265. *Peptides. Part VII.* The Preparation and Use of p-Nitrophenyl Thiolesters.†*

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Tri-*p*-nitrophenyl phosphorotrithioite (II) converts the benzyloxycarbonyl derivatives of peptides or amino-acids into the corresponding *p*-nitrophenyl thiolesters (I). These thiolesters react with aqueous solutions of peptides or amino-acids at room temperature. Both stages of this peptide synthesis proceed in good yield, but racemisation is liable to occur during the second.

WIELAND and his co-workers [1,2] have shown that peptide links can be formed in aqueous media from phenyl thiolesters, because they are stable to hydrolysis and yet react with amines, albeit rather slowly. Our experiences, described later in this paper, with *p*-nitrophenyl esters suggested that the *p*-nitrophenyl thiolesters (I) would react faster ‡ and might be even more valuable than Wieland's intermediates.

Benzyloxycarbonylglycine *p*-nitrophenyl thiolester was prepared, like the phenyl analogue,[1] from the mixed anhydride with ethyl hydrogen carbonate.[3] Its solution in dioxan reacted smoothly at 18° with aqueous solutions of phenylalanine and phenylalanylglycine, the red colour of the liberated *p*-nitrothiophenoxide anion providing a convenient indication of the reaction. The best way of buffering the reaction mixture was to stir it with magnesium carbonate.

Completely racemic *p*-nitrophenyl thiolester was obtained from benzyloxycarbonylglycyl-L-phenylalanine through the carbonic mixed anhydride. The mixed anhydride with lithium hydrogen sulphate [4,5] gave a moderate yield of optically active thiolester. That this specimen was partly racemic was suggested by the products of reaction with glycine and glycine ester, and this was confirmed later when genuine L-material became available. Both the sulphuric anhydride mentioned above and that derived from benzyloxycarbonylglycine consumed the full molar proportion of *p*-nitrothiophenol, and some of

* Part VI, preceding paper.

† A preliminary account has appeared (Farrington, Kenner, and Turner, *Chem. and Ind.*, 1955, 601).

‡ *p*-Nitrothiophenol has p*K* 6·8 in 1 : 2 (v/v) dimethylformamide–0·2M-potassium phosphate (determined from absorption at 448 mμ). In water the p*K* was estimated as 4·5; accurate determination was frustrated by the insolubility of *p*-nitrothiophenol.

[1] Wieland, Schäfer, and Bokelmann, *Annalen*, 1951, **573**, 99.

[2] Wieland and Schäfer, *ibid.*, 1952, **576**, 104.

[3] Boissonas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan and Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 676.

[4] Kenner and Stedman, *J.*, 1952, 2069.

[5] Clayton, Farrington, Kenner, and Turner, preceding paper.

this was regenerated when the aqueous liquors were boiled. Very likely lithium *S*-*p*-nitrophenyl thiosulphate is a by-product of attack on the sulphur, instead of the carbon, in the mixed anhydride.

$R{\cdot}CO{\cdot}S{\cdot}C_6H_4{\cdot}NO_2$ (I) $P(S{\cdot}C_6H_4{\cdot}NO_2)_3$ (II) $C_6H_5{\cdot}NH{\cdot}C(:N{\cdot}C_6H_5){\cdot}S{\cdot}C_6H_4{\cdot}NO_2$ (III)

A new method for converting carboxylic acids into thiolesters was clearly needed and we explored the use of tervalent phosphorus derivatives of *p*-nitrothiophenol, following the preparation of anilides from *N*-arylphosphenimidous anilides.[6] Finally a good reagent was discovered, namely, tri-*p*-nitrophenyl phosphorotrithioite (II). It is a stable crystalline solid, prepared from phosphorus trichloride and a salt of *p*-nitrothiophenol; our preferred method avoids the somewhat troublesome preparation of sodium *p*-nitrothiophenoxide by starting from *S*-*p*-nitrophenyl-*NN'*-diphenyl*iso*thiourea (III), which is readily available from *p*-nitrobenzenediazonium chloride and thiocarbanilide.[7] Excellent yields of *p*-nitrophenyl thiolesters were obtained with the reagent (II) by three methods. The carboxylic acid was allowed to react directly in warm dimethylformamide or in pyridine, or a solution of its lithium salt in dimethylformamide was shaken with the reagent at room temperature. In each instance the reaction mixture was anhydrous and the neutral product isolated after being washed with sodium hydrogen carbonate solution. The lithium salts reacted faster than the free acids, but the first method gave the most consistent yields of easily purified material. Presumably the other reaction product was either the *SS'*-di-*p*-nitrophenyl ester of phosphorodithious (or phosphonodithoic) acid or its lithium salt as the case might be, but we were unable to isolate it. It was evidently destroyed during the working-up, for the red colour of *p*-nitrothiophenoxide anion developed in the bicarbonate washings. Normally the reagent (II) and the carboxylic acid were mixed in equimolar proportions, but when 3 mol. of benzyloxycarbonylglycine lithium salt were added 2 mol. of thiolester were obtained.

The benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl thiolester obtained from the phosphorotrithioite (II) had double the optical rotation of that prepared from the sulphuric anhydride. Furthermore it gave benzyloxycarbonylglycyl-L-phenylalanylglycine of good quality in high yield when it was allowed to react with glycine in aqueous dioxan. But it had already been found in a study of the sulphuric anhydride synthesis [5] that racemisation is more likely to occur during the condensation of benzyloxycarbonylglycyl-L-alanine with L-phenylalanylglycine. The benzyloxycarbonyltetrapeptide was therefore prepared by the thiolester method and analysed by counter-current distribution.[5] This revealed the presence of almost half as much DL- as LL-isomer, a much worse result than that of the sulphuric anhydride method. Unrestricted application of the new method cannot therefore be advocated, but the relatively great tendency to crystallisation and stability of the *p*-nitrophenyl thiolesters and the unusually high yields will render it valuable in certain instances. These will be chiefly syntheses through the thiolesters of either benzyloxycarbonylamino-acids or derivatives of peptides with *C*-terminal glycine, neither of which are prone to racemisation; some examples are described in the Experimental section. A special application is to the synthesis of cyclic peptides.[8]

Before working with *p*-nitrophenyl thiolesters we investigated the use of analogous oxygen compounds. These are, in principle, more directly accessible from carboxylic acids, like the cyanomethyl esters which have been so successfully used for peptide synthesis.[9] However, despite the fact that small amounts of dinitrophenylpeptides may be produced during the dinitrophenylation of amino-acids,[10] we were unable to effect reaction of benzyloxycarbonylglycine lithium salt with either fluorodinitrobenzene or

[6] Grimmel, Guenther, and Morgan, *J. Amer. Chem. Soc.*, 1946, **68**, 539.
[7] Busch and Schulz, *J. prakt. Chem.*, 1938, **150**, 180.
[8] Kenner and Turner, *Chem. and Ind.*, 1955, 602.
[9] Schwyzer and co-workers, *Helv. Chim. Acta*, 1955, **38**, 80, 83, 1508.
[10] Heikens, Hermans, and van Velden, *Nature*, 1954, **174**, 1187.

picryl chloride. Benzyloxycarbonylglycine *p*-nitrophenyl ester was prepared in moderate yield from *p*-nitrophenol and the mixed anhydrides with both ethyl and lithium hydrogen sulphate; in the latter instance reaction was notably easier in aqueous than in anhydrous dimethylformamide. The reactivity of this *p*-nitrophenyl ester towards alanine was greater than that of the corresponding phenyl thiolester but less than that of the *p*-nitrophenyl thiolester (ratio of rates, 16 : 1 : 140). The *p*-nitrophenyl ester obtained from benzyloxycarbonylglycyl-L-phenylalanine through the sulphuric anhydride had a small optical rotation but it gave the DL-tripeptide derivative on reaction with glycine. Consequently our work along these lines [11] was abandoned. Very recently a *p*-nitrophenyl ester has been prepared by a new route and applied to the synthesis of gramicidin-S.[12]

EXPERIMENTAL

M. p.s are corrected. Evaporations were carried out under reduced pressure.

Tri-p-nitrophenyl Phosphorotrithioite.—(*a*) A solution of lithium hydroxide (2·21 g.; commercial dihydrate) in water (25 c.c.) was added to a solution of *S-p*-nitrophenyl-*NN'*-diphenyl*iso*-thiourea [7] (17·5 g.; m. p. 133°) in dimethylformamide (75 c.c.), which was immediately coloured deep red. The water was removed, under 15 mm. of nitrogen, through a 6 in. column of steel gauze rings in 50 c.c. of distillate. The solution was cooled to 0° and stirred under nitrogen while phosphorus trichloride (1·46 c.c.; freshly distilled from diethylaniline) was added dropwise. As the addition was completed the mixture changed from red to yellow. It was stirred for 15 min. further at 0°, and the *trithioite* (5·7 g., 70%) was then collected, washed with dimethyl formamide (6 c.c.), and dried at 80°/3 mm. before being recrystallised from ethylene dichloride in yellow needles, m. p. 166—167° (Found : C, 43·8; H, 2·6; N, 8·8. $C_{18}H_{12}O_6N_3S_3P$ requires C, 43·8; H, 2·5; N, 8·5%). It may be dimorphic since analytically pure products of different preparations had m. p.s as much as 18° lower.

(*b*) A solution of partly hydrated sodium *p*-nitrothiophenoxide (60 g.) in pyridine (600 c.c.) was dried by concentration to 500 c.c. under a Fenske fractionating column. After a small amount of brown material had been removed at a centrifuge, the solution was stirred and cooled in ice. Phosphorus trichloride diluted with 10 vols. of pyridine was added slowly until the intense red colour suddenly faded to orange-pink. The colloidal salt was separated centrifugally and the liquors were set aside for a week at −40°. The trithioite which crystallised (35 g.) was collected and a further crop (10 g.) was obtained by evaporation of the liquor. Recrystallisation from chlorobenzene afforded 27 g., of m. p. 155°.

General Procedures for Preparation of p-*Nitrophenyl Thiolesters from Tri-*p-*nitrophenyl Phosphorotrithioite.*—(a) *In dimethylformamide.* Tri-*p*-nitrophenyl phosphorotrithioite (20 mmoles; dried at 40°/0·5 mm. for 8 hr.) was added to a solution, which had been dried by concentration at 50°/11 mm., under a 6 in. column packed with steel gauze, of the carboxylic acid (20 mmoles) in dimethylformamide (40 c.c.). The mixture was stirred under nitrogen at 85° for 20 min., during which the trithioite dissolved. The solution was evaporated and the residue, apart from some di-*p*-nitrophenyl disulphide, was taken up in ethyl acetate (200 c.c.) and water (50 c.c.). The ethyl acetate solution was washed with saturated sodium hydrogen carbonate solution (3 × 50 c.c.) and water (3 × 50 c.c.) before being evaporated. The almost colourless residue was taken up again in ethyl acetate (15 c.c.), which was then filtered from a little bisnitrophenyl disulphide and evaporated, yielding the product. The small amount of unchanged acid could be recovered from the alkaline washings, after they had been treated with a few drops of 30% hydrogen peroxide, filtered, and washed with ethyl acetate, by acidification and extraction with ethyl acetate.

(b) *In pyridine.* Tri-*p*-nitrophenyl phosphorotrithioite (10 mmoles) was added to a solution of the carboxylic acid (9·5 mmoles) in dry pyridine (15 c.c.). The mixture was kept at 60° for 5 min. so that all had dissolved, and then left overnight at 18°. The residue from evaporation of the pyridine was taken up in ethyl acetate (100 c.c.), which was washed successively with 50 c.c. portions of N-sulphuric acid, water, saturated sodium hydrogen carbonate solution

[11] Cf. Bodánszky, *Nature*, 1955, **175**, 685; Bodánszky, Szelke, Tömörkeny, and Weisz, *Chem. and Ind.*, 1955, 1517; Wieland and Jaenicke, *Annalen*, 1956, **599**, 125.
[12] Schwyzer and Sieber, *Angew. Chem.*, 1956, **68**, 518.

(twice), and water. The ethyl acetate was combined with back-extracts of the aqueous washings and evaporated to yield the product. Any unchanged acid could be recovered from alkaline washings as in (a).

(c) *Through the lithium salt.* A solution of the carboxylic acid (5 mmoles) in dimethylformamide (65 c.c.) was neutralised with methanolic lithium methoxide and then dried by concentration at 50°/11 mm., under a 6 in. column of steel gauze rings. The solution (50 c.c.) was cooled to 20° and treated with tri-*p*-nitrophenyl phosphorotrithioite (5 mmoles), which dissolved on shaking. The solution was kept overnight and then either poured into water or evaporated and worked up as in (b).

Benzyloxycarbonylglycine p-*Nitrophenyl Thiolester.*—(a) *From tri*-p-*nitrophenyl phosphorotrithioite.* Procedure (a) described above gave 96%, procedure (b) gave 99%, and procedure (c) gave 96% of the *thiolester*, which recrystallised from methanol, benzene, or aqueous acetone in colourless needles, m. p. 112·5° (Found: C, 55·7; H, 4·2; N, 8·3. $C_{16}H_{14}O_5N_2S$ requires C, 55·5; H, 4·1; N, 8·1%).

(b) *From the carbonic anhydride.* Ethyl chloroformate (0·48 c.c., 5 mmoles) was added dropwise to a stirred solution of benzyloxycarbonylglycine (1·045 g., 5 mmoles) and triethylamine (0·675 c.c., 5 mmoles) in dimethylformamide (50 c.c.) at −5°. After 10 min. a solution of sodium *p*-nitrothiophenoxide (0·885 g., 5 mmoles) in dimethylformamide (15 c.c.) was added, and the mixture was left overnight at 18°. Working according to the phosphorotrithioite procedure (b) then furnished 65% of orange crystalline thiolester.

(c) *From the sulphuric anhydride.* Sodium *p*-nitrothiophenoxide (1·77 g., 10 mmoles) was dissolved in a mixture 0·5M-potassium dihydrogen phosphate (93 c.c.), 0·5M-dipotassium hydrogen phosphate (40 c.c.), and dimethylformamide (67 c.c.). The solution was added to a solution of lithium benzyloxycarbonylglycyl sulphate [5] (10 mmoles) in dimethylformamide (100 c.c.), and the mixture was stirred at 18° during 15 hr. while the thiolester precipitated. The red colour faded to about half its intensity in ½ hr. and was regularly pale orange after 1½ hr. The pH was brought to 4 with 3N-sulphuric acid, and the solvents were evaporated before working-up as before; the yield was 70%. When the reaction was carried out in anhydrous dimethylformamide the yield was 30% after 4 hr., 50% after 19 hr., 67% after 66 hr.

*Benzyloxycarbonylglycyl-*L-*phenylalanine* p-*Nitrophenyl Thiolester.*—(a) *From tri*-p-*nitrophenyl phosphorotrithioite.* Procedure (c), followed by recrystallisation from methanol, afforded 79% of the *thiolester* as colourless needles, m. p. 154—155°, $[\alpha]_D^{16}$ −67·0° (±0·4°) (*c* 4·6 in dioxan) (Found: C, 61·0; H, 4·5; N, 8·6. $C_{25}H_{23}O_6N_3S$ requires C, 60·9; H, 4·7; N, 8·5%).

(b) *From the carbonic anhydride.* The preparation followed the plan of the glycine series except that *p*-nitrothiophenol itself was added instead of the sodium salt, and it afforded 81% of thiolester, which had, after recrystallisation, m. p. 154—155°, $[\alpha]_D^{17}$ +0·6° (*c* 3·7 in dioxan).

(c) *From the sulphuric anhydride.* The preparation followed the plan of the glycine series except that the precipitated thiolester (53%) was collected directly. After recrystallisation it had m. p. 152—153°, $[\alpha]_D^{17}$ −35·7° (*c* 3·2 in dioxan).

*Benzyloxycarbonyl-*L-*leucine* p-*Nitrophenyl Thiolester.*—Procedure (a) afforded 97% of the *thiolester*, which recrystallised from methanol in colourless needles, m. p. 107° (Found: C, 59·9; H, 5·2; N, 6·8. $C_{20}H_{22}O_5N_2S$ requires C, 59·7; H, 5·5; N, 7·0%).

*Benzyloxycarbonyl-*L-*leucylglycine* p-*Nitrophenyl Thiolester.*—Procedure (a) afforded 96% of the *thiolester*, which recrystallised from methanol or benzene–light petroleum (b. p. 40—60°) in colourless needles, m. p. 118° (Found: C, 57·7; H, 5·2; N, 9·5. $C_{22}H_{25}O_6N_3S$ requires C, 57·5; H, 5·5; N, 9·2%).

*Benzyloxycarbonylglycyl-*L-*leucylglycine* p-*Nitrophenyl Thiolester.*—Procedures (a) and (b) both gave 98% of the *thiolester*, which had, after recrystallisation from aqueous acetone, m. p. 159° (Found: C, 55·6; H, 5·2; N, 10·8. $C_{24}H_{28}O_7N_4S$ requires C, 55·8; H, 5·5; N, 10·8%).

*Benzyloxycarbonylglycyl-*L-*leucylglycyl-*L-*leucylglycine* p-*Nitrophenyl Thiolester.*—Procedure (a) gave 93%, while procedure (b) gave 98%, of the *thiolester*, which had, after recrystallisation from aqueous acetone, m. p. 184° (Found: C, 55·9; H, 6·1; N, 12·3. $C_{32}H_{42}O_9N_6S$ requires C, 56·0; H, 6·2; N, 12·2%).

L-2-p-*Nitrophenylthiocarbonyl-5-oxo-1-toluene-p-sulphonylpyrrolidine.*—This *thiolester* was prepared in quantitative yield from 5-oxo-1-toluene-*p*-sulphonylpyrrolidine-2-carboxylic acid [13] by procedure (b). It was recrystallised from aqueous acetone and had m. p. 174° (Found: C, 51·0; H, 4·0; N, 6·7. $C_{18}H_{16}O_6N_2S_2$ requires C, 51·4; H, 3·8; N, 6·7%).

[13] Rudinger, *Coll. Czech. Chem. Comm.*, 1954, **19**, 375.

Benzyloxycarbonyl-L-leucylglycine.—A solution of benzyloxycarbonyl-L-leucine *p*-nitrophenyl thiolester (8·0 g.) in tetrahydrofuran (80 c.c.) and one of glycine (3·0 g.) in water (60 c.c.) were shaken together with magnesium carbonate (8 g.) at 18° during 40 hr. The solvents were evaporated and the residue was partitioned between ethyl acetate (200 c.c.) and N-sulphuric acid (60 c.c.). The ethyl acetate was extracted with sodium hydrogen carbonate solution (2 × 100 c.c.); the extract was rendered colourless by a little hydrogen peroxide before being filtered and washed with ethyl acetate. The product (6·3 g., 98%) was recovered from the sodium hydrogen carbonate solution by acidification, extraction with ethyl acetate, and evaporation. It recrystallised from ethyl acetate–light petroleum (b. p. 40—60°) in colourless prisms, m. p. 117—118°.[14]

L-Leucylglycine Hydrobromide.—Benzyloxycarbonyl-L-leucylglycine (1·61 g.), in acetic acid (20·5 c.c.), was treated with 6N-hydrogen bromide in acetic acid (4·5 c.c.). The mixture was kept for 2 hr. at 18°. The residue obtained by evaporation was triturated with ether, which left colourless crystals (1·34 g.). These were used directly for further preparations, but the pure *hydrobromide* was obtained by recrystallisation from ethereal methanol in colourless prisms, m. p. 194—195° (Found: C, 35·8; H, 6·7; N, 10·2. $C_8H_{17}O_3N_2Br$ requires C, 35·7; H, 6·4; N, 10·4%).

Benzyloxycarbonylglycyl-L-leucylglycine.—(*a*) The corresponding ethyl ester [5] (9·58 g.) was shaken overnight with 0·5N-sodium hydroxide (50 c.c.). The solution was washed with ethyl acetate and then acidified with N-sulphuric acid. The precipitated oil was extracted into ethyl acetate (100 c.c.) before being purified by counter-current distribution (14 transfers; 100 c.c. phases) between ethyl acetate and 0·82M-KH_2PO_4–0·18M-K_2HPO_4. Tubes 1—7 contained 8·6 g. of highly crystalline material (*K* 0·25), recrystallisation of which from ethyl acetate furnished pure *benzyloxycarbonylglycyl-L-leucylglycine* (7·99 g., 89%), m. p. 110°, $[\alpha]_D^{19}$ −14·7° (*c* 4 in dimethylformamide) (Found: C, 57·2; H, 6·3; N, 11·1. $C_{18}H_{25}O_6N_3$ requires C, 57·0; H, 6·6; N, 11·1%).

(*b*) Magnesium carbonate (3 g.) and solutions of benzyloxycarbonylglycine *p*-nitrophenyl thiolester (1·73 g.) in tetrahydrofuran (40 c.c.) and L-leucylglycine hydrobromide (1·345 g.) in water (40 c.c.) were used in a preparation like that of benzyloxycarbonyl-L-leucylglycine. The yield of material, m. p. 104—105°, was 1·77 g. (93%).

Benzyloxycarbonylglycyl-L-leucylglycyl-L-leucylglycine.—A preparation, like the foregoing preparation (b), from benzyloxycarbonylglycyl-L-leucylglycine *p*-nitrophenyl thiolester (1·32 g.) and L-leucylglycine hydrobromide (0·72 g.) gave an amorphous product (1·31 g., 93%), m. p. 205—206°, which was characterised as its crystalline *p*-nitrophenyl thiolester (see above). On counter-current distribution (14 transfers; 100 c.c. phases) between ethyl acetate and 0·20M-KH_2PO_4–0·05M-K_2HPO_4 it appeared homogeneous (*K* 0·12), but it would not have been separated from benzyloxycarbonylglycyl-L-leucylglycine (*K* 0·15).

Benzyloxycarbonylglycyl-L-phenylalanine.—A solution of L-phenylalanine (3·14 g., 19 mmoles) in water (190 c.c.) was added to a solution of benzyloxycarbonylglycine *p*-nitrophenyl thiolester (6·58 g., 19 mmoles) in dioxan (285 c.c.). The clear yellow solution had pH 5·8 (to indicator paper) and this was raised to and maintained at 7·5 by gradual addition during 1 hr. of N-sodium hydroxide (38 c.c.). The mixture was left overnight at 18° and it was then treated in the usual way. The product (6·31 g., 93%) had m. p. 124—125°.[15]

Benzyloxycarbonylglycyl-DL-phenylalanylglycine.—DL-Phenylalanylglycine (0·111 g., 0·5 mmole) was dissolved in 0·5M-potassium dihydrogen phosphate (3 c.c.) and 0·5M-dipotassium hydrogen phosphate (7 c.c.) and then stirred at 18° during 16 hr. with a solution of benzyloxycarbonylglycine *p*-nitrophenyl thiolester (0·173 g., 0·5 mmole) in dioxan (20 c.c.). The product (0·185 g., 90%), obtained in the usual way, had m. p. 140·5—142°.[16]

Benzyloxycarbonylglycyl-L-phenylalanylglycine.—(*a*) A preparation, similar to those described above, from benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl thiolester, glycine, and magnesium carbonate in aqueous dioxan, furnished a quantitative yield of the tripeptide derivative,[16, 5] m. p. 156—157°, $[\alpha]_D^{18}$ −14·6° (±0·3°) (*c* 3·8 in EtOH).

(*b*) A mixture of glycine ethyl ester hydrochloride (0·070 g., 0·25 mmole), triethylamine (0·068 c.c., 0·5 mmole), and dimethylformamide (4 c.c.) was added dropwise to a solution of the same thiolester (0·123 g., 0·25 mmole) in dimethylformamide (2 c.c.). The solution, which

[14] Bergmann, Zervas, and Fruton, *J. Biol. Chem.*, 1935, **111**, 238.
[15] Hofmann and Bergmann, *ibid.*, 1940, **134**, 225.
[16] Kenner and Stedman, *J.*, 1952, 2074.

immediately became red, was left for 16 hr. at 18° and then evaporated to dryness. The neutral product was isolated by the usual separations between ethyl acetate and 3N-sulphuric acid, saturated sodium hydrogen carbonate solution, and water. Hydrolysis of it at 18° by a slight excess of dilute sodium hydroxide in 80% methanol gave 0·099 g. (96%) of material with m. p. 156—157°, $[\alpha]_D^{18}$ −13·7° (±0·3°) (*c* 3·4 in EtOH).

*Benzyloxycarbonylglycyl-*L*-alanyl-*L*-phenylalanylglycine and the* DL*-Isomer.*—Benzyloxycarbonylglycyl-L-alanine (0·560 g., 2 mmoles) was converted by procedure (c) into the thiolester, which was isolated by extraction with ethyl acetate as an orange, partly crystalline material (1·21 g.); 9% of the starting material was recovered unchanged. The crude thiolester was dissolved in dioxan (20 c.c.) and then stirred with magnesium carbonate and a solution of L-phenylalanylglycine (0·480 g. of the hydrate, 2 mmoles) in water (10 c.c.) during 12 hr. at 20°. The solvents were evaporated in a current of air and the residue was partitioned between 3N-sulphuric acid (20 c.c.) and ethyl acetate (60 c.c.), which was then extracted with saturated sodium hydrogen carbonate solution (5 × 10 c.c.; first portion containing 0·7 c.c. 20-vol. hydrogen peroxide). The carbonate solution was filtered, acidified with concentrated hydrochloric acid, and extracted with ethyl acetate (100 c.c., 2 × 50 c.c.). Evaporation of the ethyl acetate yielded crystals (0·922 g.) which were handled as before.[5] The first distribution (94 transfers, 5-tube filling) separated the tetrapeptide derivatives (0·763 g., 79%; tubes 34—65) from benzyloxycarbonylglycyl-L-alanine (0·037 g., 5%; tubes 0—33) and bis-*p*-nitrophenyl-disulphide (0·141 g., tubes 66—94). The second distribution (850 transfers, 5-tube filling) of the tetrapeptide derivatives showed that 70% was the LL- (*K* 0·582) and 30% was the DL-form (*K* 0·742). In agreement with previous findings [5] the optical rotations were −31·5° and +14·9° respectively.

Benzyloxycarbonylglycine p-*Nitrophenyl Ester.*—(*a*) Ethyl chloroformate (0·96 c.c., 10 mmoles) was added dropwise to a stirred solution of benzyloxycarbonylglycine (2·09 g., 10 mmoles) and triethylamine (1·35 c.c. 10 mmoles) in dry toluene (50 c.c.) at 0°, and the mixture was stirred at 0° for 10 min. further. A solution of *p*-nitrophenol (1·39 g., 10 mmoles) in toluene (20 c.c.) and dimethylformamide (10 c.c.) was added. The mixture was left for 15 hr. at 20° and then heated for 1 hr. at 50°. The toluene solution was washed with 0·1N-hydrochloric acid (2 × 50 c.c.), saturated sodium hydrogen carbonate solution (4 × 50 c.c.), and water (2 × 10 c.c.) before being evaporated. The p-*nitrophenyl ester* (1·53 g., 46%) recrystallised from methanol in colourless needles, m. p. 128° (Found: C, 58·1; H, 4·0; N, 8·7. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·3; N, 8·5%).

(*b*) A solution of *p*-nitrophenol (1·39 g., 10 mmoles) in 0·5N-sodium hydroxide (20 c.c.) was added to a solution of lithium benzyloxycarbonylglycyl sulphate [5] (10 mmoles) in dimethylformamide (28 c.c.). A precipitate was formed rapidly, but the mixture was left overnight at 18° before being worked up in the usual way. *p*-Nitrophenol was removed from the neutral product (2·03 g., 60%) by sublimation; 40% of the benzyloxycarbonylglycine was recovered. A preparation from lithium *p*-nitrophenoxide in anhydrous dimethylformamide, which was allowed 4 days at 50° for reaction, gave only a 28% yield; lithium sulphate, which is sparingly soluble in dimethylformamide, did not separate from the reaction mixture.

Relative Reactivities of Derivatives of Benzyloxycarbonylglycine.—0·1M-L-Alanine (5 c.c.) was added to a solution of the *p*-nitrophenyl ester, phenyl thiolester, or *p*-nitrophenyl thiolester of benzyloxycarbonylglycine (0·5 mmole) in dioxan (10 c.c.) at 21°. The pH of the clear solution was measured by means of a glass electrode and it was maintained at a predetermined value (7·5, 8, 8·5, 9, or 9·5) by addition of N-sodium hydroxide from a micrometer syringe. The pK_2 of alanine being taken as 10·0, the amount of sodium hydroxide used in overcoming the buffering effect of the alanine and the concentration of the alanine anion could be calculated. The relative specific rates of reaction between the anion and the several esters could then be derived from the volumes of alkali consumed in given times: they were in the ratio 16 : 1 : 140.

Other Reactions of Benzyloxycarbonylglycine p-*Nitrophenyl Ester.*—(*a*) Benzyloxycarbonylglycylglycine (90%) was obtained from reaction between the ester and glycine in ethanol and potassium phosphate buffer.

(*b*) Benzyloxycarbonylglycylglycine ethyl ester (94%) was obtained from reaction between the ester, glycine ethyl ester, and triethylamine in ethanol.

(*c*) Benzyloxycarbonylglycyl-L-phenylalanine (32%) was obtained from reaction between the ester and L-phenylalanine in dioxan and potassium phosphate buffer. Probably insufficient time had been allowed for reaction.

Benzyloxycarbonylglycylphenylalanine p-*Nitrophenyl Ester*.—A solution of *p*-nitrophenol (0·695 g., 5 mmoles) in N-sodium hydroxide (5 c.c.), 0·5M-potassium dihydrogen phosphate (70 c.c.), and 0·5M-dipotassium hydrogen phosphate (30 c.c.) was added to a solution of lithium benzyloxycarbonylglycyl-L-phenylalanyl sulphate [5] (5 mmoles) in dimethylformamide (50 c.c.) at 0°. The mixture was left overnight at 18° and the precipitated *p-nitrophenyl ester* (0·890 g., 37%) was collected and recrystallised from methanol in colourless crystals, m. p. 133°, $[\alpha]_D^{17}$ +2·0° (±0·3°) (*c* 2·6 in dioxan) (Found: C, 63·2; H, 4·7; N, 8·8. $C_{25}H_{23}O_7N_3$ requires C, 62·9; H, 4·9; N, 8·8%). It cannot be concluded from the optical rotation alone that the material is almost completely racemic because the rotations of the free acid and the *p*-nitrophenyl thiolester are +41·5° (in EtOH) and −67·0° (in dioxan) respectively. However, reaction between the *p*-nitrophenyl ester and glycine in aqueous dioxan buffered with magnesium carbonate gave benzyloxycarbonylglycyl-DL-phenylalanylglycine.

We thank Professor Sir Alexander Todd, F.R.S., for his interest, the Rockefeller Foundation and the Nuffield Trust for their generous support, the Department of Scientific and Industrial Research for Maintenance Grants (to J. A. F. and J. M. T.), and the Royal Commissioners for the 1851 Exhibition for an Overseas Studentship (to P. J. H.).

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