# 1340. *O*-Benzyl-3-nitrotyrosine and its Use in the Synthesis of Peptides Containing 3-Nitrotyrosine

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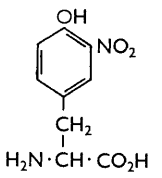
The synthesis of several derivatives of *O*-methyl-, *O*-benzyl-, and *O*-diphenylmethyl-3-nitro-L-tyrosine is described. *O*-Benzyl-3-nitro-L-tyrosine has been used in the synthesis of dipeptides containing 3-nitro-L-tyrosine.

Recent interest in peptides containing 3-nitro-L-tyrosine (I), engendered by the structural elucidation of the Rufomycins [1] (Ilamycins [2]) and by the work of Iwasaki and Witkop [3] on the selective cleavage of 3-nitrotyrosyl peptides, prompts us to report our synthetic studies with this amino-acid. Peptides of 3-nitro-L-tyrosine have been described previously. *N*-Glycyl-3-nitro-L-tyrosine and *N*-DL-leucyl-3-nitro-L-tyrosine were prepared by Abderhalden [4] by the ammonolysis of *N*-chloroacetyl-3-nitro-L-tyrosine and *N*-bromoisocaproyl-3-nitro-L-tyrosine, respectively. *N*-Benzoyl-3-nitro-L-tyrosyl-L-phenylalanine was prepared by Iwasaki and Witkop [3] by the use of the *NN'*-dicyclohexylcarbodi-imide coupling procedure.[5] Nonetheless, for the synthesis of larger and more complex peptides, the use of a suitable protecting group for the phenolic function seems desirable, both to modify the physical properties of the peptides and to avoid synthetic ambiguities.



(I)

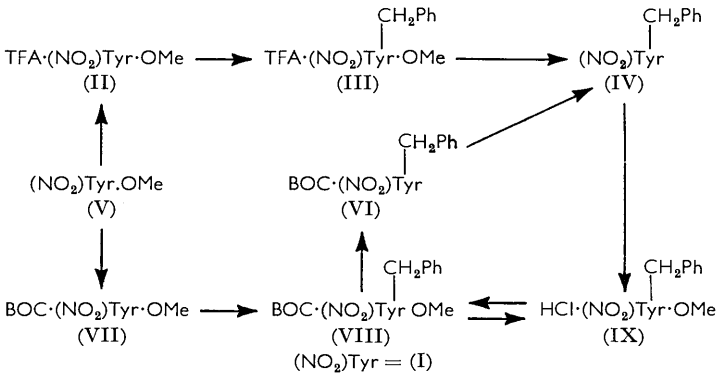
Attempts to prepare the t-butyl ether of 3-nitro-L-tyrosine by treating a suspension of the amino-acid methyl ester in dioxan or *NN*-dimethylformamide with isobutene in the presence of concentrated sulphuric acid [6] were unsuccessful. The more soluble methyl *N*-trifluoroacetyl-3-nitro-L-tyrosinate (II), prepared from methyl 3-nitro-L-tyrosinate (V) and ethyl thioltrifluoroacetate,[7] was similarly unreactive under these conditions. Nor could the *O*-tetrahydropyranyl derivative of this compound be obtained by reaction with 2,3-dihydropyran in ethyl acetate solution containing hydrogen chloride.[8]

*N*-Benzyloxycarbonyl-3-nitro-L-tyrosine was prepared in the usual way from the amino-acid and benzyloxycarbonyl chloride in the presence of three equivalents of base and was treated with diazomethane to give methyl *N*-benzyloxycarbonyl-*O*-methyl-3-nitro-L-tyrosinate. Saponification of the methyl ester and subsequent removal of the

[1] J. Ueyanagi, H. Iwasaki, M. Fujino, T. Kamiya, A. Miyake, and S. Tatsuoka, 142nd National Meeting of the American Chemical Society, Atlantic City, September 1962. Abstracts, p. 10.
[2] T. Takita, H. Naganawa, K. Maeda, and H. Umezawa, *J. Antibiotics* (*Tokyo*), 1964, **17**, 129 (*Chem. Abs.*, 1964, **61**, 9578).
[3] H. Iwasaki and B. Witkop, *J. Amer. Chem. Soc.*, 1964, **86**, 4698.
[4] E. Abderhalden and W. Schairer, *Fermentforschung*, 1931, **12**, 295.
[5] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[6] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359.
[7] M. Hauptschein, C. S. Stokes, and E. A. Nodiff, *J. Amer. Chem. Soc.*, 1952, **74**, 4005; E. E. Schallenberg and M. Calvin, *ibid.*, 1955, **77**, 2779.
[8] B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, 1956, **39**, 57.

benzyloxycarbonyl group by the use of hydrogen bromide in acetic acid solution gave *O*-methyl-3-nitro-L-tyrosine. The preparation of the amino-acid methyl ether *via* *N*-formyl-*O*-methyl-3-nitro-L-tyrosine,[9] itself prepared by the action of dimethyl sulphate on *N*-formyl-3-nitro-L-tyrosine, seems unsatisfactory. In our experience, only incomplete methylation of *N*-formyl-3-nitro-L-tyrosine could be achieved in this way and, in view of the stability of *O*-methyl-3-nitro-L-tyrosine under acid conditions, this probably accounts for the reported [9] presence of 3-nitrotyrosine in samples of *O*-methyl-3-nitro-L-tyrosine prepared by this route. Unlike *O*-benzyl-L-tyrosine [10] and *O*-methyl-L-tyrosine,[11] which are cleaved by hydrogen bromide in acetic acid solution to give the free amino-acid, *O*-methyl-3-nitro-L-tyrosine is virtually resistant to this reagent which seems to preclude its use for the preparation of 3-nitro-L-tyrosine peptides.

Attempts to prepare *O*-benzyl-3-nitro-L-tyrosine (IV) by the action of benzyl bromide on a hot methanolic suspension of the copper complex of 3-nitro-L-tyrosine (cf. *O*-benzyl-L-tyrosine [12]) were unsuccessful. The benzyl ether was prepared *via* the action of phenyldiazomethane on suitably protected derivatives of 3-nitro-L-tyrosine. Methyl *N*-trifluoroacetyl-*O*-benzyl-3-nitro-L-tyrosinate (III), prepared from methyl *N*-trifluoroacetyl-3-nitro-L-tyrosinate (II) and phenyldiazomethane, gave *O*-benzyl-3-nitro-L-tyrosine (IV) by treatment with two equivalents of alkali. An identical product was obtained by a similar series of reactions starting with methyl *N*-t-butoxycarbonyl-3-nitro-L-tyrosinate (VII), which was prepared from methyl 3-nitro-L-tyrosinate (V) and t-butyl azidoformate.[13] This route gave direct access to *N*-t-butoxycarbonyl-*O*-benzyl-3-nitro-L-tyrosine (VI) and methyl *O*-benzyl-3-nitro-L-tyrosinate hydrochloride (IX). The latter was also prepared from the benzylated amino-acid. *O*-Benzyl-3-nitro-L-tyrosine (IV), on treatment at room temperature with a 5·5N-solution of hydrogen bromide in acetic acid for 1 hr., gave 3-nitrotyrosine in quantitative yield and the use of the amino-acid benzyl ether (IV) in the synthesis of simple peptides was therefore investigated.

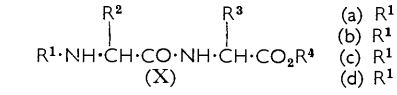



The abbreviations and conventions used throughout are those recommended by the Committee on Nomenclature which reported at the Fifth European Symposium on Peptides.[14]

Five protected dipeptides (Xa—d) and (XI), each containing an *O*-benzyl-3-nitro-L-tyrosine residue, were prepared by conventional means. All but one of these esters were readily saponified to give the corresponding crystalline acids. Methyl *NS*-ditrityl-L-cysteinyl-*O*-benzyl-3-nitro-L-tyrosinate (Xc) could not be saponified using sodium

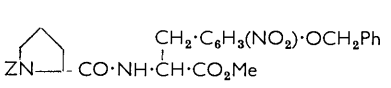
[9] G. R. Allen, B. R. Baker, A. C. Dornbush, J. P. Joseph, H. M. Kissman, and M. J. Weiss, *J. Medicin. Pharmaceut. Chem.*, 1960, **2**, 391.
[10] M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.
[11] H. D. Law and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1960, **82**, 4579.
[12] E. Wünsch, G. Fries, and A. Zwick, *Chem. Ber.*, 1958, **91**, 542.
[13] R. Schwyzer, P. Sieber, and H. Kapeller, *Helv. Chim. Acta*, 1959, **42**, 2622; L. A. Carpino, *J. Amer. Chem. Soc.*, 1960, **82**, 2725.
[14] G. T. Young, "Peptides: Proc. Fifth European Peptide Symp., Oxford September 1962," ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 261.

hydroxide in solution in ethanol or in *NN*-dimethylformamide. Steric hindrance has been invoked [15] to account for the resistance of esters of the higher *N*-trityl amino-acids to saponification, but so far as we are aware, the ditrityl dipeptide ester (Xc) provides the first example of an *N*-trityl dipeptide ester which is resistant to saponification. When heated with 50% aqueous acetic acid, the *NS*-ditrityl dipeptide ester (Xc) was converted into the piperazine-2,5-dione derivative (XIIa).

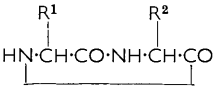


(X)

(a) $R^1$ = Z; $R^2$ = H; $R^3$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O{\cdot}CH_2Ph$; $R^4$ = Me
(b) $R^1$ = BOC; $R^2$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O{\cdot}CH_2Ph$; $R^3$ = H; $R^4$ = $Bu^t$
(c) $R^1$ = TRI; $R^2$ = $CH_2{\cdot}S{\cdot}TRI$; $R^3$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O\,CH_2Ph$; $R^4$ = Me
(d) $R^1$ = BOC; $R^2$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O{\cdot}CH_2Ph$; $R^3$ = $CH_2Ph$; $R^4$ = Me



(XI)



(XII)

(a) $R^1$ = $CH_2{\cdot}S{\cdot}TRI$; $R^2$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O{\cdot}CH_2Ph$
(b) $R^1$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}O{\cdot}CH_2Ph$; $R^2$ = $CH_2Ph$
(c) $R^1$ = $CH_2{\cdot}C_6H_3(NO_2){\cdot}OH$; $R^2$ = $CH_2Ph$

The action of hydrogen bromide in acetic acid on the other *N*-protected dipeptide benzyl ethers gave the free dipeptides in satisfactory yield and in some cases the intermediate *O*-benzyl dipeptides were isolated. *O*-Benzyl-3-nitro-L-tyrosyl-L-phenylalanine was converted into the piperazine-2,5-dione derivative (XIIb) by recrystallisation from boiling acetic acid and the same compound resulted when methyl *O*-benzyl-3-nitro-L-tyrosyl-L-phenylalaninate hydrochloride, obtained from the protected dipeptide (Xd) by treatment with methanolic hydrogen chloride, was heated in pyridine. The benzyl ether was cleaved to give the free piperazine-2,5-dione (XIIc) by the action of hydrogen bromide in acetic acid. 3-Nitro-L-tyrosyl-L-phenylalanine also gave the piperazine-2,5-dione derivative (XIIc) when heated with glacial acetic acid. The ease of formation of the piperazine-2,5-dione derivatives (XIIa—c) contrasts with the conditions necessary for similar conversions of other dipeptides.[16] However, all dipeptides containing 3-nitro-L-tyrosine are not so labile; the glycine and proline peptides were recrystallised unchanged from hot glacial acetic acid, although proline-containing dipeptides generally form piperazine-2,5-dione derivatives more readily than others.[17]

Although these experiments show that *O*-benzyl-3-nitro-L-tyrosine (IV) may be used in the synthesis of simple protected peptides of 3-nitro-L-tyrosine and that the benzyl group may subsequently be removed by the action of hydrogen bromide, it was felt that the necessarily prolonged exposure to this reagent would not be desirable with more complex peptides. *O*-Diphenylmethyl-3-nitro-L-tyrosine has therefore been prepared, in a manner comparable with that used for the *O*-benzyl derivative, from diphenyldiazomethane and methyl *N*-trifluoroacetyl-3-nitro-L-tyrosinate (II). Short exposure to a solution of hydrogen bromide in acetic acid resulted in total cleavage of the diphenylmethyl group indicating that this compound may be a suitable intermediate for the synthesis of 3-nitrotyrosine peptides.

*Note Added in Proof.*—J. P. Marsh, jun., and L. Goodman (*J. Org. Chem.*, 1965, **30**, 2491) have recently reported that benzyl ethers of various nitrophenol derivatives are cleaved by cold trifluoroacetic acid. We now find that the free phenols may similarly be liberated from *O*-benzyl-3-nitrotyrosine derivatives. The rate of cleavage is dependent on the total structure of the compound and, for example, decreases in the following series: *N*-benzyloxycarbonylglycyl-*O*-benzyl-3-nitro-L-tyrosine > *O*-benzyl-3-nitro-L-tyrosyl-L-phenylalanine ≫ *O*-benzyl-3-nitro-L-tyrosylglycine.

[15] C. G. Stelakatos, D. M. Theodoropoulos, and L. Zervas, *J. Amer. Chem. Soc.*, 1959, **81**, 2884.
[16] P. Brigl, *Ber.*, 1923, **56**, 1887; E. Abderhalden and E. Komm, *Z. Physiol. Chem.*, 1924, **193**, 147.
[17] E. Abderhalden and H. Nienburg, *Fermentforschung*, 1933, **13**, 573; E. L. Smith and M. Bergmann, *J. Biol. Chem.*, 1944, **153**, 627.

## Experimental

*Methyl 3-Nitro-L-tyrosinate* (V).—Methyl 3-nitro-L-tyrosinate hydrochloride was prepared from 3-nitro-L-tyrosine [18] in 94% yield by the method of Brenner and Huber.[19] It had m. p. 195—197° (decomp.), $[\alpha]_D^{19.5}$ +8·9° (*c* 2·75 in MeOH) (lit.,[20] m. p. 197°). The free ester, liberated in quantitative yield from an aqueous solution of the hydrochloride by the action of ammonia, had m. p. 177—179° after recrystallisation from dioxan (83% recovery) (lit.,[20] m. p. 173°).

*Methyl N-Trifluoroacetyl-3-nitro-L-tyrosinate* (II).—A suspension of methyl 3-nitro-L-tyrosinate (4·80 g.) in dry dioxan (50 ml.) was treated with ethyl thioltrifluoroacetate [7] (4 ml.) and the mixture was shaken for 4·5 days. Evaporation of the solvent gave a brown oil, which yielded a yellow solid (6·48 g.) after being triturated under light petroleum. Recrystallisation from benzene–light petroleum afforded the *N-trifluoroacetyl derivative* (6·28 g., 93%), m. p. 95·5—97°. A sample further recrystallised from aqueous ethanol had m. p. 103·5—104·5°, $[\alpha]_D^{19.5}$ −2·4° (*c* 2 in MeOH) (Found: C, 42·9; H, 3·3; N, 8·3. $C_{12}H_{11}F_3N_2O_6$ requires C, 42·7; H, 3·35; N, 8·2%).

*N-Benzyloxycarbonyl-3-nitro-L-tyrosine.*—A solution of 3-nitro-L-tyrosine (4·52 g.) in stirred, ice-cold 2N-sodium hydroxide (15 ml.) was treated alternately, in small portions over 2 hr., with benzyloxycarbonyl chloride (3·4 g.) and 2N-sodium hydroxide (15 ml.). The solution was stirred for a further 2 hr., extracted with ether (4 × 20 ml.), and poured on to a stirred mixture of crushed ice (40 g.) and concentrated hydrochloric acid (5 ml.). The yellow precipitate which appeared was dissolved in hot ethyl acetate (3 × 30 ml.) and the ethyl acetate solution was extracted with saturated sodium hydrogen carbonate solution (3 × 25 ml.). Acidification of the aqueous extract yielded the chromatographically pure *acyl amino-acid* (5·93 g., 82%), m. p. 119—124° (gels at 95°). A sample purified *via* the sodium salt had m. p. 130—134° (gels at 110°), $[\alpha]_D^{19}$ +12·5° (*c* 2·9 in MeOH) (Found: C, 56·6; H, 4·3; N, 7·6. $C_{17}H_{16}N_2O_7$ requires C, 56·9; H, 4·5; N, 7·8%).

*Methyl N-Benzyloxycarbonyl-O-methyl-3-nitro-L-tyrosinate.*—Treatment of *N*-benzyloxycarbonyl-3-nitro-L-tyrosine (12·0 g.) in dry ethanol (100 ml.) with excess ethereal diazomethane, followed, after 24 hr., by chromatography on neutral alumina and recrystallisation of the crude product from ether afforded the *protected amino-acid methyl ether methyl ester* (5·41 g., 42%), m. p. 66—68°, $[\alpha]_D^{19}$ −9·7° (*c* 1 in MeOH) (Found: C, 59·4, 59·1; H, 5·45, 5·1; N, 7·1. $C_{19}H_{20}N_2O_7$ requires C, 58·8; H, 5·2; N, 7·2%).

*N-Benzyloxycarbonyl-O-methyl-3-nitro-L-tyrosine.*—The methyl ester (4·44 g.) in acetone (50 ml.) was stirred for 30 min. with 2N-sodium hydroxide (6·3 ml.) and water (19 ml.). The acetone was removed and the aqueous solution was acidified with concentrated hydrochloric acid. The product (4·18 g.) was recrystallised from aqueous methanol, affording the *acyl amino-acid methyl ether* (3·99 g., 95%), m. p. *ca.* 100°, $[\alpha]_D^{13}$ −3·8° (*c* 1·5 in MeOH) (Found: C, 57·3; H, 4·9; N, 7·3. $C_{18}H_{18}N_2O_7$ requires C, 57·75; H, 4·85; N, 7·5%).

*O-Methyl-3-nitro-L-tyrosine Hydrobromide.*—*N*-Benzyloxycarbonyl-*O*-methyl-3-nitro-L-tyrosine (2·99 g.) was added to a stirred 5·5N-solution of hydrogen bromide in glacial acetic acid (18 ml.). After 30 min., ether (25 ml.) was added and the *amino-acid hydrobromide* (2·37 g., 92%), m. p. 227° (decomp.), was collected. A sample recrystallised from ethanol had m. p. 227°, $[\alpha]_D^{19}$ −6·0° (*c* 0·6 in MeOH) (Found: C, 37·5; H, 4·2; N, 8·7. $C_{10}H_{13}BrN_2O_5$ requires C, 37·4; H, 4·1; N, 8·7%).

*O-Methyl-3-nitro-L-tyrosine.*—A solution of the above hydrobromide (1·91 g.) in hot water (5 ml.) was neutralised with concentrated ammonia solution. The product (1·27 g.) was recrystallised from aqueous acetic acid affording the *methylated amino-acid* (1·19 g., 82%), m. p. 240—241° (decomp.), $[\alpha]_D^{20}$ +3·8° (*c* 1·5 in 0·1N-NaOH) (Found: C, 50·25; H, 5·05; N, 11·3. $C_{10}H_{12}N_2O_5$ requires C, 50·0; H, 5·0; N, 11·65%).

*Methyl N-Trifluoroacetyl-O-benzyl-3-nitro-L-tyrosinate* (III).—Methyl *N*-trifluoroacetyl-3-nitro-L-tyrosinate (8·06 g.) in dry dioxan (20 ml.) was treated with phenyldiazomethane [21] (3·75 g.) in dry dioxan (10 ml.) and the resulting solution was kept at 50° for 16 hr. The dioxan was removed and the residue was subjected to chromatography on a column of neutral alumina. The crude product, obtained from the ether eluate as a yellow oil, solidified when triturated under light petroleum. Recrystallisation of this solid (6·67 g.) from ether gave the *fully protected amino-acid* (5·50 g., 54%), m. p. 93—95°. A sample again recrystallised from ether

[18] T. B. Johnson and E. F. Kohlmann, *J. Amer. Chem. Soc.*, 1915, **37**, 1863.
[19] M. Brenner and W. Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.
[20] H. Bauer, E. Strauss, and E. Maschmann, *Ber.*, 1935, **68**, 1108.
[21] C. G. Overberger and J.-P. Anselme, *J. Org. Chem.*, 1963, **28**, 592.

had m. p. 94—95°, $[\alpha]_D^{19.5}$ −0·4° (*c* 3 in MeOH) (Found: C, 53·8; H, 4·4; N, 6·8. $C_{19}H_{17}F_3N_2O_6$ requires C, 53·5; H, 4·6; N, 6·6%).

O-*Benzyl*-3-*nitro*-L-*tyrosine* (IV).—Methyl *N*-trifluoroacetyl-*O*-benzyl-3-nitro-L-tyrosinate (3·96 g.) in acetone (20 ml.) was kept with N-sodium hydroxide (19·0 ml.) for 1 hr. at room temperature. The acetone was evaporated and the aqueous solution was extracted with ether (2 × 15 ml.). Addition of 2N-hydrochloric acid (9·5 ml.) caused the product (2·71 g.) to separate. Recrystallisation from aqueous ethanol gave the *amino-acid benzyl ether* (2·51 g., 86%), m. p. 226—228° (decomp.), $[\alpha]_D^{16}$ −63·5° (*c* 1 in glacial $CH_3{\cdot}CO_2H$) (Found: C, 60·9; H, 5·2; N, 8·7. $C_{16}H_{16}N_2O_5$ requires C, 60·7; H, 5·1; N, 8·85%).

*Methyl* O-*Benzyl*-3-*nitro*-L-*tyrosinate Hydrochloride* (IX).—The amino-acid (IV) (0·63 g.) was esterified by means of thionyl chloride and methanol [19] to give the crude *ester hydrochloride* (0·70 g., 95%), m. p. 186—189° (decomp.). Recrystallisation from aqueous acetonitrile gave material with m. p. 189—190° (decomp.), $[\alpha]_D^{17}$ +0·5° (*c* 2 in MeOH) (Found: C, 55·5; H, 5·2; Cl, 9·3; N, 7·8. $C_{17}H_{19}ClN_2O_5$ requires C, 55·65; H, 5·2; Cl, 9·7; N, 7·6%).

*Methyl* N-*t*-*Butoxycarbonyl*-3-*nitro*-L-*tyrosinate* (VII).—Methyl 3-nitro-L-tyrosinate (4·80 g.) was dissolved by warming in a mixture of dimethyl sulphoxide (75 ml.) and pyridine (25 ml.). When the solution had cooled to 45°, t-butyl azidoformate [13] (5·60 g.) was added and the solution was kept at room temperature for 2 days. The solvents were removed and the residue, in solution in ethyl acetate (40 ml.), was washed with 10% citric acid solution (2 × 10 ml.) and water (2 × 10 ml.). Evaporation of the dried ethyl acetate solution gave the *acyl amino-acid ester* (6·17 g., 91%), m. p. 98—100°. A sample recrystallised from methanol had m. p. 101—102·5°, $[\alpha]_D^{20}$ +8·9° (*c* 1·7 in MeOH) (Found: C, 52·8; H, 5·8; N, 8·2. $C_{15}H_{20}N_2O_7$ requires C, 52·9; H, 5·9; N, 8·2%). Saponification of this ester gave *N*-*t*-*butoxycarbonyl*-3-*nitro*-L-*tyrosine*, in 69% yield, m. p. 98—99° (from ethyl acetate–light petroleum), $[\alpha]_D^{17}$ +9·2° (*c* 3 in MeOH) (Found: C, 51·8; H, 5·8; N, 8·4. $C_{14}H_{18}N_2O_7$ requires C, 51·5; H, 5·7; N, 8·6%).

*Methyl* N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosinate* (VIII).—(*a*) Methyl *N*-t-butoxycarbonyl-3-nitro-L-tyrosinate (2·89 g.) was benzylated using phenyldiazomethane in solution in dioxan and the product was isolated by means of chromatography on neutral alumina. The crude product (2·14 g.) was recrystallised from ethyl acetate–light petroleum to give the *protected amino-acid* (1·67 g., 46%), m. p. 109—111°. Material further recrystallised from ethyl acetate–light petroleum had m. p. 112—114°, $[\alpha]_D^{20}$ +3·9° (*c* 3 in $CH_3{\cdot}CO_2Et$) (Found: C, 61·6; H, 6·1; N, 6·3. $C_{22}H_{26}N_2O_7$ requires C, 61·4; H, 6·1; N, 6·5%).

(*b*) Methyl *O*-benzyl-3-nitro-L-tyrosinate hydrochloride (3·69 g.) in water (20 ml.) was shaken with ethyl acetate containing triethylamine (1·01 g.). The phases were separated and the ethyl acetate was washed with water (2 × 20 ml.), dried, and evaporated. The residue was dissolved in pyridine (10 ml.) and treated with t-butyl azidoformate (4·2 ml.). After 3 days the product (4·13 g., 96%) was isolated as described above for methyl *N*-t-butoxycarbonyl-3-nitro-L-tyrosinate (VII). It had m. p. 112·5—114° and was identical with material prepared by method (*a*) above.

*Action of Hydrogen Chloride on Methyl* N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosinate*.—A solution of the protected amino-acid (0·43 g.) in ethyl acetate (4 ml.) was saturated with hydrogen chloride. After 16 hr., ether (10 ml.) was added and methyl *O*-benzyl-3-nitro-L-tyrosinate hydrochloride (0·36 g., 99%), m. p. and mixed m. p. 191—193°, was collected.

N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosine* (VI).—The methyl ester (VIII) (2·15 g.) in acetone (50 ml.) was stirred for 90 min. with N-sodium hydroxide (5·5 ml.). Isolated in the usual manner, the product (1·83 g.) was a chromatographically pure, solid foam, $[\alpha]_D^{19}$ +16·7° (*c* 3·2 in MeOH). It could not be crystallised and was used without further purification.

*Action of Hydrogen Chloride on* N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosine*.—A solution of the acyl amino-acid (1·0 g.) in ethyl acetate (5 ml.) was saturated with hydrogen chloride. After 1 hr., ether (20 ml.) was added and the product was collected and dissolved in water. *O*-Benzyl-3-nitro-L-tyrosine (0·71 g., 94%), m. p. and mixed m. p. 222—226°, $[\alpha]_D^{17}$ −63·1° (*c* 1 in glacial $CH_3{\cdot}CO_2H$) was liberated by the addition of ammonia solution.

*Action of Hydrogen Bromide on* O-*Benzyl*-3-*nitro*-L-*tyrosine*.—The amino-acid benzyl ether (0·32 g.) in glacial acetic acid (8 ml.) was treated with 5·5N-hydrogen bromide in glacial acetic acid solution (2 ml.). After 1 hr., ether (50 ml.) was added and the product (0·29 g., 95%), m. p. 240° (decomp.), was collected and identified as 3-nitro-L-tyrosine hydrobromide by comparison with an authentic specimen.

N-*Benzyloxycarbonylglycyl*-O-*benzyl*-3-*nitro*-L-*tyrosine*.—Methyl *O*-benzyl-3-nitro-L-tyrosinate

hydrochloride (IX) (4·24 g.) and benzyloxycarbonylglycine [22] (2·40 g.) in cold, stirred chloroform (20 ml.) containing triethylamine (1·17 g.) were treated with *NN'*-dicyclohexylcarbodi-imide (2·61 g.). After 20 hr., chromatographically pure *methyl* N-*benzyloxycarbonylglycyl*-O-*benzyl*-3-*nitro*-L-*tyrosinate* (Xa) (5·90 g., 98%), m. p. 70—71·5°, was isolated in the usual manner. Recrystallisation from methanol–ether gave material (5·24 g., 82%), m. p. 74—76°, raised to 77—79° by a further recrystallisation from the same solvent, $[\alpha]_D^{17}$ +11·9° (*c* 1·85 in MeOH) (Found: C, 62·0; H, 5·5; N, 8·0. $C_{27}H_{27}N_3O_8$ requires C, 62·2; H, 5·2; N, 8·1%). The same product was obtained in 56% yield by a mixed anhydride coupling using isobutyl chloroformate and in 83% yield from an " active ester " coupling using *p*-nitrophenyl *N*-benzyloxycarbonylglycinate.[23]

This product (7·27 g.), in acetone (50 ml.), was saponified using N-sodium hydroxide (15·4 ml.). The chromatographically pure *acyl-dipeptide* (6·10 g., 86%) was isolated, in the usual manner, as an amorphous solid. A small sample separated from acetonitrile–ether as micro-needles, m. p. 117—120°, $[\alpha]_D^{17}$ +30·6° (*c* 1 in MeOH) (Found: C, 61·5; H, 4·8; N, 8·3. $C_{26}H_{25}N_3O_8$ requires C, 61·6; H, 5·0; N, 8·3%).

*Glycyl*-3-*nitro*-L-*tyrosine*.—*N*-Benzyloxycarbonylglycyl-3-nitro-L-tyrosine (4·50 g.) in glacial acetic acid (5 ml.) was treated with 5·5N-hydrogen bromide in glacial acetic acid solution (20 ml.). After 1 hr., ether (500 ml.) was added. The sticky solid which separated was washed by decantation with more ether (2 × 200 ml.) and dissolved in hot ethanol (20 ml.), and the solution was treated with pyridine (6 ml.). The *dipeptide* (2·20 g., 67%) which separated had m. p. 233—235° (decomp.). A sample recrystallised from aqueous acetic acid had m. p. 259—260° (decomp.) (lit.,[4] 220°) $[\alpha]_D^{16}$ +34·4° (*c* 1 in 2N-HCl) (Found: C, 46·85; H, 4·6; N, 14·6. Calc. for $C_{11}H_{13}N_3O_6$: C, 46·7; H, 4·6; N, 14·6%).

*t-Butyl* N-*t-Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosylglycinate* (Xb).—*N*-t-Butoxycarbonyl-*O*-benzyl-3-nitro-L-tyrosine (VI) (7·08 g.) and t-butyl glycinate [24] (2·23 g.) were coupled in ethyl acetate solution (25 ml.), using *NN'*-dicyclohexylcarbodi-imide (3·85 g.). The reaction was worked up in the usual manner to yield the *protected dipeptide* (8·37 g., 91%) as a chromatographically pure, solid foam, m. p. 56—60°, $[\alpha]_D^{19.5}$ +6·45° (*c* 3 in MeOH) (Found: C, 61·6; H, 7·0; N, 7·6. $C_{27}H_{35}N_3O_8$ requires C, 61·2; H, 6·7; N, 7·9%).

*Action of Hydrogen Bromide on t-Butyl* N-*t-Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosylglycinate*.—A stream of hydrogen bromide was passed through a solution of the protected dipeptide (8·35 g.) in nitromethane (50 ml.). After 10 min. the solvent was decanted and the residue (4·74 g., 66%), m. p. 207—209° (decomp.) was washed by decantation with nitromethane and then with ether. A sample recrystallised from acetonitrile gave O-*benzyl*-3-*nitro*-L-*tyrosylglycine hydrobromide*, m. p. 210—212° (decomp.), $[\alpha]_D^{18}$ −4·5° (*c* 3 in DMF) (Found: C, 47·8; H, 4·2; Br, 18·0; N, 9·1. $C_{18}H_{20}BrN_3O_6$ requires C, 47·6; H, 4·4; Br, 17·6; N, 9·25%) from which, by the action of pyridine in ethanol, O-*benzyl*-3-*nitro*-L-*tyrosylglycine* (79% yield), m. p. 201—204° (decomp.), raised to 212—213° (decomp.) by recrystallisation from aqueous ethanol (Found: C, 57·7; H, 5·2; N, 11·0. $C_{18}H_{19}N_3O_6$ requires C, 57·9; H, 5·1; N, 11·3%), was obtained.

This dipeptide (1·72 g.) was dissolved, by brief warming, in 5·5N-hydrogen bromide in acetic acid solution (15 ml.) and was then kept at room temperature. After 1 hr., ether (200 ml.) was added and the product was treated with pyridine in ethanol as described above for glycyl-3-nitro-L-tyrosine. The resulting 3-*nitro*-L-*tyrosylglycine* (0·83 g., 64%) had m. p. 247—249° (decomp.), raised to 255—257° (decomp.) by recrystallisation from aqueous acetic acid, $[\alpha]_D^{17}$ +50·8° (*c* 1 in 2N-HCl) (Found: C, 46·55; H, 4·8; N, 14·55. $C_{11}H_{13}N_3O_6$ requires C, 46·7; H, 4·6; N, 14·8%).

*Methyl* NS-*Dityrityl*-L-*cysteinyl*-O-*benzyl*-3-*nitro*-L-*tyrosinate* (Xc).—Diethylammonium *NS*-dityrityl-L-cysteinate [25] (18·58 g.) in chloroform (100 ml.) was washed with N-hydrochloric acid (25 ml.) and then with water (2 × 25 ml.). The chloroform was dried and then evaporated to leave an oil. Methyl *O*-benzyl-3-nitro-L-tyrosinate hydrochloride (IX) (9·15 g.) was shaken with chloroform (100 ml.) containing triethylamine (2·53 g.) until a clear solution was obtained. The chloroform was washed with water (3 × 25 ml.), dried, and evaporated to leave an oil.

[22] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[23] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373; D. F. Elliot and D. W. Russell, *Biochem. J.*, 1957, **66**, 49.
[24] R. W. Roeske, *Chem. and Ind.*, 1959, 1121.
[25] L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, 1962, **84**, 3894; G. Amiard, R. Heymès, and L. Velluz, *Bull. Soc. chim. France*, 1956, 698.

These oily products, in ice-cold, stirred, ethyl acetate solution (100 ml.) were treated with *NN'*-dicyclohexylcarbodi-imide (5·66 g.) and the mixture was kept for 24 hr. at room temperature. The precipitate was removed, washed with ethyl acetate, and extracted with boiling chloroform (50 ml.). The solution was cooled to 0°, when *NN'*-dicyclohexylurea separated out and was filtered off. Evaporation of the chloroform gave material (21·8 g.), m. p. 198—200°. Two recrystallisations from acetonitrile yielded the *protected dipeptide* (17·95 g., 78%), m. p. 205—206°. A sample further recrystallised from acetonitrile had m. p. 211—213°, $[\alpha]_D^{20}$ +98·9° (*c* 1·1 in $CHCl_3$) (Found: C, 75·6; H, 5·4; N, 4·4. $C_{58}H_{51}N_3O_6S$ requires C, 75·9; H, 5·6; N, 4·6%). The ethyl acetate reaction solution, after being worked up in the usual manner, gave further product (1·30 g., 6%), m. p. 202—204°.

*Action of Acetic Acid on Methyl* NS-*Ditrityl*-L-*cysteinyl*-O-*benzyl*-3-*nitro*-L-*tyrosinate*.—The protected dipeptide (Xc) (2·0 g.) was heated with glacial acetic acid (20 ml.) on a boiling-water bath until solution occurred (*ca.* 5 min.) and then for a further 5 min. The solvent was evaporated and the residue was triturated with ether (3 × 30 ml.). Recrystallisation of the product (1·24 g.) from nitromethane afforded 3-*tritylthiomethyl*-6-(3'-*nitro*-4'-*benzyloxybenzyl*)-*piperazine*-2,5-*dione* (XIIa) (0·97 g., 69%), m. p. 198—200°. A sample further recrystallised from nitromethane had m. p. 204—205° (Found: C, 70·7; H, 5·25; N, 6·15; S, 4·9. $C_{38}H_{33}N_3O_5S$ requires C, 70·9; H, 5·2; N, 6·5; S, 5·0%).

N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosyl*-L-*phenylalanine*.—*N*-t-Butoxycarbonyl-*O*-benzyl-3-nitro-L-tyrosine (VI) (8·32 g.) and methyl L-phenylalaninate [26] (3·58 g.) were coupled in ethyl acetate solution (40 ml.) by means of *NN'*-dicyclohexylcarbodi-imide (4·53 g.). The crude product (8·38 g.), isolated in the usual manner and recrystallised from methanol–ether, afforded *methyl* N-*t*-*butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosyl*-L-*phenylalaninate* (Xd) (7·33 g., 64%), m. p. 85—87°, $[\alpha]_D^{17}$ +2·1° (*c* 1 in MeOH) (Found: C, 64·7; H, 6·3; N, 7·4. $C_{31}H_{35}N_3O_8$ requires C, 64·5; H, 6·1; N, 7·3%).

Saponification of this product (1·22 g.) in aqueous acetone solution (1 : 1·5, 25 ml.) with N-sodium hydroxide (2·2 ml.) gave the crude *acyl dipeptide* (1·09 g.). Recrystallisation from methanol–ether gave the pure product (0·91 g., 81%), m. p. 140—142°, $[\alpha]_D^{17}$ +10·5° (*c* 1 in MeOH) (Found: C, 64·0; H, 6·0; N, 7·4. $C_{30}H_{33}N_3O_8$ requires C, 63·9; H, 5·9; N, 7·5%).

3-(3'-*Nitro*-4'-*benzyloxybenzyl*)-6-*benzylpiperazine*-2,5-*dione* (XIIb).—Methyl *N*-t-butoxycarbonyl-*O*-benzyl-3-nitro-L-tyrosyl-L-phenylalaninate (Xd) (1·0 g.) was kept for 2 hr. in a 5N-solution of hydrogen chloride in dry methanol (10 ml.). The solvent was evaporated and the residue (0·87 g.) was triturated under ether and recrystallised from acetonitrile to give *methyl* O-*benzyl*-3-*nitro*-L-*tyrosyl*-L-*phenylalaninate hydrochloride* (0·83 g., 94%), m. p. 153—155°, raised to 155—156° by a further recrystallisation from the same solvent, $[\alpha]_D^{17}$ +61° (*c* 1 in MeOH) (Found: C, 60·5; H, 5·4; Cl, 7·2; N, 8·1. $C_{20}H_{28}ClN_3O_6$ requires C, 60·7; H, 5·5; Cl, 6·9; N, 8·2%). This product (0·57 g.) was heated for 2 hr. in refluxing pyridine (10 ml.). The solvent was evaporated and the residue was triturated with water. Recrystallisation of the crude product (0·455 g., 98%), m. p. 259—261°, from glacial acetic acid gave the *substituted piperazinedione*, m. p. 263°, $[\alpha]_D^{17}$ −114·8° (*c* 1 in DMF) (Found: C, 67·1; H, 5·0; N, 9·5. $C_{25}H_{25}N_3O_6$ requires C, 67·4; H, 5·2; N, 9·4%).

3-(3'-*Nitro*-4'-*hydroxybenzyl*)-6-*benzylpiperazine*-2,5-*dione* (XIIc).—The protected piperazinedione (XII*b*) (1·14 g.) was dissolved by gentle warming in glacial acetic acid (5 ml.). 5·5N-Hydrogen bromide in glacial acetic acid solution (5 ml.) was added and the solution was kept for 1 hr. a room temperature. After the solvent had been evaporated, the residue was triturated with ether and extracted with boiling ethanol (30 ml.). The insoluble material (0·64 g.) was dissolved in 5% sodium carbonate solution (25 ml.) and the solution filtered and acidified with concentrated hydrochloric acid. Recrystallisation of the product (0·62 g.) from glacial acetic acid gave the *phenolic piperazinedione* (0·57 g., 65%), m. p. 283—284°, $[\alpha]_D^{17}$ −129·3° (*c* 1 in DMF) (Found: C, 60·9; H, 4·7; N, 11·6. $C_{18}H_{17}N_3O_5$ requires C, 60·8; H, 4·8; N, 11·8%).

*Action of Hydrogen Bromide on* N-*t*-*Butoxycarbonyl*-O-*benzyl*-3-*nitro*-L-*tyrosyl*-L-*phenylalanine*.—(*a*) The acyl dipeptide (3·59 g.) in solution in the minimum quantity of glacial acetic acid was treated with 5·5N-hydrogen bromide in glacial acetic acid solution (4·5 ml.). After 1 hr., a chromatographically homogeneous, ninhydrin-positive product (1·70 g.), m. p. 228—

[26] R. A. Boissonnas, St. Guttmann, P. A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, 1956, **39**, 1421.

230° (decomp.), probably *O*-benzyl-3-nitro-L-tyrosyl-L-phenylalanine, was isolated by the method described for glycyl-3-nitro-L-tyrosine. This product yielded, after recrystallisation from glacial acetic acid, 3-(3′-nitro-4′-benzyloxybenzyl)-6-benzylpiperazine-2,5-dione (XIIb), (1·53 g., 47·5%), m. p. 257—259°, identified by comparison with an authentic specimen.

(*b*) The acyl dipeptide (0·37 g.) in glacial acetic acid (2 ml.) was kept for 2 hr. with 5·5N-hydrogen bromide in glacial acetic acid solution (8 ml.). A thin oil, obtained by evaporation of the solvent, was washed by decantation with ether (3 × 10 ml.), dissolved in water (5 ml.), and passed down a column of Amberlite IR 120 in the hydrogen cycle. The column was washed with water until the washings were neutral and was finally eluted with 10% ammonia solution. The ammonia was evaporated and the residue triturated with ethanol. The product (0·17 g.) was recrystallised from aqueous ethanol to provide *3-nitro-L-tyrosyl-L-phenylalanine* (0·15 g., 63%), m. p. 275—276° (decomp.), $[\alpha]_D^{13}$ +48·8° (*c* 0·35 in 2N-HCl) (Found: C, 58·0; H, 5·1; N, 11·1. $C_{18}H_{19}N_3O_6$ requires C, 57·9; H, 5·1; N, 11·3%).

This product (20 mg.) was heated for 30 min. in refluxing glacial acetic acid (2 ml.). Evaporation and trituration with ether gave crude 3-(3′-nitro-4′-hydroxybenzyl)-6-benzylpiperazine-2,5-dione (XIIc), (18 mg.), m. p. 266—268°, identified by comparison of its infrared spectrum with that of authentic material obtained from the benzyl ether (XIIb).

*N-Benzyloxycarbonyl-L-prolyl-O-benzyl-3-nitro-L-tyrosine.*—*N*-Benzyloxycarbonyl-L-proline [27] (1·40 g.) in dry, ice-cold chloroform (15 ml.) containing triethylamine (0·57 g.) was stirred and treated with isobutyl chloroformate (0·77 g.). After 30 min., a pre-cooled solution of methyl *O*-benzyl-3-nitro-L-tyrosinate hydrochloride (IX) (2·06 g.) in chloroform (15 ml.) containing triethylamine (0·57 g.) was added. After a further 30 min., the solution was allowed to warm to room temperature and was kept for 18 hr. The crude product (2·57 g.) was isolated in the usual manner and recrystallised from ethyl acetate to afford *methyl N-benzyloxycarbonyl-L-prolyl-O-benzyl-3-nitro-L-tyrosinate* (XI) (2·24 g., 69%), m. p. 141—142°, $[\alpha]_D^{18}$ +2·6° (*c* 3 in dioxan) (Found: C, 64·5; H, 5·6; N, 7·45. $C_{31}H_{33}N_3O_8$ requires C, 64·7; H, 5·8; N, 7·3%). Saponification of this product (1·84 g.) in solution in aqueous acetone gave the crude *acyl dipeptide* (1·76 g.) which was recrystallised from aqueous methanol to give the pure material (1·58 g., 88%), m. p. 102—104°, $[\alpha]_D^{19}$ +17·1° (*c* 3 in MeOH) (Found: C, 64·0; H, 5·5; N, 7·4. $C_{30}H_{31}N_3O_8$ requires C, 64·15; H, 5·6; N, 7·5%).

*L-Prolyl-3-nitro-L-tyrosine.*—*N*-Benzyloxycarbonyl-L-prolyl-*O*-benzyl-3-nitro-L-tyrosine (0·64 g.) was stirred and treated with 5·5N-hydrogen bromide in glacial acetic acid solution (3 ml.). The crude *dipeptide* (0·24 g., 68%), m. p. 243—246° (decomp.), was isolated in the same way as described for glycyl-3-nitro-L-tyrosine. A sample twice recrystallised from aqueous acetic acid had m. p. 273—274° (decomp.), $[\alpha]_D^{17}$ −28·5° (*c* 1 in 2N-HCl) (Found: C, 51·0, 50·5; H, 5·5, 5·3; N, 12·4. $C_{14}H_{17}N_3O_6,0{\cdot}5H_2O$ requires C, 50·6; H, 5·5; N, 12·65%).

*O-Diphenylmethyl-3-nitro-L-tyrosine.*—A solution of diphenyldiazomethane [28] (from 34·6 g. of benzophenone hydrazone) was added to a solution of methyl *N*-trifluoroacetyl-3-nitro-L-tyrosinate (II) (16·8 g.) in dry dioxan (50 ml.) and the mixture was heated on a boiling-water bath for 3 hr. After evaporation of the dioxan the residue was dissolved in ether and passed down a column of neutral alumina. The column was eluted with ether and the ether-soluble material was treated, in solution in acetone (200 ml.), with 2N-sodium hydroxide (50 ml.). After 1 hr., the acetone was evaporated, water (150 ml.) was added, and the aqueous solution was extracted with ethyl acetate (2 × 20 ml.) and acidified with 2N-hydrochloric acid (50 ml.). The light oil which separated was extracted into ethyl acetate (2 × 50 ml.) and the crude product (13·3 g.; m. p. 173—175°) was precipitated from the dried solution by the addition of an excess of dicyclohexylamine. Recrystallisation from ethanol (*ca.* 400 ml.) gave the *amino-acid ether hemihydrate* (8·14 g., 41%), m. p. 190—195° (decomp.) (Found: C, 65·1, 65·8; H, 5·4, 5·1; N, 6·9, 6·8. $C_{22}H_{20}N_2O_5,0{\cdot}5H_2O$ requires C, 65·85; H, 5·3; N, 7·0%).

*Action of Hydrogen Bromide on O-Diphenylmethyl-3-nitro-L-tyrosine.*—The amino-acid ether (10 mg.) was kept with 5·5N-hydrogen bromide in glacial acetic acid solution (0·5 ml.). After 5 min., chromatography of the solution indicated that quantitative formation of 3-nitrotyrosine had occurred.

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[27] A. Berger, J. Kurtz, and E. Katchalski, *J. Amer. Chem. Soc.*, 1954, **76**, 5552.
[28] W. Schroeder and L. Katz, *J. Org. Chem.*, 1954, **19**, 718.