341. *Cyto-active Amino-acids and Peptides. Part III.*[1,2] *Synthesis of* para-*Substituted Phenyl and Alkoxymethyl Ethers of Tyrosine.*

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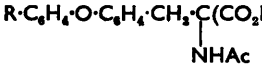
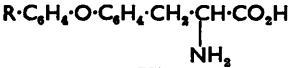
O-[*p*-Di-(2-chloroethyl)aminophenyl]-DL-tyrosine has been synthesised by two routes. Etherification of *N*-acyl-L-tyrosine esters with *p*-fluoronitrobenzene led to racemisation. Attempts to resolve *N*-acetyl-*O*-*p*-nitrophenyl-DL-tyrosine failed. Methoxymethyl and ethoxymethyl ethers of L-tyrosine have been prepared.

In Parts I [1] and II [2] of this series the preparation of novel *para*-substituted phenylalanines was described. One of them, *p*-di-(2-chloroethyl)amino-L-phenylalanine (CB 3025) was found to be very active against an experimental animal tumour,[3] to cause mutations in *Drosophila melanogaster*,[4] chromosome damage in tumour cells,[5] and inhibition of phenylalanine uptake in *Staph. aureus*.[6] In continuation of this work, syntheses of *para*-substituted phenyl and alkoxymethyl ethers of tyrosine were undertaken as a step towards the study of corresponding iodinated derivatives.

O-[*p*-Di-(2-chloroethyl)aminophenyl]-DL-tyrosine [II; R = $(Cl\cdot CH_2\cdot CH_2)_2N$] was obtained from diethyl acetamido-*p*-(*p*'-nitrophenoxy)benzylmalonate [7] (I; R = NO_2) *via* the *p*'-amino-compound [7] (I; R = NH_2), the preparative sequence being continued with hydroxyethylation, chlorination, and hydrolysis, in a manner published previously.[1] The reduction stage was improved and the di-(2-hydroxyethyl)amino-intermediate [I; R = $(HO\cdot CH_2\cdot CH_2)_2N$] was obtained crystalline. However, no attempt was made to isolate the malonate [I; R = $(Cl\cdot CH_2\cdot CH_2)_2N$], the chlorination product from the dialcohol [I; R = $(HO\cdot CH_2\cdot CH_2)_2N$] being hydrolysed directly to the required amino-acid [II; R = $(Cl\cdot CH_2\cdot CH_2)_2N$].



(I)



(II)

Attempts to synthesise the optically active forms of the tyrosine [II; R = $(Cl\cdot CH_2\cdot CH_2)_2N$], in particular the L-isomer, in view of the biological results in the phenylalanine series,[3] failed in every instance when *p*-fluoronitrobenzene was used with a number of *N*-acyl-L-tyrosine esters. Although etherification was achieved in reasonable yields, in presence of anhydrous potassium carbonate in ethyl methyl ketone, it was always accompanied by racemisation of the tyrosine ethers produced. In consequence, only the DL-forms of *N*-formyl-, *N*-benzoyl-, and *N*-phthaloyl-*O*-(*p*-nitrophenyl)tyrosine ethyl esters were obtained. That this was due to the vigorous conditions necessary to effect etherification was shown by the results with fluoro-2 : 4-dinitrobenzene which, when condensed with *N*-formyl-L-tyrosine ethyl esters under milder conditions, gave the (2 : 4-dinitrophenyl)-*N*-formyl-*O*-L-tyrosine ester. We also confirmed the claim by Canzanelli *et al.*[8] that 3 : 4 : 5-tri-iodo-1-nitrobenzene, condensed with *N*-benzoyl-L-tyrosine ethyl ester, yields the L-form of di-iodinated *O*-*p*-nitrophenyltyrosine. *N*-Formyl-*O*-(*p*-nitrophenyl)-DL-tyrosine ethyl ester was reduced to the amino-compound, hydroxyethylated, chlorinated, and hydrolysed to the " mustard " derivative [II; R = $(Cl\cdot CH_2\cdot CH_2)_2N$], identical with

[1] Part I, Bergel and Stock, *J.*, 1954, 2409.
[2] Part II, Bergel, Burnop, and Stock, *J.*, 1955, 1223.
[3] Bergel, Haddow, and Stock, *B.E.C.C. Ann. Report*, 1953, **31**, 6.
[4] Fahmy and Fahmy, *J. Genet.*, 1956, **54**, 146.
[5] Koller and Veronesi, *Brit. J. Cancer*, in the press.
[6] Crathorn and Hunter, *Biochem. J.*, in the press.
[7] Southwick, Foltz, and McIntyre, *J. Amer. Chem. Soc.*, 1953, **75**, 5877.
[8] Canzanelli, Harington, and Randall, *Biochem. J.*, 1934, **28**, 68.

the product from the malonic ester synthesis reported above. In the case of the *O-p*-nitrophenyl-*N*-phthaloyltyrosine ethyl ester, reduction gave the corresponding amine which, like its precursor, showed no optical activity.

Our next efforts were towards the resolution of *N*-acetyl-*O-p*-nitrophenyl-DL-tyrosine by formation of salts with optically active bases such as brucine, quinine, cinchonine, cinchonidine, strychnine, and (+)-amphetamine. In no case were satisfactory results obtained. Equally unsuccessful was the application of the enzyme acylase I to the same tyrosine derivative under conditions similar to those used by Greenstein *et al.*[9] in the resolution of *N*-acetyl-DL-*alloiso*leucine.

In addition to the above phenyl ethers we prepared alkoxymethyl ethers of tyrosine, starting with *N*-phthaloyl-L-tyrosine ethyl ester. When this compound was allowed to react with chloromethyl methyl or ethyl ether and the phthaloyl group was removed with ethanolic hydrazine, *O*-methoxymethyl- and *O*-ethoxymethyl-L-tyrosine were obtained. Both products, in contrast to tyrosine, were easily soluble in water and liberated formaldehyde in warm dilute mineral acids. It was thought possible that this characteristic would enable these compounds to release formaldehyde slowly under biological conditions and that they would act as anti-tumour agents. However, no such effect was achieved with *O*-methoxymethyl-L-tyrosine (CB 3081) when applied by A. Haddow [10] to rats carrying the implanted Walker carcinoma 256.

Negative biological results were also obtained with the compound [II; R = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$] (CB 3051). This is surprising in view of the high activity of the *p*-di-(2-chloroethyl)aminophenylalanine.[3] On the other hand, Fahmy and Fahmy [11] observed point mutations with CB 3051 in *Drosophila melanogaster*.

Experimental

O-[p-*Di*-(2-*chloroethyl*)*aminophenyl*]-DL-*tyrosine* [II; R = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$].—*Method* (1). (*a*) The nitro-compound (I; R = NO_2), prepared as described by Southwick *et al.*,[7] had m. p. 154° (lit., 153·5—154°); it (8·88 g.) was hydrogenated in ethyl acetate (200 ml.) and methanol (50 ml.) at atmospheric pressure in presence of palladised calcium carbonate (0·5 g.). After filtration, the solvent was evaporated *in vacuo* and the residue dissolved in warm benzene (charcoal), which was filtered again. The cool solution on admixture with a small amount of light petroleum yielded, almost quantitatively, colourless crystals of the amine (I; R = NH_2) which, recrystallised from ethanol–light petroleum, had m. p. 150° (lit., m. p. 147—148°).

(*b*) Ethylene oxide (12 ml.) was added to a solution of the amino-compound (I; R = NH_2) (5·0 g.) in water (75 ml.) and glacial acetic acid (45 ml.). The mixture was kept at 20° for 24 hr., then neutralised by solid sodium hydrogen carbonate. The precipitated gum was extracted with ethyl acetate, and the solution dried (Na_2SO_4), treated with charcoal, and, after filtration, evaporated *in vacuo* to dryness. The residue (yield 65%), crystallised from benzene–light petroleum, gave the required *di*(*hydroxyethyl*)*amino-compound* [I; R = $(HO{\cdot}CH_2{\cdot}CH_2)_2N$] as colourless needles, m. p. 104—105° (Found: C, 62·3; H, 7·1; N, 5·7. $C_{26}H_{34}O_8N_2$ requires C, 62·2; H, 6·8; N, 5·6%).

(*c*) The foregoing compound (5 g.) was dissolved in sodium-dried benzene (200 ml.) and *ca.* 50 ml. of the solvent were distilled off, in order to remove any traces of water. To the cooled solution was added phosphorus oxychloride (15 ml.), and the whole then heated under reflux for 25 min. The benzene was removed *in vacuo*, and the residue dissolved in concentrated hydrochloric acid (120 ml.), refluxed for 6 hr., and concentrated to a thick syrup *in vacuo*. This was dissolved in water (40 ml.), and the solution treated with charcoal, filtered, and neutralised by saturated sodium acetate solution, whereupon the O-[p-*di*-(2-*chloroethyl*)*aminophenyl*]-DL-*tyrosine* was deposited as a heavy off-white precipitate (35%). Recrystallised from aqueous methanol, it had m. p. 159—160° (decomp.) (Found: C, 57·2; H, 5·9; N, 7·0; Cl, 18·2. $C_{19}H_{22}O_3N_2Cl_2$ requires C, 57·4; H, 5·6; N, 7·1; Cl, 17·8%).

Method (2). (*a*) *N*-Formyltyrosine ethyl ester was mentioned by Haas *et al.*[12] but no details

[9] Greenstein, Birnbaum, and Levintow, *Biochem. Preparations*, 1953, **3**, 87.
[10] Haddow, personal communication.
[11] Fahmy and Fahmy, personal communication.
[12] Haas, Sizer, and Loofbourow, *Biochim. Biophys. Acta*, 1951, **6**, 1589.

were given. In the present investigation it was prepared by treating L-tyrosine ethyl ester (5 g.) with 98% formic acid (25 ml.) and acetic anhydride (4 ml.) at 40—50° for ¾ hr. The solvent was removed *in vacuo* and the residue diluted with water (250 ml.). The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and concentrated to a small volume. Addition of ether precipitated *N*-formyl-L-tyrosine ethyl ester (75%), m. p. 105—106° (lit., 105—106°), $[\alpha]_D^{23}$ +37·2° (*c* 4·65 in EtOH) (Found: C, 60·5; H, 6·4; N, 5·9. Calc. for $C_{12}H_{15}O_4N$: C, 60·8; H, 6·4; N, 5·9%).

(*b*) This material (11·8 g.) and *p*-fluoronitrobenzene (7·0 g.) in freshly dried, redistilled ethyl methyl ketone (200 ml.) were refluxed for 48 hr. over finely powdered anhydrous potassium carbonate (3·5 g.), and the solution was filtered, and taken to dryness *in vacuo*. The residue was dissolved in ethyl acetate, and the solution washed with water, dried (Na_2SO_4), and evaporated to a small volume. After dilution with ether, addition of a small amount of pentane and storage in the refrigerator overnight, gave colourless N$^{\alpha}$-*formyl*-O-p-*nitrophenyl*-DL-*tyrosine ethyl ester* (40%), m. p. 105°, α 0° (Found: C, 60·4; H, 5·4; N, 7·8. $C_{18}H_{18}O_6N_2$ requires C, 60·3; H, 5·4; N, 7·8%).

(*c*) The nitro-compound (4·78 g.) in ethyl acetate (100 ml.) and methanol (25 ml.) was hydrogenated in the presence of palladised calcium carbonate (0·2 g.). The filtered solution was washed with water, dried (Na_2SO_4), and taken to dryness. *N*$^{\alpha}$-Formyl-*O*-*p*-aminophenyl-DL-tyrosine ethyl ester was left as oil (yield 70%).

It gave an *acetyl derivative* which crystallised from aqueous ethanol as a monohydrate, m. p. 85·5° (Found: C, 61·8; H, 6·3; N, 7·1. $C_{20}H_{22}O_5N_2,H_2O$ requires C, 61·8; H, 6·2; N, 7·2%). The amine and its acetyl derivative were optically inactive.

(*d*) The amine was hydroxyethylated and chlorinated as for the malonic ester intermediate. After acid hydrolysis the *O*-[*p*-di-(2-chloroethyl)aminophenyl]-DL-tyrosine was obtained, identical in m. p. and mixed m. p. with the product obtained by method (1).

O-p-*Nitrophenyl*-N-*phthaloyl*-DL-*tyrosine Ethyl Ester*.—(*a*) Equivalent amounts of L-tyrosine ethyl ester and phthalic anhydride were dissolved in benzene, the solvent was removed, and the reactants were heated as a thick oil on the water-bath for 30 min. The product was refluxed in 2·5N-ethanolic hydrogen chloride for 1½ hr., then evaporated to dryness *in vacuo*, and ethyl acetate added to the residual oil. While the phthaloyl compound was dissolved by the ethyl acetate, unchanged amino-ester was deposited as the solid hydrochloride. The ethyl acetate solution was filtered, washed with aqueous sodium carbonate, and, after drying (Na_2SO_4), concentrated to a small volume. Addition of pentane precipitated oily N-*phthaloyl*-L-*tyrosine ethyl ester* which solidified (yield 75%). A small portion was recrystallised from *cyclo*hexane, giving white needles, m. p. 104—105°, $[\alpha]_D^{20}$ −192° (*c* 2·01 in EtOH) (Found: C, 67·1; H, 5·2; N, 4·2. $C_{19}H_{17}O_5N$ requires C, 67·3; H, 5·1; N, 4·1%).

(*b*) This material was etherified by *p*-fluoronitrobenzene under the conditions used for the *N*-formyl compound. After evaporation of the filtered ketonic solution, an oil (*ca.* 60%) was obtained which crystallised readily upon addition of ethanol. Recrystallisation from ethanol gave O-p-*nitrophenyl*-N-*phthaloyl*-DL-*tyrosine ethyl ester* as colourless prisms, m. p. 116°, α 0° (Found: C, 65·1; H, 4·4; N, 6·1. $C_{25}H_{20}O_7N_2$ requires C, 65·2; H, 4·4; N, 6·1%).

O-p-*Aminophenyl*-N$^{\alpha}$-*phthaloyl*-DL-*tyrosine Ethyl Ester*.—The nitro-compound (15 g.) in ethyl acetate (200 ml.) and methanol (50 ml.) was hydrogenated over palladised calcium carbonate (0·5 g.). The filtered solution was shaken with water to remove methanol, separated, dried (Na_2SO_4), and taken to dryness. The residual oil crystallised overnight (yield 80%). It recrystallised from propan-2-ol as light yellow needles, m. p. 121—122°, α 0° (Found: C, 70·2; H, 5·1; N, 6·6. $C_{25}H_{22}O_5N_2$ requires C, 69·8; H, 5·2; N, 6·5%).

N-*Benzoyl*-O-p-*nitrophenyl*-DL-*tyrosine Ethyl Ester*.—*N*-Benzoyl-L-tyrosine ethyl ester was prepared according to Canzanelli *et al.*;[8] it had m. p. 122—123°, $[\alpha]_D^{20}$ −15·3° (*c* 3·0 in ethyl methyl ketone), $[\alpha]_D^{20}$ −23·7° (*c* 3·7 in EtOH). It was treated with *p*-fluoronitrobenzene as above. The oil obtained after filtration and evaporation of the ketone crystallised in *ca.* 60% yield on addition of ethanol. Recrystallisation from the same solvent gave colourless needles, m. p. 145—146°, α 0° (Found: C, 66·5; H, 5·2; N, 6·1. $C_{24}H_{22}O_6N_2$ requires C, 66·4; H, 5·1; N, 6·5%).

N$^{\alpha}$-*Formyl*-O-(2 : 4-*dinitrophenyl*)-L-*tyrosine Ethyl Ester*.—*N*-Formyl-L-tyrosine ethyl ester in an exact equivalent of aqueous N-sodium hydroxide was heated with an equivalent amount of 1-fluoro-2 : 4-dinitrobenzene in ethanol on the water-bath for 30 min.; water was added to precipitate the *dinitrophenyl tyrosine ether* (75%). The product solidified and, recrystallised

from benzene–pentane, had m. p. 92—93°, $[\alpha]_D^{18}$ +18° (*c* 1·8 in EtOH) (Found : C, 53·4; H, 4·3; N, 10·6. $C_{18}H_{17}O_8N_3$ requires C, 53·6; H, 4·3; N, 10·4%).

Attempts to Resolve N-*Acetyl*-O-p-*nitrophenyl*-DL-*tyrosine*.—This compound, prepared by the method of Southwick *et al.*,[7] had m. p. 162°. (*a*) It was mixed with equimolecular proportions of brucine, cinchonine, cinchonidine, quinine, strychnine, or (+)-amphetamine in ethanol; *e.g.*, brucine (3·94 g.) in ethanol (10 ml.) was mixed with the tyrosine derivative (3·44 g.) in ethanol (15 ml.). No crystals were deposited during several days' storage at 20°. Evaporation gave a gum. When strychnine (3·34 g.) was used, the insolubility of the base required larger amounts of ethanol (75 ml.). In no case, including the use of methanol, chloroform, or aqueous ethanol (70%) as solvents, were satisfactory results obtained.

(*b*) The tyrosine derivative was treated in the manner described by Greenstein *et al.*[9] for acetyl-DL-*alloiso*leucine, in an aqueous solution of lithium hydroxide with dry acylase I powder. No preferential enzymic hydrolysis was observed.

O-*Methoxymethyl*-L-*tyrosine*.—*N*-Phthaloyl-L-tyrosine ethyl ester (10 g.) and an excess of chloromethyl methyl ether (25 ml.) in dry benzene (200 ml.) were refluxed over dry potassium carbonate (5 g.) for 13 hr. The solution was filtered and evaporated *in vacuo*. The residual gum was extracted with a very large volume of boiling light petroleum (b. p. 60—80°) from which slowly crystallised, on cooling, O-*methoxymethyl*-N-*phthaloyl*-L-*tyrosine ethyl ester* (75%) as colourless needles, m. p. 75—76°, $[\alpha]_D^{20}$ −191° (*c* 3·01 in C_6H_6) (Found : C, 65·7; H, 5·75; N, 3·9. $C_{21}H_{21}O_6N$ requires C, 65·8; H, 5·5; N, 3·7%).

This compound was refluxed in an equivalent of M-ethanolic hydrazine for 2 hr., then cooled and filtered to remove phthalhydrazide, and the filtrate evaporated to dryness. The residue was passed in benzene down a column of alumina to remove any traces of phthalhydrazide. The eluate on evaporation gave an oil which was hydrolysed to the amino-acid by shaking it with a slight excess of N-sodium hydroxide at 20° for 2—3 hr. Most of the oil dissolved in the alkaline solution, which was then filtered and neutralised with an exact equivalent of N-acetic acid. The solution was then evaporated *in vacuo* at 40—50° until solid material was deposited. A large excess of ethanol was then added to precipitate completely the O-*methoxymethyl*-L-*tyrosine* (yield 40%). Recrystallised from aqueous ethanol, it had m. p. 185—190° (decomp.) on slow heating, $[\alpha]_D^{23}$ −27·5° (*c* 2·00 in H_2O) (Found : C, 58·5; H, 6·9; N, 6·2. $C_{11}H_{15}O_4N$ requires C, 58·65; H, 6·7; N, 6·2%).

On warming a solution of the compound (0·25 g.) in N-hydrochloric acid (5 ml.) in a stoppered tube for 1—2 min., an odour of formaldehyde was produced. Neutralisation with N-sodium hydroxide precipitated L-tyrosine. The filtrate on addition of ethanolic dimedone deposited the formaldehyde derivative (m. p. and mixed m. p.).

O-*Ethoxymethyl*-L-*tyrosine*.—In the same manner chloromethyl ethyl ether and *N*-phthaloyl-L-tyrosine ethyl ester gave O-*ethoxymethyl*-L-*tyrosine* (*ca.* 10%) without crystallisation of the intermediate *N*-phthaloyl ethyl ester. The compound, recrystallised from aqueous ethanol, had m. p. 188—190° (decomp.), $[\alpha]_D^{21}$ −20·5° (*c*, 1·00 in H_2O) (Found : C, 59·8; H, 7·0; N, 6·0. $C_{12}H_{17}O_4N$ requires C, 60·2; H, 7·2; N, 5·9%).

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