822. *Peptides. Part IX.* Preparation of* L-*Phenylalanine from* L-*Tyrosine.*

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Reduction by sodium in liquid ammonia of the diethyl phosphate of L-tyrosine, prepared through the cupric salt, gives L-phenylalanine, conveniently isolated as its *N*-acetyl derivative which may be converted directly by ethanolic hydrogen chloride into the ethyl ester of L-phenylalanine hydrochloride.

In studies of synthetic peptides L-phenylalanine finds frequent application but it is expensive. In contrast, its near relative, L-tyrosine, is readily available from casein and other natural proteins, and the current retail prices of the two amino-acids are in a ratio of 35 : 1. Consequently, when we discovered an efficient method of reducing phenols to

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aromatic hydrocarbons,[1] L-tyrosine was an obvious subject for it.* The complications introduced by the presence of the amino- and carboxyl groups have now been mastered, and we are able to describe a preparative method which gives consistently a 42% over-all conversion of L-tyrosine into L-phenylalanine.

Our general reductive method is based on the reaction between the aryl diethyl phosphate and sodium in liquid ammonia, and hence the phenylalanine must be separated from alkali-metal salts. On a preparative scale, it is most convenient to extract the amino-acid as its *N*-acetyl derivative, from which it can be regenerated in excellent yield; moreover, after acetylation, the contaminating tyrosine, which is an inevitable by-product of the reduction,[1] can be destroyed by permanganate. If the ethyl ester of phenylalanine is required, its hydrochloride can be prepared directly from *N*-acetylphenylalanine. Alcoholysis has not apparently been combined with esterification before in just this way, but the process may be generally useful with α-acetamido-acids. Unfortunately, it fails with benzyl alcohol.

$$ArO{\cdot}PO(OEt)_2 \xrightarrow[NH_3]{Na-} ArH$$

The phenolic hydroxyl group of L-tyrosine can be phosphorylated cleanly in aqueous alkali with tetraethyl pyrophosphate when the amino- and the carboxyl group are masked in the cupric salt, just as in the familiar ω-acylation of αω-diamino-carboxylic acids.[3] Provided that the cupric ions are removed carefully with hydrogen sulphide, the reduction is smooth and 43% of the tyrosine is converted into *N*-acetyl-L-phenylalanine. It is noteworthy that, as judged by the small consumption of permanganate, only a little tyrosine is regenerated during the reduction; most of the losses probably occur before this stage.

An alternative way, which attracted us, of masking the amino-group is to acetylate it before phosphorylation of the phenolic hydroxyl group, instead of after the reduction. The methyl ester of *N*-acetyl-L-tyrosine, a very accessible intermediate, gives a nicely crystalline diethyl phosphate, which however is converted by sodium in liquid ammonia into a complex mixture. Reduction of the analogous benzyl ester is much more satisfactory, presumably because the carboxyl group is liberated quickly, but the process is not competitive. On the other hand these difficulties do not arise with *N*-acetyl-L-tyrosine itself and, since this intermediate need not be isolated before phosphorylation and the over-all yield is almost as good, this route would be preferable to that *via* the cupric salts, were it not for a small amount of racemisation which is apparently unavoidable. Racemisation can occur easily during the acetylation of α-amino-acids but it is avoided when the solution is alkaline;[4] we confirmed that the stage of acetylation was not responsible in the present instance by comparing the *N*-acetyl-L-tyrosine with a sample prepared from the benzyl ester by hydrogenolysis. Clearly, the mixed anhydrides of *N*-acetyl-L-tyrosine and its diethyl phosphate with diethyl hydrogen phosphate are responsible, because they can have transient existence during the treatment with tetraethyl pyrophosphate. In this connection we were able to show, by means of a Carlsberg recording titrator (" pH-stat "),[5] that acetate ions accelerate the alkaline hydrolysis of tetraethyl pyrophosphate, presumably through formation of acetyl diethyl phosphate.

As racemisation is not usually encountered with α-toluene-*p*-sulphonamido-acids and they are cleaved by sodium in liquid ammonia,[6] we prepared the crystalline diethyl phosphate of *N*-toluene-*p*-sulphonyl-L-tyrosine, but this route is inconvenient. A rather

* The method has also been used elsewhere.[2]

[1] Kenner and Williams, *J.*, 1955, 522.
[2] Pelletier and Locke, *J. Amer. Chem. Soc.*, 1957, **79**, 4531; *J. Org. Chem.*, 1958, **23**, 131; Wenkert and Jackson, *J. Amer. Chem. Soc.*, 1958, **80**, 217.
[3] Goodman and Kenner, *Adv. Protein Chem.*, 1957, **12**, 508.
[4] Neuberger, *Adv. Protein Chem.*, 1948, **4**, 357.
[5] Jacobsen, Léonis, Linderstrøm-Lang, and Ottesen, " Methods of Biochemical Analysis " (ed. D. Glick), Interscience Publishers, New York, Vol. IV, 1957, p. 171.
[6] Du Vigneaud and Behrens, *J. Biol. Chem.*, 1937, **117**, 27.

similar, but more direct, route begins with reaction between L-tyrosine itself and tetraethyl pyrophosphate, but in this instance the reduction gives a complex mixture, apparently containing peptides, and the over-all yield is only 8%.

Experimental

Paper chromatograms were run on Whatman No. 1 paper in the system butan-1-ol–acetic acid–water (4 : 1 : 5 v/v).

Tetraethyl Pyrophosphate.—When commercial material was not available, the following procedure [7] was convenient. A mixture of diethyl phosphite (51 c.c.), carbon tetrachloride (71·2 c.c.), and ether (200 c.c.) was stirred at 0° during the dropwise addition of triethylamine (27·8 c.c.) and then for a further hour before addition of triethylamine (second portion of 27·8 c.c.) and water (6·5 c.c.). The mixture was kept overnight at 5° and then filtered. The triethylamine hydrochloride was washed with ether. Vacuum-distillation of the combined liquors afforded 41·5 g., b. p. 126—128°/0·4 mm., n_D^{22} 1·4170.

N-*Acetyl*-L-*Phenylalanine.*—A solution of hydrated cupric sulphate (12·43 g.) in hot water (20 c.c.) was added gradually to a stirred solution of L-tyrosine (18·1 g.) in hot 2N-sodium hydroxide (50 c.c.). The mixture was cooled to room temperature and the cupric salt was dissolved by the addition of 2N-sodium hydroxide (50 c.c.) and water (80 c.c.). Tetraethyl pyrophosphate (40 g.) was added in one portion to this solution (pH 11·0), and then the pH was maintained at 11·0 by dropwise addition of 2N-sodium hydroxide (55 c.c.) with rapid stirring. The pH was reduced to 7·0 with hydrochloric acid. The light blue precipitate was collected, washed with water (100 c.c.), and dissolved in 4N-hydrochloric acid. Hydrogen sulphide was bubbled through the solution until precipitation of cupric sulphide was complete and then the filtered liquor was neutralised (pH 7·0) and evaporated under reduced pressure. The residual solid was dried by azeotropic distillation with benzene and dissolved in liquid ammonia (100 c.c.) before being added rapidly to a solution of sodium (9·2 g.) in liquid ammonia (50 c.c.). The remaining blue colour was discharged with ammonium chloride, and the ammonia was allowed to evaporate. A solution of the residue in water (100 c.c.) was made alkaline, boiled to remove the last of the ammonia, cooled, and then brought to pH 11·0. Acetic anhydride (24 c.c.) and 2N-sodium hydroxide (240 c.c.) were added in eight equal portions to the solution which was stirred at 0°, After being allowed to reach room temperature during 1 hr., the solution was acidified and extracted with ethyl acetate (10 × 50 c.c.). When the bulk of the ethyl acetate (450 c.c.) had been distilled, most (8·0 g.) of the product crystallised. The liquors were extracted with aqueous sodium carbonate solution, to which potassium permanganate solution was added until the colour persisted for 10 min. It was discharged with sodium sulphite and then extraction of the acidified solution with ethyl acetate afforded a further crop of *N*-acetyl-L-phenylalanine. The total yield was 8·7 g. (43% from tyrosine), and the product had m. p. 170—171°, $[\alpha]_D^{22}$ +47·6° (*c* 1 in ethanol) (lit.,[8] +47·5°, m. p. 171—172°).

L-*Phenylalanine Ethyl Ester Hydrochloride.*—A solution of *N*-acetyl-L-phenylalanine (2·0 g.) in ethanol (50 c.c.), originally saturated with hydrogen chloride, was boiled for 5 hr. and then evaporated. Crystallisation of the residue from ethanol–ether afforded 1·75 g. (79%) of the ester hydrochloride, m. p. 150—151°, $[\alpha]_D^{22}$ −7·7° (*c* 4 in water) (Found: C, 57·4; H, 7·0. Calc. for $C_{11}H_{16}O_2NCl$: C, 57·5; H, 7·0%).

N-*Acetyl*-O-(*di*-O-*ethylphosphoryl*)-L-*tyrosine Methyl Ester.*—Triethylamine (2·75 c.c.) and diethyl phosphorochloridate [9] (3·35 g.) were added to a solution of *N*-acetyl-L-tyrosine methyl ester [10] (4·5 g.) in dry acetonitrile (35 c.c.). After 30 hr. the precipitated triethylamine hydrochloride was removed and the solvent was evaporated. The gum (5 g.) which was obtained after removal of some more salt and unchanged ester (0·8 g.) crystallised from ether in colourless needles, m. p. 83° (Found: C, 51·3; H, 6·1; N, 3·8. $C_{16}H_{24}O_7NP$ requires C, 51·4; H, 6·5; N, 3·8%). This *phosphate* (1·0 g.) was reduced with sodium (0·13 g.) in liquid ammonia (20 c.c.), and the product was hydrolysed with concentrated hydrochloric acid before examination by paper chromatography; ninhydrin revealed seven spots with R_F 0·08 (weak), 0·24 (strong), 0·37 (strong), 0·43 (strong, tyrosine), 0·58 (strong, phenylalanine), 0·67 (weak), 0·84 (strong).

[7] Cf. Atherton and Todd, *J.*, 1947, 674; Steinberg, *J. Org. Chem.*, 1950, **18**, 637.
[8] Overby and Ingersoll, *J. Amer. Chem. Soc.*, 1951, **73**, 3365.
[9] McCombie, Saunders, and Stacey, *J.*, 1945, 381.
[10] Jackson, *J. Amer. Chem. Soc.*, 1952, **74**, 838.

N-*Acetyl*-L-*tyrosine Amide*.—A solution of *N*-acetyl-L-tyrosine methyl ester (8 g.) in ammonia (200 c.c.) was kept at −40° for 5 days. The *amide* recrystallised from ethanol in prisms (5·5 g., 73%), m. p. 220—225° (Found: C, 59·1; H, 6·0; N, 12·8. $C_{11}H_{14}O_3N_2$ requires C, 59·45; H, 6·35; N, 12·6%). Phosphorylation and subsequent reduction of the gummy phosphate followed the pattern of the foregoing experiments and the final result was apparently identical.

N-*Acetyl*-L-*tyrosine Benzyl Ester*.—The preparation from L-tyrosine benzyl ester hydrochloride [11] (8·88 g.), sodium carbonate (8·2 g.), and acetyl chloride (3·45 g.) was like that of the methyl ester,[10] and crystallisation from ether–benzene gave 7·8 g. (86%) of the *benzyl ester*, m. p. 93—94°, $[\alpha]_D^{22}$ +27·9 (*c* 4 in ethanol) (Found: C, 69·0; H, 6·0; N, 4·4. $C_{18}H_{19}O_4N$ requires C, 69·0; H, 6·1; N, 4·5%). Phosphorylation and reduction with sodium in liquid ammonia was carried out as in the two foregoing instances, but the final acidic hydrolysis was omitted; instead *N*-acetyl-L-phenylalanine, m. p. 170°, $[\alpha]_D^{25}$ +47·5° (*c* 1 in ethanol) (36%), was isolated after destruction of *N*-acetyltyrosine with alkaline permanganate.

Phosphorylation and Reduction of N-*Acetyl*-L-*tyrosine*.—Hydrogenolysis of its benzyl ester with palladised charcoal in methanol containing a trace of acetic acid yielded *N*-acetyl-L-tyrosine, m. p. 153—154°, $[\alpha]_D^{25}$ +53·1° (*c* 3 in methanol), identical with that prepared in the usual way.[12] A solution of this compound (6·0 g.) in 2N-sodium hydroxide (27 c.c.) and water (50 c.c.) was brought to pH 10·0 with 2N-hydrochloric acid and then tetraethyl pyrophosphate (7·5 c.c.) was added all at once. By means of an automatic titrator delivering 2N-sodium hydroxide, the pH was maintained at 10·0 during 4 hr. Extraction of the acidified solution with ethyl acetate (3 × 40 c.c.) furnished a colourless syrup which was dissolved in dry ether (15 c.c.) and liquid ammonia (15 c.c.). This solution was added dropwise during 5 min. to a stirred solution of sodium (2·5 g.) in ammonia (50 c.c.). The *N*-acetylphenylalanine (2·05 g., 37%) which was isolated in the usual way after destruction of the *N*-acetyltyrosine with alkaline permanganate, had consistently on repetition m. p. 168—169°, $[\alpha]_D^{22}$ +45·2° (*c* 5 in ethanol). Runs under slightly varied conditions gave the same result.

O-(*Di*-O-*ethylphosphoryl*)-N-*toluene*-p-*sulphonyl*-L-*tyrosine*.—Tetraethyl pyrophosphate (0·6 c.c.) was added to a solution of *N*-toluene-*p*-sulphonyl-L-tyrosine [13] (0·55 g.) in N-sodium hydroxide, (6·0 c.c.). After 5 min. the solution was acidified and extracted with ethyl acetate (2 × 20 c.c.), which was dried, concentrated to 10 c.c., and then kept for 2 days at −5° while starting material (0·2 g.) separated. Crystallisation of the remainder from ethyl acetate–light petroleum yielded colourless needles (0·22 g., 30%) of the *phosphate*, m. p. 107°, $[\alpha]_D^{22}$ +10° (*c* 2 in ethanol) (Found: C, 51·0; H, 5·6; N, 3·15. $C_{20}H_{26}O_8NSP$ requires C, 51·0; H, 5·6; N, 3·0%). This compound (0·22 g.) was reduced with sodium (0·045 g.) in liquid ammonia (20 c.c.), and the product was desalted by anion- and cation-exchange columns; judged by paper chromatography it was entirely phenylalanine.

Phosphorylation and Reduction of L-*Tyrosine*.—Tetraethyl pyrophosphate (8·0 g.) was added in one portion to a stirred solution (pH 10·5) of L-tyrosine (3·62 g.) in 2N-sodium hydroxide (10 c.c.) and water (50 c.c.). By means of an automatic titrator delivering 2N-sodium hydroxide, the pH was maintained during 1 hr. at 10·5 before being brought to 7·0. The residue from evaporation of the solution was dissolved in liquid ammonia (50 c.c.) and added gradually to a solution of sodium (1·38 g.) in liquid ammonia (50 c.c.). The blue colour was discharged with ammonium chloride before the ammonia was allowed to evaporate. A solution of the residue in 5N-hydrochloric acid (100 c.c.) was boiled during 8 hr., made alkaline, and boiled again to drive off ammonia. The solution was then brought to pH 11·0 and acetylated in the usual way. After destruction of the *N*-acetyltyrosine, 0·35 g. (8·4%) of *N*-acetyl-L-phenylalanine, m. p. 169—170°, $[\alpha]_D^{21}$ +47·1° (*c* 2 in ethanol), was isolated.

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[11] Erlanger and Hall, *J. Amer. Chem. Soc.*, 1954, **76**, 5781.
[12] Du Vigneaud and Meyer, *J. Biol. Chem.*, 1932, **98**, 305.
[13] Fischer and Lipschitz, *Ber.*, 1915, **48**, 375.