573. *The Synthesis of Actinomycin Analogues. Part I. 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine and Related Compounds*

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The synthesis of actinocinyl-bis-glycine ethyl ester and some derivatives of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine, used as intermediates and model compounds in the synthesis of actinomycin analogues, is described.

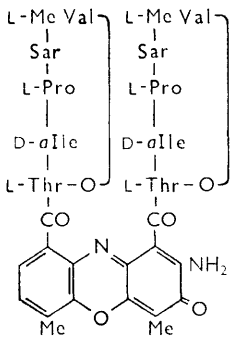
THE actinomycins,[1] a series of peptide antibiotics of closely-related chemical structure,[2] have aroused interest in recent years because of their pronounced anti-tumour activity, and some members of this group of compounds have been used clinically in the treatment

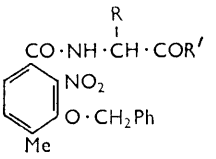
[1] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exp. Biol. Med.*, 1940, **45**, 609.
[2] (*a*) H. Brockmann, *Ann. N.Y. Acad. Sci.*, 1960, **89**, 323; (*b*) A. W. Johnson, *ibid.*, p. 336; H. Brockmann, *Fortschritte Chem. Org. Naturstoffe.*, 1960, **18**, 1.

of malignant disease.[3] The search for less toxic agents of related structure is being pursued by controlled biosynthesis,[4] partial synthesis,[5] and total synthesis.[6]

In the synthesis of actinomycin C_3 (I) by Brockmann and Lackner,[7] the " chromophoric precursor " is the 3-benzyloxy-4-methyl-2-nitrobenzoyl group, which with its attached peptide, is reduced to a 3-hydroxy-4-methylanthraniloyl group capable of oxidation to the " actinocyl " chromophore. In planning synthetic routes to actinomycin-like structures, the mode of construction of the peptide lactones is open to wider variation than that of the chromophore, for which 3-benzyloxy-4-methyl-2-nitrobenzoic acid [8] remains the most obvious starting material.

The synthesis of actinomycin C_3, which was reported with few experimental details,[7] began with the *N*-acylation of L-threonine by means of 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride. Elsewhere [9] this reaction was reported not to give the desired product (II; R = CH_3·CHOH, R′ = OH), which was prepared instead *via* reaction of *p*-nitrophenyl 3-benzyloxy-4-methyl-2-nitrobenzoate with L-threonine methyl ester followed by saponification of the product. We have synthesised 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine (II; R = CH_3·CHOH, R′ = OH) and its methyl ester (II; R = CH_3·CHOH,





R′ = OMe) by reaction of 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride with L-threonine and L-threonine methyl ester respectively using conditions appropriate to the acylation of hydroxyamino acids, *viz.*, mildly alkaline aqueous solution. The same procedure was used to prepare *o*-nitrobenzoyl-DL-threonine and 3-benzyloxy-2-nitrobenzoyl-L-threonine.

3-Benzyloxy-4-methyl-2-nitrobenzoylglycine ethyl ester (II; R = H, R′ = OEt) was synthesised by the mixed carbonic–carboxylic anhydride method [10] and on saponification gave the corresponding acid (II; R = H, R′ = OH). The construction of the " actinocyl " chromophore by the now classical method [8] was illustrated by the preparation of actinocyl-bis-glycine ethyl ester (III) by ferricyanide oxidation of 3-hydroxy-4-methylanthraniloylglycine ethyl ester, itself made by catalytic hydrogenation of the ester (II; R = H, R′ = OEt).

In the synthesis [7] of actinomycin C_3, 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine was coupled with a tripeptide benzyl ester by means of dicyclohexylcarbodi-imide.[11]

[3] See review in *Ann. N.Y. Acad. Sci.*, 1960, **89**.
[4] G. Schmidt-Kastner, *Ann. N.Y. Acad. Sci.*, 1960, **89**, 299; E. Katz, *ibid.*, p. 304.
[5] H. Brockmann, ref. 2 (*a*); J. H. Burchenal, H. F. Oettgen, J. A. Reppert, and V. Coley, *Ann. N.Y. Acad. Sci.*, 1960, **89**, 399; W. Müller, *Naturwiss.*, 1962, **49**, 156.
[6] H. Brockmann and V. Graef, *Naturwiss.*, 1962, **49**, 540.
[7] H. Brockmann and H. Lackner, *Naturwiss.*, 1960, **47**, 230.
[8] H. Brockmann and H. Muxfeldt, *Chem. Ber.*, 1958, **91**, 1242; W. G. Hanger, W. C. Howell, and A. W. Johnson, *J.*, 1958, 496.
[9] B. Weinstein, O. P. Crews, M. A. Leaffer, B. R. Baker, and L. Goodman, *J. Org. Chem.*, 1962, **27**, 1389.
[10] In J. P. Greenstein and M. Winitz, " Chemistry of the Amino-acids," Wiley, New York, 1961, Vol. 2, p. 978.
[11] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.

Since this procedure is known to be accompanied by racemisation [12] in cases where the amino-acid being activated is not protected by the benzyloxycarbonyl group or a related group, it was apparent that the azide coupling procedure [13] would be safer. This was confirmed by means of model reactions, in which 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonyl-L-leucine ethyl ester (II; R = CH_3·CHOH, R′ = Leu·OEt) and 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-phenylalanine ethyl ester (II; R = CH_3·CHOH, R′ = Phe·OEt) were each prepared first by an azide coupling and then by means of dicyclohexylcarbodi-imide. 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine hydrazide (II; R = CH_3·CHOH, R′ = $NH·NH_2$) was prepared from the corresponding ester (II; R = CH_3·CHOH, R′ = OMe) and converted into the azide for coupling with L-leucine ethyl ester and with L-phenylalanine ethyl ester; for the dicyclohexylcarbodi-imide couplings the corresponding acid (II; R = CH_3·CHOH, R′ = OH) was employed. The results (see Experimental section) indicate considerable epimerisation of the threonine residue during couplings with L-phenylalanine ethyl ester using dicyclohexylcarbodi-imide, although the corresponding coupling in the case of leucine was apparently satisfactory. Consequently it was decided that the azide method would be more reliable in this respect.

3-Hydroxy-4-methyl-2-nitrobenzoylsarcosine *p*-nitrophenyl ester was required as a model compound for testing whether an *o*-nitrophenolic group would interfere during a *p*-nitrophenyl ester coupling with a secondary amino-group. Sarcosine *p*-nitrophenyl ester hydrobromide was prepared by the action of hydrogen bromide in acetic acid [14] upon benzyloxycarbonylsarcosine *p*-nitrophenyl ester. Reaction of this product with 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride in the presence of aqueous sodium hydrogen carbonate unexpectedly gave 3-benzyloxy-4-methyl-2-nitrobenzoic acid *p*-nitrophenyl ester. The action of hydrogen bromide in acetic acid upon this product readily cleaved the benzyl ether to give 3-hydroxy-4-methyl-2-nitrobenzoic acid *p*-nitrophenyl ester, which upon reaction with morpholine gave N-(3-hydroxy-4-methyl-2-nitrobenzoyl)morpholine with no appreciable side reaction.

An attempted preparation of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine thiophenyl ester (II; R = CH_3·CHOH, R′ = PhS) by reaction of the azide with thiophenol in the presence of triethylamine gave a highly crystalline product with the expected elementary analysis, but the changes in its melting-point and optical rotation which were observed upon repeated recrystallisation indicated that some epimerisation had occurred during the reaction.

Reaction of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine azide with aniline failed to produce the expected anilide. Instead the product, obtained by working up as usual, was the oxazolidone derived *via* rearrangement to the isocyanate followed by intramolecular reaction with the hydroxyl group (cf. the case of benzyloxycarbonylserine azide [15]). On the other hand azide coupling with cyclohexylamine gave 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine cyclohexylamide (II; R = CH_3·CHOH, R′ = $NH·C_6H_{11}$) required as a model compound for a study of various methods for *O*-peptidisation of the threonine residue in related intermediates. These and other studies in the synthesis of actinomycin will be described in future publications.

Experimental

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine (II; R = CH_3·CHOH, R′ = OH).—A solution of 3-benzyloxy-4-methyl-2-nitrobenzoic acid [8] (6·02 g.) in benzene (120 ml.) containing thionyl chloride (24 ml.) was heated under reflux for 1 hr. The solvent was removed and the yellow solid acid chloride (6·55 g.) was dissolved in dioxan (40 ml.) and added dropwise to a

[12] N. A. Smart, G. T. Young, and M. W. Williams, *J.*, 1960, 3902.
[13] T. Curtius, *Ber.*, 1902, **35**, 3226.
[14] D. Ben-Ishai, *J. Org. Chem.*, 1954, **19**, 62.
[15] J. S. Fruton, *J. Biol. Chem.*, 1942, **146**, 463; A. Stoll and T. Petrzilka, *Helv. Chim. Acta*, 1952, **35**, 594.

solution of L-threonine (2·50 g.) and sodium hydrogen carbonate (2·75 g.) in water (22 ml.), containing 2N-sodium hydroxide (11 ml.) and dioxan (13 ml.), with stirring and cooling in ice. The solution was kept for 1 hr. at room temperature and then concentrated to one-third volume under reduced pressure, diluted with water (50 ml.), and washed with ethyl acetate (2 × 60 ml.). The solution was acidified with hydrochloric acid and extracted with ethyl acetate (150 and 60 ml.). The combined extracts were washed and dried (Na_2SO_4), and the solvent removed, affording a solid which crystallised as plates, m. p. 170—172° (lit.,[4] 161·5—162·5), from ethyl acetate–ligroin (yield 6·07 g.); $[\alpha]_D^{25} = -55°$ (*c* 1 in EtOH) (lit.,[9] −50·4° in EtOH) (Found: N, 7·4. Calc. for $C_{19}H_{20}N_2O_7$; N, 7·2%).

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine Methyl Ester (II; R = CH_3CHOH, R′ = OMe).—3-Benzyloxy-4-methyl-2-nitrobenzoyl chloride (prepared as above from 6·57 g. of the acid), dissolved in ethyl acetate (40 ml.), was stirred vigorously with a solution of L-threonine methyl ester hydrochloride (3·87 g.) and sodium hydrogen carbonate (5·0 g.) in water (40 ml.) at room temperature for 2 hr. The organic phase was separated, diluted with ethyl acetate (40 ml.), and washed with N-hydrochloric acid (2 × 75 ml.), aqueous sodium hydrogen carbonate (2 × 75 ml.), and water (60 ml.). After drying (Na_2SO_4), the solvent was removed giving a solid which was re-crystallised as needles, m. p. 118—119° (lit., 114—115°) from benzene (yield, 6·45 g.); $[\alpha]_D^{25} = -49°$ (*c* 1% in EtOH) (lit., −43·6°).

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine Hydrazide (II; R = CH_3·CHOH, R′ = $NHNH_2$).—The foregoing ester (4·55 g.) was dissolved in methanol (25 ml.) and hydrazine (1·6 ml.) was added. After 1 hr. at room temperature the crystalline precipitate was filtered off, washed with water, and dried (yield, 4·65 g.). The hydrazide crystallised as plates, m. p. 206—207° (decomp.) from methanol; $[\alpha]_D^{25} = 11°$ (*c* 1% in AcOH). (Found: C, 57·0; H, 5·7; N, 14·2. $C_{19}H_{22}N_4O_6$ requires C, 56·7; H, 5·5; N, 13·9%).

N-o-*Nitrobenzoyl-DL-threonine.*—A solution of *o*-nitrobenzoyl chloride (3·70 g.) in dioxan (40 ml.) was added dropwise, with stirring and cooling in ice, to a solution of DL-threonine (2·38 g.) and sodium hydrogen carbonate (2·0 g.) in water (20 ml.) containing 2 N-sodium hydroxide (10 ml.) and dioxan (16 ml.). After 1 hr. at room temperature the solution was concentrated under reduced pressure, diluted with water (40 ml.), washed with ethyl acetate (2 × 50 ml.), acidified with hydrochloric acid, and extracted with ethyl acetate (150 and 50 ml.). The extracts were washed with water, dried (Na_2SO_4), and the solvent removed. The residual gum solidified under ethyl acetate. The *product* recrystallised as needles, m. p. 148—151°, from ethyl acetate–ligroin (yield, 3·47 g.) (Found: C, 49·4; H, 4·6; N, 10·3. $C_{11}H_{12}N_2O_6$ requires C, 49·3; H, 4·5; N, 10·5%).

3-Benzyloxy-4-methyl-2-nitrobenzoylglycine Ethyl Ester (II; R = H, R′ = OEt). A solution of 3-benzyloxy-4-methyl-2-nitrobenzoic acid (2·87 g.) in chloroform (20 ml.) containing triethylamine (1·6 ml.) was cooled below 5° and isobutyl chloroformate (1·3 ml.) was added with stirring. After 20 min. below 5° a solution of glycine ethyl ester hydrochloride (1·39 g.) in chloroform (20 ml.) containing triethylamine (1·4 ml.) was added and the solution kept overnight at room temperature. After removal of the solvent the residue was dissolved in ethyl acetate (50 ml.) and washed with N-hydrochloric acid (50 ml.), aqueous sodium hydrogen carbonate (75 ml.), and water (50 ml.) and dried (Na_2SO_4). The solution was concentrated, brought onto a column (8 × 2 cm.) of alumina (Spence type H), and eluted with ethyl acetate. Removal of the solvent gave a syrup which crystallised under ligroin (yield, 2·05 g.). The *product* recrystallised as prisms (1·85 g.), m. p. 108°, from ethyl acetate–ligroin (Found: C, 61·5; H, 5·7; N, 7·8. $C_{19}H_{20}N_2O_6$ requires C, 61·3; H, 5·4; N, 7·5%).

3-Benzyloxy-4-methyl-2-nitrobenzoylglycine (II; R = H, R′ = OH).—The foregoing ester (6·02 g.) was dissolved in ethanol (240 ml.) and N-sodium hydroxide (24 ml.) was added. The solution was kept at 30° for 1½ hr. then concentrated to 50 ml., diluted with water (180 ml.), and washed with ethyl acetate (120 ml.). After acidification with hydrochloric acid and extraction with ethyl acetate (2 × 100 ml.), the combined extracts were washed with water (100 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a crystalline *product* (4·96 g.) which recrystallised as needles, m. p. 191—193°, from ethyl acetate–ligroin (yield, 3·98 g.) (Found: C, 59·3; H, 4·9; N, 7·9. $C_{17}H_{16}N_2O_6$ requires C, 59·3; H, 4·7; N, 8·1%).

3-Hydroxy-4-methylanthraniloylglycine Ethyl Ester Hydrochloride.—3-Benzyloxy-4-methyl-2-nitrobenzoylglycine ethyl ester (1·71 g.) in ethanol (35 ml.) containing 5% palladium–charcoal (0·3 g.) was shaken in an atmosphere of hydrogen until uptake ceased. The solution was filtered through Hyflo, concentrated to 10 ml., diluted with ether (40 ml.), and hydrogen chloride

was passed through the solution. The crystalline *product* (1·11 g.) was collected and recrystallised as needles, m. p. 194—195° (decomp.) from ethanol–ether (Found: C, 50·0; H, 6·0; Cl, 12·2; N, 9·4. $C_{12}H_{17}ClN_2O_4$ requires C, 49·9; H, 5·9; Cl, 12·3; N, 9·7%).

Actinocyl-bis-(glycine Ethyl Ester) (III).—The foregoing hydrochloride (0·76 g.) was dissolved in phosphate buffer, pH 7·4 (800 ml.), and the solution warmed to 40° while potassium ferricyanide (2·0 g.) in water (80 ml.) was added with stirring. After 1 hr. the orange precipitate was collected, washed with water, and dried (yield, 0·58 g.). After recrystallisation from acetic acid the *product* formed needles, m. p. 264° (decomp.) (Found: C, 57·5; H, 5·1; N, 11·2. $C_{24}H_{26}N_4O_8$ requires C, 57·8; H, 5·3; N, 11·2%).

3-Benzyloxy-2-nitrobenzoyl-L-threonine Ethyl Ester.—3-Benzyloxy-2-nitrobenzoic acid [16] (1·39 g.) was heated under reflux in benzene (20 ml.) containing thionyl chloride (5 ml.) for 1 hr. The solvent was removed and the residual solid acid chloride was dissolved in ethyl acetate (20 ml.). The solution was stirred briskly at room temperature with a solution of L-threonine ethyl ester hydrochloride (1·12 g.) in water (20 ml.) while sodium hydrogen carbonate (1·0 g.) was added gradually. After 2 hr. the organic layer was separated, washed with water (20 ml.), dried (Na_2SO_4), and the solvent removed, affording a crystalline *product* which recrystallised as needles, m. p. 163—164°, from ethyl acetate–ligroin (yield, 1·40 g.) (Found: C, 59·8; H, 5·7; N, 7·1. $C_{20}H_{22}N_2O_7$ requires C, 59·7; H, 5·5; N, 7·0%).

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonyl-L-leucine Ethyl Ester (II; R = CH_3·CHOH, R′ = NH·CH(Bu^i)CO_2Et).—(a) *Azide route.* 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine hydrazide (1·86 g.) was dissolved in water (15 ml.) containing concentrated hydrochloric acid (1·5 ml.) and acetic acid (25 ml.). The solution was cooled below 2° and a solution of sodium nitrite (0·70 g.) in water (2 ml.) was added. After dilution with ice-cold water (50 ml.) the precipitated azide was extracted into ethyl acetate (2 × 60 ml.), the organic layer washed with aqueous sodium hydrogen carbonate until the washings were neutral, and then with water (75 ml.). Pre-cooled reagents were used and ice was added to the mixture throughout this procedure. The solution was dried (Na_2SO_4) and a suspension of L-leucine ethyl ester hydrochloride (0·90 g.) in ethyl acetate (15 ml.) containing triethylamine (0·65 ml.) was added. The mixture was refrigerated overnight, then washed with N-hydrochloric acid (75 ml.), aqueous sodium hydrogen carbonate (75 ml.), and water (75 ml.), and dried (Na_2SO_4). The solvent was removed and the residual syrup (2·45 g.) was passed in ethyl acetate through a column (8 × 2 cm.) of alumina. Removal of the solvent gave a solid product (yield, 1·31 g.). The *dipeptide derivative* recrystallised as needles, m. p. 121—123°, from ethyl acetate–ligroin; $[\alpha]_D^{25} = -34°$ (*c* 1% in ethanol) (Found: C, 61·4; H, 6·6; N, 8·0. $C_{27}H_{35}N_3O_8$ requires C, 61·2; H, 6·7; N, 7·9%).

(b) *Carbodi-imide method.* A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine (1·24 g.) in ethyl acetate (40 ml.) was mixed with a suspension of L-leucine ethyl ester hydrochloride (0·65 g.) in ethyl acetate (30 ml.) containing triethylamine (0·5 ml.) and a solution of dicyclohexylcarbodi-imide (0·69 g.) in ethyl acetate (15 ml.) was added. The mixture was stirred at room temperature overnight, treated with acetic acid (0·5 ml.), then after 1 hr. filtered, washed with N-hydrochloric acid (2 × 70 ml.), aqueous sodium hydrogen carbonate (2 × 70 ml.), and water (2 × 70 ml.), and dried (Na_2SO_4). Removal of the solvent gave a gum which solidified on rubbing under ligrion. The *product* recrystallised as needles, m. p. 120—121°, from ethyl acetate–ligroin; $[\alpha]_D^{25} = -33°$ (*c* 1% in EtOH).

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonyl-L-phenylalanine Ethyl Ester (II; R = CH_3·CHOH, R′ = NH·CH(CH_2Ph)CO_2Et).—(a) *Azide method.* The coupling procedure described in (a) above was used. 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine hydrazide (1·86 g.) and L-phenylalanine ethyl ester hydrochloride (1·07 g.) gave 2·26 g. of solid *product*, which crystallised as needles, m. p. 149°, from ethyl acetate–ligroin; $[\alpha]_D^{25} = -24°$ (*c* 1% in ethanol) (Found: C, 63·5; H, 6·0; N, 7·3. $C_{30}H_{33}N_3O_8$ requires C, 63·9; H, 5·9; N, 7·5%).

(b) *Carbodi-imide method.* The coupling procedure described in (b) above was used. 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine and L-phenylalanine ethyl ester hydrochloride gave 2·55 g. of semicrystalline *product*, which crystallised as needles. m. p. 85—90°, from ethyl acetate–ligroin; $[\alpha]_D^{25} = -10°$ (*c* 1% in ethanol) (Found: C, 64·2; H, 6·1; N, 7·2%). The infrared spectrum was almost identical with that of a sample of the product obtained by method (a).

[16] S. Senoh, T. Seki, and H. Kikkawa, *J. Chem. Soc. Japan*, 1953, **74**, 251.

Sarcosine p-*Nitrophenyl Ester Hydrobromide*.—Benzyloxycarbonylsarcosine[17] (11·15 g.) and *p*-nitrophenol (7·7 g.) were dissolved in ethyl acetate (120 ml.) and dicyclohexylcarbodiimide (11·3 g.) was added, causing evolution of heat and formation of a copious precipitate. After 6 hr. acetic acid (2 ml.) was added, the solid filtered off, and the solvent removed under reduced pressure. The oily residue was taken up in ether, the solution filtered from a further small quantity of dicyclohexylurea, washed with aqueous sodium hydrogen carbonate, and with water, dried (Na_2SO_4), and the solvent removed. The viscous benzyloxycarbonylsarcosine *p*-nitrophenyl ester (13·9 g.) resisted attempts at crystallisation, so was dissolved in acetic acid (15 ml.) and the solution added to 50% hydrogen bromide in acetic acid (45 ml.). The solution was warmed at 40—50° for 15 min., then allowed to stand at room temperature for 1 hr. Ether (400 ml.) was added, the precipitate filtered off and washed well with ether. The *product* recrystallised as needles, m. p. 195—197°, from ethanol or methanol–ether (yield, 8·1 g.) (Found: C, 37·2; H, 3·9; Br, 26·6; N, 9·5. $C_9N_{11}BrN_2O_4$ requires C, 37·1; H, 3·8; Br, 27·4; N, 9·6%).

3-*Benzyloxy*-4-*methyl*-2-*nitrobenzoic Acid* p-*Nitrophenyl Ester*.—A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride (prepared as described above from 5·00 g. of the acid) in ethyl acetate (60 ml.) was stirred briskly with a solution of sarcosine *p*-nitrophenyl ester hydrobromide (4·85 g.) in water (60 ml.) while sodium hydrogen carbonate (1·0 g.) was gradually added. After 2 hr. at room temperature the layers were separated, the aqueous phase was extracted with ethyl acetate (50 ml.) and the combined ethyl acetate extracts were washed with 2N-hydrochloric acid (120 ml.) and water (100 ml.) and dried (Na_2SO_4). The solution was concentrated, passed through a column of alumina, and the solvent removed. The *product* crystallised as plates, m. p. 120—122°, from ethyl acetate–ligroin (yield, 4·78 g.) (Found: C, 61·9; H, 3·9; N, 6·9. Calc. for $C_{21}H_{16}N_2O_7$: C, 61·8; H, 4·0; N, 6·9%).

3-*Hydroxy*-4-*methyl*-2-*nitrobenzoic Acid* p-*Nitrophenyl Ester*.—The foregoing benzyl ether was dissolved in acetic acid (40 ml.) and 45% hydrogen bromide in acetic acid (20 ml.) was added. The solution was kept at 40° for 1 hr. and then evaporated to dryness *in vacuo*. The solid *product* recrystallised as yellow needles, m. p. 118—119°, from ethyl acetate–light petroleum (b. p. 60—80°) (yield, 2·79 g.) (Found: C, 53·0; H, 3·1; N, 9·0. $C_{14}H_{10}N_2O_7$ requires C, 52·8; H, 3·2; N, 8·8%).

N-(3-*Hydroxy*-4-*methyl*-2-*nitrobenzoyl*)*morpholine*.—3-Hydroxy-4-methyl-2-nitrobenzoic acid p-nitrophenyl ester (0·19 g.) was heated under reflux in ethyl acetate (25 ml.) containing morpholine (0·10 ml.) for 20 hr. The solution was washed with 2N-hydrochloric acid (20 ml.) and water (20 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a solid *product* which crystallised as yellow needles, m. p. 153°, from ethyl acetate–ligroin (Found: C, 54·1; H, 5·3; N, 10·5. $C_{12}H_{14}N_2O_3$ requires C, 53·7; H, 5·3; N, 10·2%).

3-*Benzyloxy*-4-*methyl*-2-*nitrobenzoylthreonine Thiophenyl Ester* (II; R = CH_3·CHOH, R′ = SPh).—To a solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine azide (prepared as above from 1·86 g. of the hydrazide) in ethyl acetate (100 ml.) below 5° was added thiophenol (0·45 ml.) and triethylamine (0·65 ml.), and the solution was refrigerated overnight. The highly crystalline precipitate was collected and washed with a little ethyl acetate. The *product* recrystallised as needles, m. p. 186—188°, from ethanol (yield, 1·12 g.); $[\alpha]_D^{25} = -75°$ (*c* 1% in dimethylformamide). After recrystallisation from ethyl acetate it had m. p. = 184—196°, $[\alpha]_D^{25} = -94°$, and after a further recrystallisation, m. p. = 192—199°, $[\alpha]_D^{25} = -95°$ (Found: C, 63·4; H, 4·4; N, 5·8; S, 6·7. $C_{25}H_{24}N_2O_6S$ requires C, 63·2; H, 4·6; N, 6·1; S, 6·9%).

3-*Benzyloxy*-4-*methyl*-2-*nitrobenzoyl*-L-*threonine Cyclohexylamide* (II; R = CH_3·CHOH, R′ = NH·C_6H_{11}).—A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine azide (prepared as above from 3·72 g. of the hydrazide) in ethyl acetate (100 ml.) was mixed with cyclohexylamine (1·3 ml.) below 5° and then refrigerated for two days. The solution was washed with 2N-hydrochloric acid (100 ml.), aqueous sodium hydrogen carbonate (100 ml.), and water (75 ml.), and dried (Na_2SO_4). Removal of the solvent afforded the *product* as a crystalline solid, needles, m. p. 165—166° from ethanol (yield, 3·77 g.) (Found: C, 64·2; H, 6·7; N, 8·9. $C_{25}H_{31}N_3O_6$ requires C, 63·9; H, 6·7; N, 9·0%).

4-(3′-*Benzyloxy*-4′-*methyl*-2′-*nitrobenzamido*)-5-*methyloxazolid*-2-*one*.—A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-threonine azide (prepared as above from 3·72 g. of the hydrazide) in ethyl acetate (150 ml.) was mixed below 5° with aniline (1·0 ml.). The solution was refrigerated for two days, then washed with 2N-hydrochloric acid (100 ml.), aqueous sodium

[17] D. Ben-Ishai and E. Katchalski, *J. Amer. Chem. Soc.*, 1952, **74**, 3688.

hydrogen carbonate (100 ml.), and water (50 ml.), and dried (Na_2SO_4). Removal of the solvent gave a white solid (2·89 g.) which was dissolved in boiling ethanol. On cooling, the *product* separated as needles containing alcohol of crystallisation (Found: C, 58·7; H, 5·2; N, 9·6. $C_{21}H_{25}N_3O_7$ requires C, 58·5; H, 5·8; N, 9·7%). After drying *in vacuo* at 110° the oxazolidone was obtained free from solvation and had m. p. 237—238° (Found: C, 59·1; H, 5·0; N, 11·3. $C_{19}H_{19}N_3O_6$ requires C, 59·2; H, 4·9; N, 10·9%). Infrared spectrum showed maxima at 3200 (N–H), 1755 (C=O of urethane in 5-membered ring), 1650 (C=O amide), 1540 (N–H Amide II) cm.$^{-1}$.

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