evaporation with methanol and evacuation giving free acid orotidine (1.5 g.) as a white froth. This was dissolved in methanol (25 ml.) and treated with an ethereal solution of diazomethane as above. After evaporation to dryness and high-vacuum evacuation, the crude $N_3$-methylorotodine methyl ester (1.57 g.) was dissolved in dry acetone (18 ml.) containing 2,2-dimethoxypropane (2 ml.) and dried Dowex 50 ($H^+$) resin (1 g.). After 3 hours, the pale brown solution was filtered and the filtrate evaporated to dryness leaving 1.73 g. of crude $N_3$-methyl-2′,3′-O-isopropylidineorotidine methyl ester as a tan colored froth which smelled strongly of acetone polymers. This material was dissolved in dichloromethane and applied to a column containing 50 g. of neutral alumina (activity 1). Brown, oily acetone polymers were removed with dichloromethane, while elution with ether–methanol (1:1) gave fairly pure $N_3$-methyl-2′,3′-O-isopropylidineorotidine methyl ester (0.6 g.) as an oil which crystallized from benzene–petroleum ether giving chromatographically homogeneous white needles of m.p. 110–111°[15]; $\lambda_{max}$ (MeOH) 272 mμ, $\epsilon_{max}$ 7060; $\lambda_{min}$ (MeOH) 232 mμ.

*Anal.* Calcd. for $C_{18}H_{20}N_2O_8$: C, 50.60; H, 5.66; N, 7.86. Found: C, 50.88; H, 5.92; N, 7.90.

Nothing was eluted from the column with methanol, but water removed 0.7 g. of an oil which was electrophoretically shown to have a free carboxyl group. The *p*H of the eluate was, however, neutral. This material was dissolved in water and rapidly passed through a cold column containing Dowex 50 ($H^+$) resin (5 ml.) into an excess of aqueous cyclohexylamine. The eluate was evaporated to dryness leaving a crystalline residue which was recrystallized from isopropyl alcohol giving 600 mg. of chromatographically pure cyclohexylammonium $N_3$-methyl-2′,3′-O-isopropylidineorotidine, m.p. 210–211°; $\lambda_{max}$ (MeOH) 266 mμ, $\epsilon_{max}$ 9320; $\lambda_{max}$ (0.1 *N* NaOH–MeOH) 265 mμ, $\epsilon_{max}$ 9100.

*Anal.* Calcd. for $C_{17}H_{20}N_2O_8$: C, 54.60; H, 7.08; N, 9.52. Found: C, 54.11; H, 7.33; N, 9.52.

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

# Transformation of Serine to Cysteine. β-Elimination Reactions in Serine Derivatives[1,2]

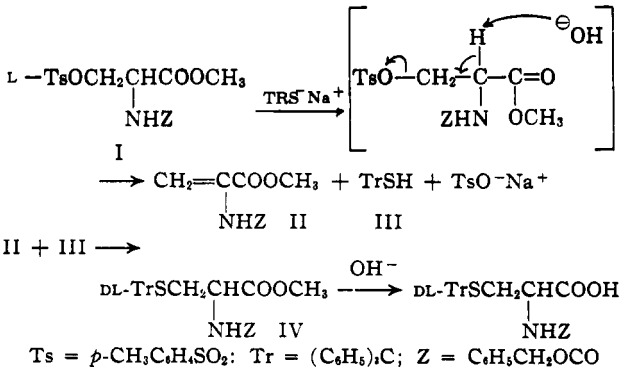
BY IPHIGENIA PHOTAKI[3]

RECEIVED NOVEMBER 16, 1962

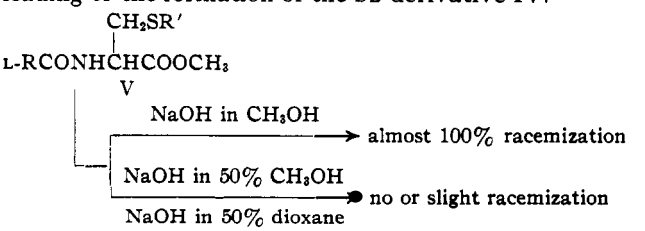
O-Tosylated and O-diphenylphosphorylated serine derivatives undergo a β-elimination reaction on being treated with 0.1 *N* alkali or diethylamine in non-polar solvents, resulting in the formation of dehydroalanine derivatives. N-Carbobenzoxy-O-tosyl-L-serine methyl ester is converted to N-carbobenzoxy-S-trityl-DL-cysteine by reaction with tritylthiocarbinol sodium salt, followed by saponification. It has been observed that N-acyl-S-alkyl-L-cysteine esters are extensively racemized by saponification with alcoholic alkali, but little or no racemization is produced by alkali in 50% aqueous methanol or aqueous dioxane.

## Introduction

The synthesis of unsymmetrical cystine peptides containing two or more –S–S– bridges is in progress in this Laboratory. For this purpose, cysteine residues bearing different S-protecting groups which may be removed selectively have been introduced.[4] We thought that this problem might also be solved by distributing L-serine residues in a peptide chain and converting these to cysteine residues. Serine has been transformed to cysteine through formation of phenylthiazolines[5] or phenyloxazolines,[6] but these conversions are not suitable for our purposes. An alternative method of conversion has been realized in the case of N-carbobenzoxy-O-tosyl-L-serine methyl ester (I). As has been stated in a preliminary report,[1a] this ester reacts very quickly with the sodium salt of tritylthiocarbinol to form the corresponding S-trityl-N-carbobenzoxycysteine methyl ester (IV). This route has the advantage that the S-trityl group can be cleaved very smoothly even at 0° either by means of 0.2 *N* HBr in acetic acid or with an equivalent amount of silver nitrate–pyridine.[4] In the meantime, however, it has been found that complete racemization occurs during the replacement reaction. As saponification of the reaction product either by methanolic alkali or by alkali in 50% aqueous methanol or aqueous dioxane leads to fully racemized acid, it is concluded that the loss of the optical activity occurs prior to the saponification, during the replacement of the O-tosyl group with the trityl-thio group.[7] This conclusion is based on the fact that N-carbobenzoxy-S-trityl-L-cysteine methyl ester (Vc) and in general N-acyl-S-alkyl-L-cysteine esters are extensively racemized by saponification with alcoholic alkali, but little or no racemization is produced by alkali dissolved in aqueous methanol or aqueous dioxane. Apparently in the above replacement reaction, a β-elimination is first brought about by the action of the alkaline sodium mercaptide on compound I and this is followed by the addition of tritylthiocarbinol to the dehydroalanine derivative II,[8] leading to the formation of the DL-derivative IV.




Ts = *p*-$CH_3C_6H_4SO_2$: Tr = $(C_6H_5)_3C$; Z = $C_6H_5CH_2OCO$




Va, R = $C_6H_5$, R′ = $(C_6H_5)_2CH$
Vb, R = $C_6H_5CH_2O$, R′ = $C_6H_5CH_2$
Vc, R = $C_6H_5CH_2O$, R′ = $(C_6H_5)_3C$

(1) (a) A summary of a part of this paper was presented at the 3rd European Peptide Symposium, Basle, Switzerland, September, 1960; L. Zervas and I. Photaki, *Chimia*, **14**, 375 (1960). (b) A summary of this paper was presented at the 5th European Peptide Symposium, Oxford, England, September, 1962.

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

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(4) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962); L. Zervas, I. Photaki and N. Gheiis, *ibid.*, in press.

(5) D. F. Elliot, *Nature*, **162**, 658 (1948).

(6) E. Fry, *J. Org. Chem.*, **15**, 438 (1950).

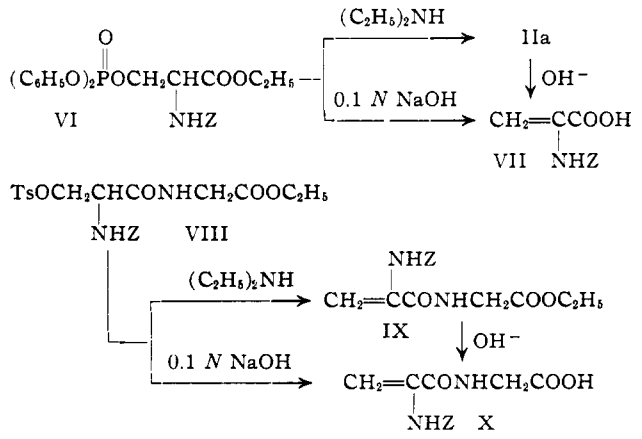
(7) For examples of exchange of O-tosyl groups to mercapto groups *cf.* H. Gilman and N. J. Beaber, *J. Am. Chem. Soc.*, **47**, 1449 (1925); A. L. Raymond, *J. Biol. Chem.*, **107**, 85 (1934); C. King, R. Dodson and L. Subluskey, *J. Am. Chem. Soc.*, **70**, 1176 (1948); J. H. Chapman and L. N. Owen, *J. Chem. Soc.*, 579 (1950); A. M. Michelson, *ibid.*, 979 (1962); N. F. Blau and C. G. Stuckwish, *J. Org. Chem.*, **25**, 1611 (1960); R. Ireland and J. A. Marshall, *ibid.*, **27**, 1615 (1962).

(8) For addition reactions of mercaptans to dehydroalanine derivatives *cf.* B. H. Nicolet, *Science*, **81**, 181 (1935).

The above findings provide an explanation for the controversial statements in the literature concerning the saponification of N-acyl-S-alkyl-L-cysteine esters. Iselin and Schwyzer[9] found no racemization when N-carbobenzoxy-S-benzyl-L-cysteine cyanomethyl ester was saponified, whereas Maclaren[10] reported racemization in the case of the saponification of N-carbobenzoxy-glycyl-S-benzyl-L-cysteine ethyl ester. The explanation may be that Iselin and Schwyzer used alkali dissolved in 30% alcohol, whereas Maclaren used solutions of alkali in 90% alcohol.

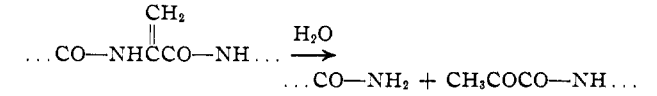
It is clear that the above method of transforming O-tosyl-L-serine residues of a peptide chain to cysteine residues would lead to the formation of diastereoisomers. Experiments are now in progress for carrying out the transformation of O-tosylserine to S-tritylcysteine under conditions which do not affect the existing configuration of the serine residue.

Experiments with O-tosylated serine derivatives revealed that these compounds rapidly undergo $\beta$-elimination under the action of bases, forming dehydroalanine derivatives. When N-carbobenzoxy-O-tosyl-L-serine methyl ester (I) is treated with 0.1 *N* alkali at room temperature, N-carbobenzoxydehydroalanine (VII) is formed to an extent of 65% in 8 minutes and to more than 90% within 15 minutes.[11] It is assumed that the $\beta$-elimination reaction proceeds much faster than the saponification of the ester group. $\beta$-Elimination is also produced by dissolving I in ethyl acetate or other non-polar solvents and adding 1–2 equiv. of diethylamine. *p*-Toluenesulfonic acid diethylammonium salt precipitates out almost instantaneously and quantitatively, whereas the N-carbobenzoxydehydroalanine ester II remains in the solution. The saponification of this ester leads to the formation of the corresponding free acid VII but also of small amounts of carbobenzoxy amide. This is further evidence that the carbobenzoxydehydroalanine is cleaved to pyruvic acid and carbobenzoxy amide under the action of very dilute alkalies at room temperature.[12]




It is known from the literature[12] that N-carbobenzoxy-O-diphenylphosphoryl-DL-serine ethyl ester (VI) undergoes $\beta$-elimination with 2 *N* alkali, giving rise to carbobenzoxydehydroalanine (VII) and diphenylphosphoric acid. It has been found in this work that both 0.1 *N* alkali and diethylamine also cause rapid $\beta$-elimination from compound VI, which in this respect behaves like the corresponding O-tosyl derivative. Similarly, treatment of N-carbobenzoxy-O-tosyl-L-serylglycine ethyl ester (VIII) with 10% more than 1 equiv. of diethylamine in ethyl acetate solution leads to the formation of *p*-toluenesulfonic acid diethylammonium salt and carbobenzoxydehydroalanylglycine ethyl ester (IX) in almost quantitative yield.[13] The use of 0.1 *N* alkali leads to the formation of the corresponding free acid X which can be isolated in good yield.

It is interesting that S-cyanocysteine[14] as well as S-dinitrophenyl- and S-permethylated cysteine derivatives[15] undergo $\beta$-elimination rapidly with alkali with the formation of the same products as in the case of the O-tosyl- and O-diphenylphosphorylserine derivatives. Such dehydroalanine peptides are known to undergo hydrolysis easily under mild basic or acidic conditions[12,16] or as has been recently found by Patchornik, *et al.*,[15] even by heating with water at 100°.




More recently, Patchornik and Sokolovsky[17] have described new procedures for the selective break-down of the double bonds in the dehydroalanine peptides. It is clear that our findings on tosylated or phosphorylated serine peptides may serve as a basis for the preferential cleavage of sequences containing serine residues, through formation of dehydroalanine peptides. The work of Witkop[18] and of Patchornik[15,17] for the determination of sequences in a non-enzymatic way acquires herewith a wider application.

## Experimental

Evaporations were carried out *in vacuo* at 30–40°. The melting points are not corrected.

Prior to analysis[19] the compounds were dried at 56° under high vacuum over phosphorus pentoxide.

The expression $\epsilon^*$ is used to denote the apparent molecular coefficient of the solute after it has undergone some transformation.[12]

**N-Carbobenzoxy-O-tosyl-L-serine Methyl Ester (I).**—To a solution of 2.5 g. (0.01 mole) of dry, sirupy, N-carbobenzoxy-L-serine methyl ester[20] in 9 ml. of anhydrous pyridine, precooled to −5°, 2.1 g. of tosyl chloride was added. After being allowed to stand for 2 hours at 0° followed by 2 hours at room temperature, the solution was poured onto crushed ice. Compound I precipitated as a sirup which crystallized on standing in the refrigerator. The product was collected by filtration, dried in a desiccator and recrystallized from a small amount of methanol. The yield was 2.5 g. (60%), m.p. 119–120°, $\lambda_{max}$ 262 m$\mu$ ($\epsilon$ 758 in 1% dimethylformamide in water), $[\alpha]^{25}D$ −10.5° (*c* 5, dimethylformamide).

*Anal.* Calcd. for $C_{19}H_{21}O_7NS$: C, 56.00; H, 5.19; N, 3.44. Found: C, 55.82; H, 5.19; N, 3.49.

**$\beta$-Elimination.**—(a) To a solution of 0.8 g. (0.002 mole) of I in 5 ml. of a mixture of ethyl acetate–ether (1:1), 0.4 ml. of diethylamine was added. Diethylammonium *p*-toluenesulfonate precipitated almost instantaneously; after being allowed to stand for several hours at room temperature and for 1 hour in the refrigerator the salt was recovered by filtration and was washed with ether (0.45 g., 92%). The filtrate which contained the carbobenzoxydehydroalanine methyl ester II [$\lambda_{max}$ 240 m$\mu$ ($\epsilon^*$ 5172 in methanol–water (4:1)] was evaporated to dryness *in vacuo*, the residue was dissolved in 2 ml. of methanol and 2.1 ml.

(9) B. Iselin, M. Feurer and R. Schwyzer, *Helv. Chim. Acta*, **38**, 1508 (1955).

(10) J. A. Maclaren, *Austral. J. Chem.*, **11**, 360 (1958).

(11) For elimination reactions on tosyl esters with bases compare P. J. Hamrick and C. R. Hauser, *J. Org. Chem.*, **26**, 4199 (1961).

(12) G. Riley, J. Turnbull and W. Wilson, *J. Chem. Soc.*, 1373 (1957).

(13) N-Carbobenzoxy-L-phenylalanyl-O-tosyl-L-serylglycine ethyl ester behaves similarly under these conditions (unpublished work).

(14) J. M. Swan, *Nature*, **180**, 643 (1957).

(15) A. Patchornik, M. Sokolovsky and T. Sadeh, Vth International Congress of Biochemistry, Moscow, 1961, Section I, p. 11; M. Sokolovsky, M. Wilchek and A. Patchornik, *Bull. Research Council Israel*, **11A**, 79 (1962).

(16) M. Bergmann and K. Grafe, *Hoppe Seyler's Z. physiol. Chem.*, **187**, 187 (1930); H. T. Clarke and J. M. Inoye, *J. Biol. Chem.*, **89**, 399 (1930).

(17) A. Patchornik and W. Sokolovsky, *Bull. Research Council Israel*, **11A**, 80 (1962).

(18) B. Witkop, "Advances in Protein Chemistry," Vol. 16, Academic Press, Inc., New York, N. Y., 1961, p. 221.

(19) Microanalyses were carried out by Mr. H. Mantzos in the Analytical Laboratory of the Royal Heilenic Research Foundation.

(20) J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).

of *N* NaOH solution was added. After 15 minutes at room temperature, the solution was diluted with water and the methanol was removed by evaporation at 25° *in vacuo*. A small amount of carbobenzoxy amide (m.p. 87°) which precipitated was filtered off. Upon acidification with hydrochloric acid, carbobenzoxydehydroalanine separated out; yield 63%, m.p. 108–109°, $\lambda_{max}$ 240 mμ (ε 5098 in 1% ethanol in water), after recrystallization from ethyl acetate–petroleum ether; reported[12] m.p. 106–108°, $\lambda_{max}$ 241 mμ (ε 5300 in water).

(b) Compound I (0.8 g., 0.002 mole) was dissolved in a mixture of 2.5 ml. of dimethylformamide, 30 ml. of methanol and 30 ml. of 0.2 *N* NaOH. Approximately 6–8 minutes after dissolution, the consumption of alkali was 65% of the theoretical amount; after 15 minutes 92% had been consumed. The solution was worked up as described above under (a); the yield of carbobenzoxydehydroalanine was 60%.

**N-Carbobenzoxy-O-tosyl-L-serylglycine Ethyl Ester (VIII).**—To a solution of 3.2 g. (0.01 mole) of N-carbobenzoxy-L-serylglycine ethyl ester[20] in 10 ml. of anhydrous pyridine, precooled to −5°, 2.1 g. of tosyl chloride was added and the mixture was kept at 0° for 2 hours. After being diluted with chloroform, the solution was washed repeatedly with sufficient amounts of dilute sulfuric acid to remove the pyridine and then with water until the aqueous extract was neutral to congo red paper. The chloroform solution was dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and addition of water to this solution caused VIII to crystallize. The product was purified by a further reprecipitation from ethanol–water. The yield was 2.9 g. (60%), m.p. 95–96°, $[\alpha]^{25}D$ +5.1° (*c* 5, dimethylformamide).

*Anal.* Calcd. for $C_{22}H_{26}N_2O_8S$: C, 55.21; H, 5.47; N, 5.85; S, 6.70. Found: C, 55.43; H, 5.63; N, 6.02; S, 6.84.

**β-Elimination.**—(a) Compound VIII was treated with diethylamine in the same manner as described for I. Diethylammonium *p*-toluenesulfonate was filtered off (yield 94%) and the filtrate was evaporated to dryness *in vacuo* to give crystalline N-carbobenzoxydehydroalanylglycine ethyl ester (IX) which was recrystallized from ethanol–water. The yield was 52%, m.p. 84° after recrystallization from ether–petroleum ether.

*Anal.* Calcd. for $C_{15}H_{18}O_6N_2$: C, 58.81; H, 5.92; N, 9.14. Found: C, 58.78; H, 5.98; N, 8.93.

From the mother liquor of IX a small amount of carbobenzoxy amide was obtained, m.p. 87°, undepressed upon mixing with an authentic sample of this product.

Upon saponification of the above ester, **N-carbobenzoxydehydroalanylglycine (X)** was obtained; the yield was 50%, m.p. 104–105° after recrystallization from ethyl acetate–petroleum ether.

*Anal.* Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.10; H, 5.07; N, 10.07. Found: C, 56.29; H, 5.21; N, 9.99.

(b) Compound VIII (0.48 g., 0.001 mole) was dissolved in a mixture of 4 ml. of ethanol and 2.2 ml. of 1 *N* NaOH. The solution was allowed to stand at room temperature for 15 minutes and was then acidified with hydrochloric acid. Concentration of the solution *in vacuo* gave 0.17 g. (55%) of N-carbobenzoxydehydroalanylglycine (X).

**N-Carbobenzoxy-L-phenylalanyl-L-serine Methyl Ester.**—To a solution of 4.65 g. (0.03 mole) of L-serine methyl ester hydrochloride and 4.2 ml. of triethylamine in chloroform, 9.4 g. of N-carbobenzoxy-L-phenylalanine (0.0315 mole) and 6.6 g. of dicyclohexylcarbodiimide were added. The reaction mixture was allowed to stand overnight at room temperature. A few drops of 50% acetic acid was then added and the precipitate of dicyclohexylurea was removed by filtration. The filtrate was washed successively with dilute hydrochloric acid, potassium hydrogen carbonate solution and water, dried over sodium sulfate and evaporated to dryness. The residue was heated in a small volume of ethyl acetate. Undissolved material (dicyclohexylurea) was filtered off and the filtrate was cooled to room temperature. After several hours the above dipeptide derivative separated out. It was collected by filtration, washed thoroughly with petroleum ether and recrystallized twice from a small volume of ethyl acetate. The yield was 2.4 g. (60%), m.p. 119–120°, $[\alpha]^{30}D$ −8.1° (*c* 7, dimethylformamide).

*Anal.* Calcd. for $C_{21}H_{24}N_2O_5$: C, 62.98; H, 6.04; N, 6.99. Found: C, 63.15; H, 5.91; N, 7.07.

**N-Carbobenzoxy-L-phenylalanyl-L-serine Hydrazide.**—A solution of 4 g. (0.01 mole) of the above dipeptide ester in 30 ml. of methanol and 0.8 ml. of hydrazine hydrate was allowed to stand at room temperature for 24 hours during which time the corresponding hydrazide crystallized out. The yield was 3.4 g. (85%), m.p. 183–184°, and 184–185° after recrystallization from ethanol; $[\alpha]^{30}D$ −6.5° (*c* 4, dimethylformamide).

*Anal.* Calcd. for $C_{20}H_{24}N_4O_5$: C, 59.98; H, 6.04; N, 13.99. Found: C, 59.78; H, 6.19; N, 14.07.

**N-Carbobenzoxy-L-phenylalanyl-L-serylglycine Ethyl Ester.**—To a solution of 3.2 g. (0.008 mole) of the above hydrazide in a mixture of 40 ml. of water, 4 ml. of acetic acid and 1.2 ml. of concd. hydrochloric acid, a mixture of 100 ml. of ether–ethyl acetate (1:1), was added. To this mixture, precooled to −5°, a solution of 0.95 g. of sodium nitrite in 6 ml. of water was added dropwise during a few minutes, with shaking and cooling. The mixture was transferred to a separating funnel where the organic phase was repeatedly washed with cold water and finally with cold potassium hydrogen carbonate solution. The ether–ethyl acetate solution was dried briefly over sodium sulfate before being added to an ethereal solution of glycine ethyl ester. After the reaction mixture had stood for several hours in the refrigerator, the above tripeptide derivative separated out. It was collected by filtration, washed with ether and recrystallized from ethyl acetate–ether. The yield was 2.3 g. (61%), m.p. 117–119°, $[\alpha]^{28}D$ −6.0° (*c* 2, dimethylformamide).

*Anal.* Calcd. for $C_{24}H_{29}N_3O_7$: C, 61.13; H, 6.19; N, 8.91. Found: C, 61.40; H, 6.38; N, 9.10.

**N-Benzoyl-S-diphenylmethyl-L-cysteine Methyl Ester (Va).**—To a solution of 1.7 g. (0.005 mole) of S-diphenylmethyl-L-cysteine methyl ester hydrochloride[4] in 5 ml. of anhydrous pyridine, precooled to −5°, 0.7 ml. of benzoyl chloride was added and the mixture was kept for 1 hour at room temperature. The solution was poured onto crushed ice and the compound Va which precipitated was recrystallized from methanol. The yield was 1.7 g. (84%), m.p. 135–136°, $[\alpha]^{30}D$ −73.5° (*c* 2, dimethylformamide).

*Anal.* Calcd. for $C_{24}H_{23}NO_3S$: C, 71.08; H, 5.71; N, 3.45; S, 7.90. Found: C, 71.20; H, 5.84; N, 3.77; S, 7.80.

**Saponification.**—(a) A solution of 0.4 g. (0.001 mole) of the ester Va in 4.5 ml. of 60% aqueous dioxane containing 0.0011 mole of sodium hydroxide was left to stand for several hours at room temperature and was then diluted with water. The unsaponified material (0.06 g.) which precipitated was removed by filtration. Most of the organic solvent was removed *in vacuo;* the solution was extracted with ethyl acetate and then acidified with concd. hydrochloric acid. **N-Benzoyl-S-diphenylmethyl-L-cysteine** separated out; the yield was 0.3 g. (75%), m.p. 135–140°, and 138–140° after recrystallization from ethanol; $[\alpha]^{30}D$ −69.9° (*c* 2.5, dimethylformamide).

*Anal.* Calcd. for $C_{23}H_{21}NO_3S \cdot 1/2H_2O$: C, 68.97; H, 5.53; N, 3.49; S, 8.00; O, 13.98. Found: C, 68.85; H, 5.77; N, 3.29; S, 8.07; O, 13.70.

(b) The saponification of the ester Va and the isolation of the free acid was carried out in the same manner as described above, except that a mixture of 3 ml. of 99% methanol and 1.5 ml. of dimethylformamide was used as solvent instead of 60% aqueous dioxane. The free acid thus obtained (0.31 g., 78%) was almost completely racemized ($[\alpha]^{30}D$ −0.8° in dimethylformamide, instead of $[\alpha]D$ −69.9° for the L-acid), m.p. 186–187°, unchanged after recrystallization from ethanol. The recrystallized acid was optically inactive.

*Anal.* Calcd. for $C_{23}H_{21}NO_3S$: C, 70.56; H, 5.40; N, 3.57; S, 8.19. Found: C, 70.59; H, 5.55; N, 3.51; S, 8.38.

**N-Carbobenzoxy-S-benzyl-L-cysteine Methyl Ester (Vb).**—To a mixture of 15 ml. of chloroform and 15 ml. of saturated potassium hydrogen carbonate solution, precooled to 0°, 2.6 g. (0.01 mole) of S-benzyl-L-cysteine methyl ester hydrochloride[21] and 2 ml. of carbobenzoxy chloride were added. The reaction mixture was shaken for 45 minutes at about 5°, and then the aqueous layer was discarded. Pyridine (1 ml.) was added to the chloroform layer and this solution was washed successively with dilute hydrochloric acid and water. Evaporation of the organic layer *in vacuo* and recrystallization of the residue from methanol gave 2.9 g. (87%) of the product, m.p. 62–63°, unchanged after further recrystallization; reported[22] m.p. 66–67°.

*Anal.* Calcd. for $C_{19}H_{21}NO_4S$: N, 3.89; S, 8.92. Found: N, 3.99; S, 8.64.

**Saponification.**—(a) A suspension of the above ester (1.1 g., 0.003 mole) in 8 ml. of 50% methanol containing 0.0033 mole of sodium hydroxide was stirred for about 90 minutes until the ester dissolved. Removal of methanol *in vacuo* at 25–30°, followed by dilution of the residual solution with water and acidification with concd. hydrochloric acid, gave 0.835 g. (80%) of N-carbobenzoxy-S-benzyl-L-cysteine, m.p. 85–87°, $[\alpha]^{22}D$ −38.2° (*c* 2, ethanol). After recrystallization from methylene chloride–petroleum ether the m.p. was 88–89°, $[\alpha]^{22}D$ −38.2° (*c* 4, ethanol); reported[9] m.p. 95–97°, $[\alpha]D$ −43° (in ethanol).

(b) The saponification and the isolation of the free acid was carried out in the same manner as described above, except that 99% methanol was used as solvent instead of 50% methanol. The free acid thus obtained (0.9 g., 85%) melted at 76–77° and possessed $[\alpha]^{22}D$ −4.9° (*c* 4, ethanol); *i.e.*, it was racemized to about 90%. The m.p. and $[\alpha]D$ were practically unchanged

(21) R. Boissonnas, S. Guttmann, P. Jaquenoud and J. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

(22) B. Hegedüs, *ibid.*, **31**, 737 (1948).

after recrystallization of the mixture from methylene chloride–petroleum ether.

**N-Carbobenzoxy-S-trityl-L-cysteine methyl ester (Vc)** was prepared by carbobenzoxylation of S-trityl-L-cysteine methyl ester hydrochloride[4] in the same manner as that described for the corresponding S-benzyl derivative. The yield of the sirupy product was 80%.

**Saponification.**—(a) The above ester (1.5 g., 0.003 mole) was dissolved in 10 ml. of 50% aqueous dioxane (or acetone) containing 0.0033 mole of sodium hydroxide. After being allowed to stand for 1 hour at room temperature, the solution was diluted with water. Most of the organic solvent was removed *in vacuo*, the solution was acidified with sulfuric acid and extracted with ether. The ethereal extract was washed repeatedly with water until the aqueous layer was neutral to congo red paper, and finally dried over sodium sulfate. Upon addition of diethylamine to the filtrate N-carbobenzoxy-S-trityl-L-cysteine diethylammonium salt separated out. The yield was 1.4 g. (81%), m.p. 164–166°, $[\alpha]^{25}D$ 18.6° (*c* 4, methanol); reported[4] m.p. 168°, $[\alpha]D$ 21.4° (in methanol); *i.e.*, the product was contaminated with about 10% of the DL-form.

(b) The saponification and the isolation of the free acid were carried out in the same manner as described in (a) except that 99% methanol was used as solvent instead of 50% dioxane. **N-Carbobenzoxy-S-trityl-DL-cysteine** was obtained as the diethylammonium salt (83%), m.p. 161–162°.

*Anal.* Calcd. for $C_{34}H_{38}N_2O_4S$: N, 4.91; S, 5.61. Found: N, 4.98; S, 5.72.

**β-Elimination in N-Carbobenzoxy-O-diphenylphosphoryl-DL-serine Ethyl Ester.**[12]—(a) Trituration of a solution of 0.2 ml. of diethylamine and 0.5 g. (0.001 mole) of the above ester in 4 ml. of ether led to the precipitation of diethylammonium O-diphenylphosphate. The mixture was left to stand for several hours at room temperature before the salt was collected by filtration and washed with ether. The yield was 85%, m.p. 123–124°.

*Anal.* Calcd. for $C_{16}H_{22}NO_4P$: N, 4.33; P, 9.58. Found: N, 4.58; P, 9.65.

From the filtrate, N-carbobenzoxydehydroalanine (60%, m.p. 108–109°) was obtained in the same manner as that described for the β-elimination in I.

(b) The above ester was treated with 3 equiv. of 0.1 *N* NaOH in 50% ethanol and within 30 minutes the β-elimination and the saponification were complete. N-Carbobenzoxydehydroalanine (60%, m.p. 108–109°) was obtained and, in addition, a small amount of carbobenzoxy amide (m.p. 87°).

**Conversion of N-Carbobenzoxy-O-tosyl-L-serine Methyl Ester (I) to N-Carbobenzoxy-S-trityl-DL-cysteine.**—Triphenylthiocarbinol (0.7 g., 0.0025 mole) was dissolved in anhydrous acetone, 2.4 ml. of methanolic 1 *N* sodium methoxide was added and the solution was rapidly evaporated to dryness at 25–30° *in vacuo*. The sodium mercaptide thus formed was dissolved in 12 ml. of anhydrous acetone and this solution was added, in 4 equal portions, to 1 g. of I dissolved in 12 ml. of anhydrous acetone, within a period of 10 minutes. Sodium tosylate precipitated almost instantaneously and after several hours at 4° it was filtered off. The filtrate was evaporated to dryness. The residue was dissolved in ether and the solution was washed with potassium hydrogen carbonate solution. Upon concentration of the ethereal solution *in vacuo*, 0.25–0.3 g. of the starting material I was recovered. The filtrate was evaporated to dryness to give a sirupy residue consisting mostly of N-carbobenzoxy-S-trityl-DL-cysteine methyl ester (II). Saponification of the product in the same manner as that described for the corresponding L-derivative in both 50% dioxane and 99% methanol afforded N-carbobenzoxy-S-trityl-DL-cysteine which was isolated as the diethylammonium salt. The yield was 65% calculated on the basis of I used; m.p. 161–162°, undepressed upon mixing with an authentic sample of the product.

**Acknowledgment.**—The author wishes to thank Professor Leonidas Zervas for many helpful suggestions and encouragement.

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[CONTRIBUTION FROM THE INSTITUTE OF APPLIED MICROBIOLOGY, UNIVERSITY OF TOKYO, HONGO, BUNKYO-KU, TOKYO, JAPAN; THE TAKAMINE LABORATORY, SANKYO CO., LTD., NISHISHINAGAWA, SHINAGAWA-KU, TOKYO, JAPAN; AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

# The Anthrasteroid Rearrangement. VIII.[1] The Rearrangement of Dehydroergosterol and $\Delta^{5,7,9(11)}$-Cholestatriene-3β-ol to $\Delta^{5,7,9,22}$-Anthraergostatetraen-x-ols and $\Delta^{5,7,9}$-Anthracholestatrien-x-ols

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RECEIVED APRIL 12, 1962

The acid-catalyzed rearrangement of dehydroergosteryl acetate leads in fair yields to two isomeric ring A-hydroxylated $\Delta^{5,7,9,22}$-tetraenes when *p*-toluenesulfonic acid monohydrate is employed in place of HCl gas. The secondary hydroxyl group is located either in position 2 or 3. By dehydration, two isomeric pentaene hydrocarbons are formed which contain a conjugated double bond in ring A. By catalytic hydrogenation of these $\Delta^{1(or\ 3),5,7,9,22}$-pentaenes two isomeric (at $C_{14}$) $\Delta^{5,7,9}$-anthraergostatrienes are formed. One of them is identical with the triene obtained previously by catalytic hydrogenation of $\Delta^{5,7,9,14,22}$-anthraergostapentaene. In an analogous manner the rearrangement of $\Delta^{5,7,9(11)}$-cholestatrien-3β-ol acetate, catalyzed by *p*-toluenesulfonic acid monohydrate, leads to two ring A-hydroxylated $\Delta^{5,7,9}$-anthracholestatrienols in a total yield of approximately 30%. The secondary alcohols were converted *via* the $\Delta^{1(or\ 3),5,7,9}$-tetraenes to 14α- and 14β-5,7,9-trienes. The former proved to be identical with the hydrocarbon obtained previously by catalytic hydrogenation of $\Delta^{5,7,9,14}$-anthracholestatetraene. By boiling of the steroid $\Delta^{5,7,9(11)}$-trienol acetates (s.ch. = $C_8H_{17}$) in glacial acetic acid the corresponding 14a-$\Delta^{5,7,9}$-trienols are obtained almost exclusively in yields of about 30%.

Tsuda and Hayatsu[3] have reported the photochemical conversion of several polyunsaturated cholesterol and ergosterol derivatives to ring A-hydroxylated anthrasteroids. This discovery appeared significant in view of the suggested mechanism of the anthrasteroid rearrangement in which the initial step is presumed to involve the creation of a double bond in ring A by loss of the hydroxyl group.[4] We have repeated the photochemical experiments according to the published directions and have consistently failed to detect any anthrasteroid material, either by ultraviolet spectral analysis or by isolation; the only identifiable products were dehydrocholesterol peroxide and ergosterol peroxide.

However, the use of *p*-toluenesulfonic acid as the rearrangement catalyst[3] (rather than hydrogen chloride) enabled us to isolate ring A-hydroxylated anthrasteroids (in *ca.* 30% yield) from the rearrangement of steroid $\Delta^{5,7,9(11)}$-trienol acetates.

The work-up of the crude reaction mixture proved to be exceedingly difficult, primarily because it involved the separation of two isomeric anthrasteroid secondary alcohols which themselves, as well as their various esters, are quite similar in their physical behavior and are rather unstable insofar as they tend to eliminate their oxygen function from ring A (with the creation of a double bond) in the process of crystallization or chromatography over alumina. Therefore in the chromatographic separation Florisil was used with great advan-

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