate–petroleum ether; yield 0.3 g (70%); mp 99°; $[\alpha]^{23}D$ −39° (*c* 2, dioxane) [lit.[12] mp 99–100°; $[\alpha]^{32}D$ −39.7° (*c* 4.32, dioxane)].

N-Carbobenzoxy-S-acetyl-L-cysteinyl-L-phenylalanine Benzyl Ester (VII).—This compound was prepared from N-carbobenzoxy-L-β-chloroalanyl-L-phenylalanine benzyl ester (V) (0.49 g, 1 mmole) in the same manner as described for the preparation of compound II (methods A and B); yield 0.45 g (84%); mp 152°; $[\alpha]^{25}D$ −38.4° (*c* 1, dimethylformamide) [lit.[5] mp 150–152°; $[\alpha]^{23}D$ −39° (*c* 1, dimethylformamide)].

Anal. Calcd for $C_{29}H_{30}N_2O_6S$: C, 65.15; M, 5.65; N, 5.24; S, 6.00. Found: C, 65.23; H, 5.55; N, 5.48; S, 6.00.

A neutral equivalent of 541 was determined by iodine titration after alcoholysis of the S-acetyl group.

(11) G. B. Brown and V. du Vigneaud, *J. Biol. Chem.*, **140**, 767 (1941).

(12) J. A. Maclaren, *Australian J. Chem.*, **11**, 360 (1958).

N-Carbobenzoxy-S-acetyl-L-cysteinylglycylglycine Ethyl Ester (VIII).—This compound was prepared from N-carbobenzoxy-L-β-chloroalanylglycylglycine ethyl ester (VI) (0.4 g, 1 mmole) in the same manner as described for the preparation of compound (II) (method A); yield 0.4 mg (91%); mp 128°; $[\alpha]^{25}D$ −42.4° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{19}H_{25}N_3O_7S$: C, 51.93; H, 5.73; N, 9.56; S, 7.28. Found: C, 52.23; H, 5.88; N, 9.25; S, 7.23.

A neutral equivalent of 435 was found by iodine titration after alcoholysis of the S-acetyl group.

N-Carbobenzoxyglycyl-S-acetyl-L-cysteinylglycine Ethyl Ester (XI).—To a solution of 0.4 ml of thioacetic acid in 5 ml of ethyl acetate, 0.7 ml of triethylamine and 0.4 g (1 mmole) of compound (IX) were added. The solution was allowed to stand for 3 hr at 50°, and was diluted with ethyl acetate. The triethylamine hydrochloride (131 mg, 96%) was filtered and the solution was washed with 0.5 *N* hydrochloric acid, water and 1 *N* sodium hydrogen carbonate, water, and dried over sodium sulfate. Removal of the solvent yielded a crystalline residue which was triturated with ether and recrystallized from ethyl acetate–petroleum ether; yield 0.35 g (80%); mp 94°; $[\alpha]^{25}D$ −29° (*c* 1.5, dimethylformamide) [lit.[2] mp 92–95°; $[\alpha]^{19}D$; −28.6° (*c* 1.5, dimethylformamide)].

Anal. Calcd for $C_{19}H_{25}N_3O_7S$: C, 51.93; H, 5.73; N, 9.56; S, 7.28. Found: C, 51.75; H, 5.51; N, 9.53; S, 7.39.[13]

N-Carbobenzoxyglycylglycyl-S-acetyl-L-cysteinylglycylglycine Ethyl Ester (XII).—This compound was prepared from N-carbobenzoxyglycylglycyl-L-β-chloroalanylglycylglycine ethyl ester (X, 0.25 g, 0.5 mmole) in the same manner as described for the preparation of compound XI (methods A, B). The product was recrystallized from ethyl acetate; yield 0.24 g (87%); mp 172°; $[\alpha]^{25}D$ −17.2° (*c* 1, dimethylformamide).

(13) A yield of 78% was found when the reaction was performed by method IIB.

Anal. Calcd for $C_{23}H_{31}N_5O_9S$: C, 49.90; H, 5.60; N, 12.66; S, 5.79. Found: C, 49.58; H, 5.90; N, 12.82; S, 5.99.

A neutralization equivalent of 568 was determined by iodine titration after alcoholysis of the S-acetyl group.

Nonaqueous Titration of Chloroalanyl Peptides.—The chloroalanyl peptide (0.1–0.2 mmole) is treated with a known volume of standardized 0.1 *M* sodium methoxide solution in methanol–benzene (1:3) in the presence of 1 drop of thymol blue (0.5% in dioxane). The mixture is stirred until all peptide dissolves. Sometimes light heating is recommended to facilitate the reaction. After 3 min the solution is titrated back to the red end point of the indicator with a standard solution of 0.1 *N* perchloric acid in dioxane. The molecular weight of the compound is calculated according to the formula: mol wt = mg × 10/ml; mg = weight of sample, ml = milliliters of 0.1 *N* sodium methoxide consumed. The accuracy of the method is 2–3%. The method is based on the formation of sodium chloride during the β-elimination reaction which chloroalanine peptides undergo when treated with sodium methoxide.

Argentometric Determination of Chloroalanyl Peptides.—The chloroalanyl peptide (0.02–0.1 mmole) is treated with 1 *N* NaOH (2 ml) with light heating. After 3 min, 2 ml of 6 *N* nitric acid and a known volume of standard 0.01 *N* silver nitrate are added. The mixture is shaken and warmed to coagulate and 0.05 ml of saturated ferric alum solution is added. The residual silver nitrate is titrated back with 0.01 *N* thiocyanate solution. The molecular weight calculation is performed as described above. The accuracy of the method is 2–4%.

Acknowledgment.—This investigation was supported by Grants No. AM-5098 and GM-11628 of the National Institute of Health, U. S. Public Health Service. The authors thank Professor Ephraim Katchalski for his interest in this work.

A New Synthesis of Unsaturated Acids. IV. Further Aspects of the Scope and Mechanism of the Conversion of Halopyrazolones to α,β-Acetylenic and Olefinic Acids[1]

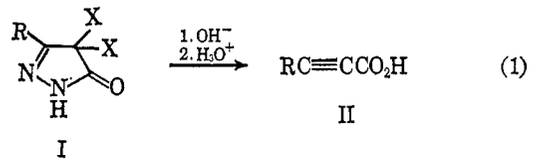
LOUIS A. CARPINO, PAUL H. TERRY, AND S. D. THATTE

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

Received March 29, 1966

Additional examples are reported illustrating the generality of a new synthesis of α,β-acetylenic and α,β-olefinic acids which involves treatment of 3-substituted 4,4-dihalo- and 3,4-disubstituted 4-halopyrazolones with aqueous sodium hydroxide. By substituting triethylamine for sodium hydroxide it has been possible to demonstrate the transient formation of a diazacyclopentadienone intermediate which can be trapped in the presence of butadiene or cyclopentadiene as the appropriate Diels–Alder adducts. The structures of the adducts have been established by alternate syntheses.

Several years ago we reported a new method for the synthesis of α,β-acetylenic and α,β-olefinic acids which involved the alkaline degradation of 4,4-dihalo- and 4-substituted 4-halo-2-pyrazolin-5-ones, respectively (eq 1 and 2).[3] An interesting feature of this reaction in the case of its application to α,β-olefinic acids was the observation that in the three cases examined the labile isomer (*cis*-IVb) predominated in the mixture of *cis* and *trans* acids obtained. This was true for both α-phenyl- and α-methylcinnamic acid and for α-phenylcrotonic acid. In the present study we have further investigated the application of these



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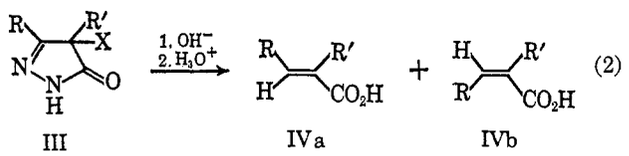

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reactions as a general synthetic route to α,β-acetylenic acids and particularly to the labile isomer of an α,β-olefinic pair. In addition it was hoped that at least the gross aspects of the mechanism of this novel reaction could be uncovered.

Three additional examples of the use of the method in the case of aliphatic α,β-acetylenic acids involved

(1) Supported in part by grants from the U. S. Army Research Office (Durham). A portion of this work has appeared in preliminary form.[2] Taken in part from the Ph.D. thesis of P. H. T., University of Massachusetts, 1963.

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(3) (a) L. A. Carpino, *J. Am. Chem. Soc.*, **80**, 599 (1958); (b) *ibid.*, **80**, 601 (1958); (c) *ibid.*, **80**, 5796 (1958).