Cyto-active Amino-acids and Peptides. Part II. Resolution of* para-*Substituted Phenylalanines and Synthesis of* p-*Di*-(2-*chloroethyl*)*amino*-DL-*phenyl*[β-^{14}C]*alanine.*

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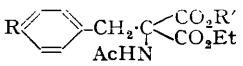
Resolution procedures for *para*-substituted phenylalanines, leading from appropriate diethyl aminomalonate derivatives to *p*-di-(2-chloroethyl)amino-L- and -D-phenylalanine, are described. DL-Phenyl[β-^{14}C]alanine and *p*-di-(2-chloroethyl)amino-DL-phenyl[β-^{14}C]alanine have been prepared.

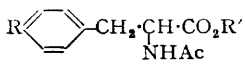
In Part I * the syntheses of DL- (CB 3007), L- (CB 3025), and D-forms (CB 3026) of *p*-di-(2-chloroethyl)aminophenylalanine (IV) were reported. In absence of a resolution procedure the optically active compounds were made from L- and D-phenylalanine respectively; the racemic compound was prepared either from the DL-amino-acid or by the aminomalonic ester method. In view of the outstanding anti-Walker-tumour effect of

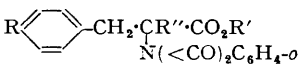
* Part I, Bergel and Stock, *J.*, 1954, 2409.

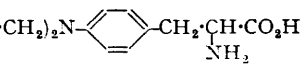
the L-isomer (cf. *Brit. Emp. Cancer Camp. Ann. Rep.*, 1953, **31**, 6), representing one of the first examples in this field of selectivity of action through optical isomerism, it became desirable to produce larger amounts of this compound for further trials, and also to synthesise a ^{14}C-labelled phenylalanine derivative for study *in vivo*.

Since the route employing the malonic ester synthesis seemed better suited to large-scale work than that starting with optically active material it was necessary to find the means of resolving one of the resulting intermediate substituted phenylalanines. It was, however, inadvisable to attempt the resolution of the final product (CB 3007) itself since halogen is readily lost from the di-(2-chloroethyl)amino-group especially under alkaline

(I) 

 (II)

(III) 

 (IV)

conditions. We therefore first prepared N^{α}-acetyl-*p*-di-(2-hydroxyethyl)amino-DL-phenylalanine [II; R = $(HO{\cdot}CH_2{\cdot}CH_2)_2N$, R′ = H] by mild alkaline hydrolysis and subsequent decarboxylation of the corresponding malonic ester [I; R = $(HO{\cdot}CH_2{\cdot}CH_2)_2N$, R′ = Et] (cf. Albertson, *J. Amer. Chem. Soc.*, 1950, **72**, 1396). The gummy product did not form a crystalline derivative with brucine or strychnine.

Attention was next directed to the possibility of resolving ethyl N^{α}-acetyl-*p*-amino-DL-phenylalaninate (II; R = NH_2, R′ = Et) with the aid of an optically active acid. A possible route to the amine lay in the decarboxylation of the half-ester (I; R = NH_2, R′ = H), but application of the sodium ethoxide method (Skinner and Huber, *ibid.*, 1951, **73**, 3321) to the malonate (I; R = NH_2, R′ = Et) did not yield the desired acid (I; R = NH_2, R′ = H), nor did pyridine hydrochloride in pyridine. We therefore had recourse to the alkaline hydrolysis and subsequent decarboxylation of the diester (I; R = NH_2, R′ = Et) (cf. Snyder, Shekleton, and Lewis, *ibid.*, p. 3321), which yielded N^{α}-acetyl-*p*-amino-DL-phenylalanine (II; R = NH_2, R′ = H) as a gum. This with ethanolic hydrogen chloride gave the crystalline ethyl ester (II; R = NH_2, R′ = Et) which readily formed a crystalline salt with α-bromocamphor-π-sulphonic acid, but not with tartaric or dibenzoyltartaric acid. However, the bromocamphorsulphonate did not effect a resolution.

We then prepared, from the ester (II; R = NH_2, R′ = Et), the corresponding *N*-*o*′-carboxybenzoyl derivative (II; R = o-$HO_2C{\cdot}C_6H_4{\cdot}CO{\cdot}NH$, R′ = Et) which was easily reconverted into the parent amine by hot ethanolic hydrazine. While it formed a crystalline salt with brucine, attempts to liberate the acid from this salt led to the loss of the *N*-*o*′-carboxybenzoyl group as phthalic acid. As the brucine salt itself was difficult to purify, this was not a practicable procedure. We then found that *N*-acetyl-*p*-nitro-DL-phenylalanine (II; R = NO_2, R′ = H), obtained by Albertson's (*loc. cit.*) treatment of the diester (I; R = NO_2, R′ = Et), was resolved readily with brucine in ethanol; the salt of the L-acid crystallised, leaving that of the D-isomer in the mother-liquor. The salt of the D-acid was recovered, and crystallised readily from water. *N*-Acetyl-*p*-nitro-L- and -D-phenylalanine were obtained from their salts by means of ammonia and subsequent acidification after removal of the brucine. The configurations were established by conversion of the acids into the ethyl esters (II; R = NO_2, R′ = Et), of which the L-isomer had been prepared from L-phenylalanine. Catalytic hydrogenation of the L- and D-nitro-esters gave the amino-derivatives (II; R = NH_2, R′ = Et) as gums which were characterised as their crystalline 2 : 4-dinitrophenyl derivatives. With ethylene oxide the amines gave the crystalline L- and D-di(hydroxyethyl) derivatives (II; R = $(HO{\cdot}CH_2{\cdot}CH_2)_2N$, R′ = Et). The next step towards the L-chloro-compound (IV) caused total or partial racemisation, whether phosphorus oxychloride, phosphorus pentachloride, or thionyl chloride was used as chlorinating agent. This difficulty was overcome by replacement of the *N*-acetyl by the *N*-phthaloyl group. This was done in two ways: first, by hydrolysing *N*-acetyl-*p*-nitro-L- or -D-phenylalanine (II; R = NO_2, R′ = H) to *p*-nitro-L- or -D-phenylalanine, esterifying the carboxyl group with ethanolic hydrogen

chloride, and allowing the ester to react with phthalic anhydride as described in Part I (*loc. cit.*) to form the nitro-ester (III; R = NO_2, R′ = Et, R″ = H). Reduction of the nitro-group, hydroxyethylation, chlorination, and hydrolysis led to the desired D- and L-forms (IV). The second approach began with the synthesis of diethyl α-4-nitrobenzyl-α-phthalimidomalonate. *p*-Nitrobenzyl chloride could not be condensed with diethyl phthalimidomalonate by use of sodium ethoxide in ethanol or the sodio-derivative of the phthalimidomalonic ester [prepared by means of powdered sodium in toluene or by Barger and Weichselbaum's method (*Org. Synth.*, 1943, Coll. Vol. II, p. 384)] in dioxan or in a melt containing an equivalent or an excess of halide, but the reactants gave a 85—90% yield in ethyl methyl ketone in the presence of sodium iodide. The resulting crystalline malonate (III; R = NO_2, R′ = Et, R″ = CO_2Et) was hydrolysed by Albertson's method to *N-o'*-carboxybenzoyl-*p*-nitro-DL-phenylalanine which was cyclised with acetic anhydride to *p*-nitro-*N*-phthaloyl-DL-phenylalanine (III; R = NO_2, R′ = R″ = H). This was not fully resolved by way of the brucine salt; but mixing equimolar amounts of cinchonidine and the DL-acid in methanol led to precipitation of the salt of the D-acid, whilst that of the L-acid was recoverable from the mother-liquor. Isolation of the isomeric acids and esterification gave the D- and the L-ester (III; R = NO_2, R′ = Et, R″ = H) previously described. Their rotations established the configurations of the parent acids and salts. From these esters, the optically active chloroethyl derivatives (IV) were obtained as described in Part I (*loc. cit.*).

In the following the synthesis of *p*-di-(2-chloroethyl)amino-DL-phenyl[β-^{14}C]alanine is reported. [*carboxy*-^{14}C]Benzoic acid, from phenylmagnesium bromide and ^{14}C-carbon dioxide (cf. Calvin *et al.*, " Isotopic Carbon," J. Wiley and Sons, New York, 1949, p. 180), was reduced with lithium aluminium hydride to [α-^{14}C]benzyl alcohol. With zinc chloride and hydrochloric acid this gave [α-^{14}C]benzyl chloride and thence diethyl acetamido-([α-^{14}C]benzyl)malonate (I; R = H, R′ = Et) in an overall yield of 67% from carbon dioxide. Part of this product was hydrolysed to DL-phenyl[β-^{14}C]alanine, identified by paper chromatography. Syntheses of phenylalanine labelled in other positions have been reported by Gurin and Delluva (*J. Biol. Chem.*, 1947, **170**, 545), Schepatz and Gurin (*ibid.*, 1949, **180**, 663), Lerner (*ibid.*, 1949, **181**, 281), and Henneberry *et al.* (*Canad. J. Chem.*, 1951, **29**, 229).

Nitration of the ester (I; R = H, R′ = Et) in acetic anhydride gave diethyl acetamido-(*p*-nitro[α-^{14}C]benzyl)malonate (I; R = NO_2, R′ = Et), readily isolated from the crude product by recrystallisation from ethyl methyl ketone. In experiments with unlabelled material the yield at this stage was 45%, nitration probably also occurring in the 2-position. In the ^{14}C-synthesis, the 4-nitro-compound remaining in the mother-liquors was recovered by dilution with unlabelled 4-nitro-ester. [Although there is a serious drop in yield at this stage, alternative routes to the nitro- or corresponding amino-compound were less satisfactory. *p*-Nitro-, *p*-acetamido-, and *p*-phthalimido-benzonitrile were prepared by Sandmeyer reactions with ^{14}C-cyanide, but with a deficiency of the cyanide in place of the conventional excess for economic reasons. Yields (on cyanide) were then low and the products contaminated with highly coloured by-products.] The nitro-compound was converted into the amino-derivative and *p*-di-(2-chloroethyl)amino-DL-phenyl[β-^{14}C]-alanine (IV) by the methods described in Part I adapted to a smaller scale, the final yield from the nitro-compound being 79% and the radioactive yield from carbon dioxide 22%.

Experimental

N-*Acetyl*-p-*nitro*-DL-, -L- *and* -D-*phenylalanine* (II; R = NO_2, R′ = H).—Diethyl α-acetamido-α-*p*-nitrobenzylmalonate (50 g.) was refluxed for 24 hr. with sodium carbonate (50 g.) in water (500 ml.) (cf. Albertson, *loc. cit.*). To the stirred, hot, filtered solution was slowly added concentrated hydrochloric acid (110 ml.). Solid separated, the solution frothed vigorously, and its temperature rose. The mixture was boiled with stirring for a few minutes, cooled, and left at 0° overnight. The pale brown crystalline product (28 g., 78%) was filtered off and had m. p 205—209°. Recrystallisation from water (charcoal) gave colourless needles of N-*acetyl*-p-*nitro*-DL-*phenylalanine*, m. p. 207—209° (Found: C, 52·7; H, 5·0; N, 10·7. $C_{11}H_{12}O_5N_2$ requires C, 52·4; H, 4·8; N, 11·1%).

To a solution of the DL-acid (23·55 g.) in hot ethanol (200 ml.) was added one of brucine (36·9 g., 1·00 mol.) in hot ethanol (200 ml.), and the mixture left overnight at room temperature. Pale yellow prisms were deposited. The mixture was cooled in ice-water for 1 hr., and the product, m. p. 203—208° (25·35 g., 0·42 mol.) filtered off. Recrystallisation from ethanol gave pale yellow prisms of the *brucine salt*, m. p. 207—209·5°, $[\alpha]_D^{21}$ +19·2° ± 0·5° (*c*, 1·59 in 1 : 1 H_2O–dioxan), of *N*-acetyl-*p*-nitro-L-phenylalanine. Recrystallisation did not significantly affect the rotation or m. p. (Found : C, 61·1; H, 5·85; N, 8·6. $C_{34}H_{38}O_9N_4,H_2O$ requires C, 61·0; H, 6·0; N, 8·4. Found, after drying at 100°/1 mm. for 4 hr. : C, 62·7; H, 6·2; N, 8·6. $C_{34}H_{38}O_9N_4$ requires C, 63·1; H, 5·9; N, 8·7%).

Treatment of an aqueous solution of the salt with ammonia or sodium hydroxide, removal of the brucine by filtration, acidification of the filtrate, and recrystallisation of the product from water yielded colourless prisms of *N-acetyl*-p-*nitro*-L-*phenylalanine*, initial m. p. 170—172°, resolidification, final m. p. 206—209°, $[\alpha]_D^{24}$ +49·7° ± 1° (*c*, 1·55 in EtOH) (Found : C, 52·5; H, 4·8; N, 11·0. $C_{11}H_{12}O_5N_2$ requires C, 52·4; H, 4·8; N, 11·1%).

The ethanolic mother-liquors from the brucine salt were evaporated to dryness under a vacuum, and the residual gum was taken up in hot water. Crystallisation set in on cooling. After an hour at 0°, the product (36·95 g.) was collected and recrystallised from water, yielding yellow prisms of the *brucine salt pentahydrate*, m. p. 98—99°, $[\alpha]_D^{21}$ −36·9° ± 0·5° (*c*, 1·63 in 1 : 1 H_2O–dioxan), of *N*-acetyl-*p*-nitro-D-phenylalanine. Recrystallisation raised the $[\alpha]_D^{21}$ to −37·6° ± 0·5° (*c*, 1·60) (Found, on a sample dried in a vacuum-desiccator over H_2SO_4 : C, 55·3; H, 6·6; N, 7·9. $C_{34}H_{38}O_9N_4,5H_2O$ requires C, 55·4; H, 6·6; N, 7·6. Found, on a sample dried to constant wt. at 80° in a high vacuum : C, 62·8; H, 5·65; N, 9·0. $C_{34}H_{38}O_9N_4$ requires C, 63·1; H, 5·9; N, 8·7%). Treatment of an aqueous solution of the salt with aqueous ammonia, removal of the precipitated brucine, acidification with hydrochloric acid, and recrystallisation of the product from water gave colourless prisms of N-*acetyl*-p-*nitro*-D-*phenylalanine*, initial m. p. 170—172°, resolidification, final m. p. 205—206°, $[\alpha]_D^{23}$ −44° ± 0·5° (*c*, 1·45 in EtOH) (Found : C, 52·3; H, 4·8; N, 11·2%).

N-*Acetyl*-p-*nitro*-L- *and* -D-*phenylalanine Ethyl Ester* (II; R = NO_2, R′ = Et).—(*a*) *p*-Nitro-L-phenylalanine ethyl ester hydrochloride (2·0 g.) prepared from L-phenylalanine (Bergel and Stock, *loc. cit.*) was acetylated in 75% yield by 10 minutes' heating with acetic anhydride–potassium carbonate. Crystallisation of the product from water gave colourless needles of the L-*acetyl compound*, m. p. 115—117°, $[\alpha]_D^{19}$ +13·5° ± 0·5° (*c*, 1·32 in EtOH) (Found : C, 55·9; H, 5·8; N, 10·2. $C_{13}H_{16}O_5N_2$ requires C, 55·7; H, 5·75; N, 10·0%).

(*b*) *N*-Acetyl-*p*-nitro-L-phenylalanine (70 mg.) from the brucine salt was esterified with 2N-ethanolic hydrogen chloride (2 days at room temperature). Evaporation of the solvent gave a colourless gum which crystallised from water in tiny colourless needles (90%) of the L-acetyl compound, m. p. 115—117°, unchanged on admixture with product obtained as in (*a*); the $[\alpha]_D^{19}$ +13·0° ± 0·5° (*c*, 1·33 in EtOH) established that the compound belonged to the L-series.

The D-ester was obtained similarly in comparable yield from the D-acid. Recrystallisation from water gave a *product* of m. p. 116—118°, $[\alpha]_D^{23}$ −11·0° ± 1·0° (*c*, 1·58 in EtOH) (Found : C, 55·9; H, 5·5; N, 9·9%).

N$^{\alpha}$-*Acetyl*-p-*amino*-DL-, -L-, *and* -D-*phenylalanine Ethyl Ester* (II; R = NH_2, R′ = Et).—(*a*) Diethyl acetamido-4-aminobenzylmalonate (2·75 g.) was refluxed for 4 hr. with 2·65N-sodium hydroxide (14 ml.), and the solution acidified with 3·06N-hydrochloric acid (12·2 ml.) and refluxed for 1 hr. (cf. Snyder *et al.*, *loc. cit.*). Evaporation *in vacuo*, extraction with ethanol, and evaporation of the solvent gave a gum which was kept in 3N-ethanolic hydrogen chloride (10 ml.) at room temperature for 3 days. Treatment of an aqueous solution of the product with ammonia, extraction into ethyl acetate, and crystallisation from benzene or water gave colourless prisms of the *ester*, m. p. 107—108°, raised to 109—110° on recrystallisation from benzene (Found : C, 62·2; H, 7·30; N, 11·3. $C_{13}H_{18}O_3N_2$ requires C, 62·4; H, 7·25; N, 11·2%). The *picrate* crystallised from benzene–ethanol in yellow needles, m. p. 161—162° (Found : N, 14·7. $C_{19}H_{21}O_{10}N_5$ requires N, 14·6%).

(*b*) *N*-Acetyl-*p*-nitro-L-phenylalanine ethyl ester (0·56 g.) was completely hydrogenated in methanol over 1% palladium–calcium carbonate (0·2 g.) in 15 min. The product was a gum. A little of the amine was heated on a steam-bath for 20 min. with a molar equivalent of 2 : 4-dinitrofluorobenzene. The N$^{\alpha}$-*acetyl*-p-(2 : 4-*dinitrophenyl*)*amino*-L-*phenylalanine ethyl ester* crystallised from ethanol in tiny yellow needles, m. p. 153—156°, $[\alpha]_D^{23}$ +54° ± 1° (*c*, 0·86 in dioxan) (Found : C, 54·6; H, 4·7; N, 13·3. $C_{19}H_{20}O_7N_4$ requires C, 54·8; H, 4·8; N, 13·5%).

N$^{\alpha}$-Acetyl-*p*-amino-D-phenylalanine ethyl ester was obtained similarly as a gum which gave

a 2 : 4-*dinitrophenyl*-D-*derivative*, m. p. 155—159°, $[\alpha]_D^{23}$ −54·5° ± 1·0° (*c*, 0·88 in dioxan) (Found : C, 55·0; H, 4·7; N, 13·1%).

The DL-amine (1·50 g., 0·006 mole) and ammonium α-bromocamphor-π-sulphonate (0·987 g., 0·003 mole) were dissolved in warm methanol (10 ml.), and N-hydrochloric acid (3 ml.) was added. The solution was taken to dryness (water-pump; steam-bath), then to dryness with methanol. The residue crystallised from methanol in colourless prisms (1·685 g.). Two further crystallisations from methanol gave the *bromocamphorsulphonate*, m. p. 236—238° (decomp.), $[\alpha]_D^{21}$ +53° ± 1° (*c*, 0·94 in H_2O), of N^{α}-acetyl-*p*-aminophenylalanine ethyl ester (Found : C, 49·4; H, 6·1; N, 5·1. $C_{23}H_{33}O_7N_2BrS$ requires C, 49·2; H, 5·9; N, 5·0%). Treatment of the aqueous solution of this salt with ammonia extraction with ethyl acetate, evaporation of the solvent, and crystallisation of the residue from benzene yielded the free base (73%), m. p. 109—111°, of negligible optical activity.

N^{α}-*Acetyl*-p-N-o′-*carboxybenzamido*-DL-*phenylalanine Ethyl Ester* (II ; R = *o*-$HO_2C{\cdot}C_6H_4{\cdot}CO{\cdot}NH$, R′ = Et).—$N^{\alpha}$-Acetyl-*p*-amino-DL-phenylalanine ethyl ester (0·84 g.) and phthalic anhydride (0·50 g., 1·00 mol.) were refluxed for 15 min. in ethyl acetate (10 ml.). The solid remaining after evaporation crystallised from ethanol in colourless needles (97%) of the monosolvated *carboxybenzoyl compound*, m. p. 177—179° (Found : C, 62·15; H, 6·3; N, 6·5. $C_{21}H_{22}O_6N_2$,EtOH requires C, 62·15; H, 6·35; N, 6·3%).

The sodium salt of the carboxybenzoyl compound and brucine hydrochloride formed a *salt* from water. Recrystallisation from water usually gave a product melting over a range, but the best crystals obtained had m. p. 131—132° (Found, on sample dried at 100° in a high vacuum : C, 63·85; H, 6·0; N, 6·75. $C_{21}H_{22}O_6N_2$,$C_{23}H_{26}O_4N_2$,$2H_2O$ requires C, 63·75; H, 6·3; N, 6·8%).

The salt (*ca.* 50 mg.) was dissolved in warm 2N-hydrochloric acid, and the solution cooled and set aside overnight. The colourless prisms formed were recrystallised from water. They were clearly phthalic acid since they contained no nitrogen (sodium fusion test), gave the fluorescein reaction with resorcinol and concentrated sulphuric acid, and had m. p. 204—205°, mixed m. p. with phthalic acid (of m. p. 207—210°) 205—207°.

Hydroxyethylation of N^{α}-*Acetyl*-p-*amino*-DL-, -L-, *and* -D-*phenylalanine Ethyl Ester*.—To a solution of the DL-amine (2·0 g.) in 2N-aqueous acetic acid (16 ml.), ethylene oxide (4 ml.) was added (cf. Everett, Roberts, and Ross, *J.*, 1953, 2386). The mixture was set aside for 24 hr., and then evaporated to dryness under a vacuum. The product was a gum.

Similarly, the gummy L-amine gave a good yield of N^{α}-*acetyl*-p-*di*-(2-*hydroxyethyl*)*amino*-L-*phenylalanine ethyl ester*, which, after two crystallisations from chloroform formed colourless prisms, m. p. 142—144°, $[\alpha]_D^{25}$ +43° ± 1° (*c*, 1·76 in EtOH) (Found : C, 60·0; H, 7·5; N, 8·2. $C_{17}H_{26}O_5N_2$ requires C, 60·3; H, 7·7; N, 8·3%). The D-amine gave, in the same way, the corresponding D-*di*-(2-*hydroxyethyl*)*amino-compound*, m. p. 140—142°, $[\alpha]_D^{24}$ −35° ± 1° (*c*, 1·65 in EtOH) increased to −36° ± 1° by a further crystallisation (Found : C, 60·4; H, 7·4; N, 8·2%).

Chlorination and Hydrolysis of N^{α}-*Acetyl*-p-*di*-(2-*hydroxyethyl*)*amino*-L-, -D-, *and* -DL-*phenylalanine Ethyl Ester*.—(*a*) The DL-compound (362 mg. of gum) was refluxed for 15 min. with dry benzene (5 ml.) and freshly distilled phosphorus oxychloride (1·0 ml.). Two phases were present throughout. The mixture was evaporated to dryness (water-pump) and the residue heated with concentrated hydrochloric acid (5 ml.) for 4 hr. Evaporation to small bulk and addition of concentrated sodium acetate solution to the residual solution gave a near-white precipitate which crystallised from methanol (charcoal) in colourless crystals of *p*-di-(2-chloroethyl)amino-DL-phenylalanine (51%), m. p. and mixed m. p. 181—182° (decomp.).

In another experiment the hydroxyethylated product from the DL-amine (0·3 g.) was refluxed for 15 min. with dry chloroform (5 ml.) and phosphorus oxychloride (1·5 ml.). The mixture became homogeneous after 10 minutes' heating. Evaporation to dryness under a vacuum, refluxing the residue with 6N-hydrochloric acid, evaporation to small bulk, and addition of sodium acetate solution yielded a product which, after crystallisation from methanol, gave the di-(2-chloroethyl)amino-DL-compound, m. p. 179—181° (decomp.), in 12% yield.

N^{α}-Acetyl-*p*-di-(2-hydroxyethyl)amino-L-phenylalanine ethyl ester was chlorinated in the same way in benzene and chloroform severally. In each case, the final product was the racemic chloro-compound.

(*b*) N^{α}-Acetyl-*p*-di-(2-hydroxyethyl)amino-L-phenylalanine ethyl ester (0·45 g.) was refluxed for 4 min. with purified thionyl chloride (1·0 ml.) in dry chloroform (5·0 ml.). The dark solution was taken to dryness (vacuum) and refluxed for 2 hr. with 6N-hydrochloric acid (6 ml.). After 12 hr. the solution was concentrated to small volume. Precipitation by means of sodium

acetate and crystallisation of the dried product from methanol (charcoal) gave almost colourless *p*-di-(2-chloroethyl)aminophenylalanine (55 mg.), m. p. 182—185° (decomp.), $[\alpha]_D^{23}$ −19·5° ± 1·0° (*c*, 0·67 in MeOH) as against $[\alpha]_D^{22}$ −31·5° ± 0·5° (*c*, 0·67) for the compound prepared from L-phenylalanine (Bergel and Stock, *loc. cit.*). A second crop (40 mg.) had m. p. 182—184° (decomp.).

(*c*) The L-dihydroxy-compound (1·0 g.) in dry chloroform (10 ml.) was added with ice-cooling to powdered phosphorus pentachloride (1·25 g.) in dry chloroform (10 ml.), then allowed to warm to room temperature and to stand for 4 hr. with occasional gentle warming for a few seconds on a water-bath. The solvent was evaporated and ice-water poured into the residue. The orange resin was slowly transformed into a pale brown oil. Extraction into chloroform, washing with water, drying ($MgSO_4$), evaporation of the solvent, dissolution in benzene, and finally passage through a short alumina column with benzene and then chloroform for elution gave a pale yellow gum (0·55 g.) which had a very approximate $[\alpha]_D^{23}$ of +29° (*c*, 2·75 in $CHCl_3$). The gum was refluxed for 2·5 hr. with 6N-hydrochloric acid (10 ml.), and the product isolated in the normal way with sodium acetate. Crystallisation from methanol gave a first crop (0·120 g.) of *p*-di-(2-chloroethyl)aminophenylalanine, m. p. 183—185° (decomp.), unchanged on admixture with authentic DL-compound. The product had no detectable optical activity (*c*, 0·67 in MeOH).

p-*Nitro*-L- *and* -D-*phenylalanine Ethyl Ester Hydrochloride.*—*N*-Acetyl-*p*-nitro-L-phenylalanine (0·5 g.; $[\alpha]_D^{24}$ +50°) was refluxed for 2·5 hr. with 6N-hydrochloric acid (10 ml.). The solution was evaporated to dryness (vacuum), and the crystalline residue evaporated with ethanol (vacuum), and then refluxed for 1·5 hr. with 2N-ethanolic hydrogen chloride (8 ml.). Evaporation of the solvent and crystallisation of the residue from acetone–methanol gave the L-ester hydrochloride (0·440 g.), m. p. 203—205° (decomp.), unchanged on admixture with the L-compound, m. p. 204—205° (decomp.), prepared from *p*-nitro-L-phenylalanine (Bergel and Stock, *loc. cit.*), $[\alpha]_D^{20}$ +11·7° ± 0·5° (*c*, 2·3 in H_2O).

The D-ethyl ester hydrochloride was prepared in the same way from *N*-acetyl-*p*-nitro-D-phenylalanine ($[\alpha]_D^{22}$ −39°). It had m. p. 208—209° (mixed m. p. 205—206° with authentic specimen of m. p. 204—205°) and $[\alpha]_D^{23}$ −10·3° ± 0·5° (*c*, 2·2 in H_2O).

Diethyl p-*Nitrobenzylphthalimidomalonate* (III; R = NO_2, R′ = Et, R″ = CO_2Et).—Diethyl sodiophthalimidomalonate (Barger and Weichselbaum, *loc. cit.*) (6·52 g.) was dissolved in boiling ethyl methyl ketone (80 ml.), and a solution of *p*-nitrobenzyl chloride (3·44 g., 1·0 mol.) in the same solvent (20 ml.) was added. Sodium iodide (*ca.* 0·5 g.), dissolved in hot ketone (10 ml.), was introduced, and produced immediate precipitation. The mixture was refluxed for 1·5 hr., cooled, filtered, and evaporated under a vacuum and the residual gum crystallised from ethanol. The *malonate* formed colourless prisms (88%), m. p. 103—105°, sharpening to 104—105° on recrystallisation from ethanol (Found: C, 59·8; H, 4·5; N, 6·4. $C_{22}H_{20}O_8N_2$ requires C, 60·0; H, 4·6; N, 6·4%).

N-*Carboxybenzoyl*-p-*nitro*-DL-*phenylalanine* (cf. Albertson, *loc. cit.*).—Diethyl *p*-nitrobenzylphthalimidomalonate (70 g.) and sodium carbonate (70 g.) in water (700 ml.) were refluxed overnight with stirring (to avoid bumping). The clear brown solution was acidified with hydrochloric acid, and refluxing and stirring were continued for a further 40 min. The mixture was cooled and the colourless precipitate (31 g.) collected. A second crop (18·5 g.) was obtained on evaporation of the mother-liquors. Crystallisation from aqueous ethanol gave the *compound* as small needles, m. p. 198—200° (Found, in sample dried at 100° in a high vacuum: C, 56·8; H, 4·1; N, 7·8. $C_{17}H_{14}O_7N_2$ requires C, 57·0; H, 3·9; N, 7·8%).

Ring-closure to the Acid (III; R = NO_2, R′ = R″ = H).—The *N*-carboxybenzoyl compound (2·7 g.) was refluxed for 30 min. with acetic anhydride (10 ml.), the mixture taken to dryness (vacuum), and the residue heated with water. The cooled gummy product became granular when rubbed and crystallised from ethyl methyl ketone–light petroleum or aqueous ethanol in almost colourless needles, m. p. 184—186°, of *p*-nitro-*N*-phthaloyl-DL-phenylalanine.

The same compound, prepared from *p*-nitrophenylalanine and phthalic anhydride and reported in Part I as having m. p. 180—181°, had m. p. 185—186° after recrystallisation from aqueous ethanol, unchanged on admixture with the foregoing material.

Resolution. Solutions of *p*-nitro-*N*-phthaloyl-DL-phenylalanine (1·0 g.) in methanol (25 ml.) and of cinchonidine (0·865 g., 1·00 mol.) in methanol (30 ml.) were mixed. Crystallisation soon set in. The mixture was left overnight, and the colourless needles (0·97 g.), m. p. 209—210°, were collected. After two recrystallisations from methanol the *cinchonidine salt* of the D-acid had m. p. 211° and $[\alpha]_D^{21}$ +82° ± 1° (*c*, 0·84 in dioxan) (Found, after drying at 100° in a high vacuum: C, 67·0; H, 5·8; N, 8·8. $C_{36}H_{34}O_7N_4$,MeOH requires C, 66·7; H, 5·7; N, 8·4%).

To the salt (2·9 g.) in warm ethanol (50 ml.) was added water (50 ml.) and a slight excess (*ca.* 10 ml.) of N-aqueous sodium hydroxide. The mixture was diluted with water, cooled, and filtered from the precipitated base, and the filtrate acidified with hydrochloric acid. The tiny needles of p-*nitro*-N-*phthaloyl*-D-*phenylalanine* (1·05 g.) had, after recrystallisation from ethanol, m. p. 207—208°, $[\alpha]_D^{20}$ +240° ± 2° (*c*, 1·01 in EtOH) (Found: C, 60·1; H, 3·7; N, 8·2. $C_{17}H_{12}O_6N_2$ requires C, 60·0; H, 3·55; N, 8·2%). Refluxing with 2N-ethanolic hydrogen chloride yielded *p*-nitro-*N*-phthaloyl-D-phenylalanine ethyl ester, m. p. 82—83°, $[\alpha]_D^{20}$ +206° ± 1° (cf. Part I).

Evaporation of the mother-liquors from the original cinchonidine experiment gave a gum which crystallised readily from aqueous ethanol in almost colourless needles (0·73 g.), m. p. 191—192·5°. Two recrystallisations from aqueous ethanol gave the L-acid cinchonidine *salt*, m. p. 192·5—194°, $[\alpha]_D^{20}$ −170° ± 1° (*c*, 1·32 in EtOH) (Found, after drying at 100° in a high vacuum: C, 66·2; H, 5·6; N, 8·6. $C_{36}H_{34}O_7N_4,H_2O$ requires C, 66·3; H, 5·5; N, 8·6%).

The acid was isolated as for the D-isomer. The recrystallised p-*nitro*-N-*phthaloyl*-L-*phenylalanine* had m. p. 209—211° and $[\alpha]_D^{21}$ −233° ± 2° (Found: C, 60·3; H, 3·8; N, 8·4%).

Conversion into the L-ethyl ester gave, after two recrystallisations, a product of m. p. and mixed m. p. 84—85°, $[\alpha]_D^{20}$ −212° ± 2° (cf. Part I).

[α-^{14}C]*Benzyl Chloride.*—[*carboxy*-^{14}C]Benzoic acid was obtained (89% yield) by treating the carbon dioxide from $Ba^{14}CO_3$ (8 mc, 4·0 millimoles) with an approximately M-solution of phenylmagnesium bromide (6 ml.) at −60° and proceding in the usual way (Calvin, *loc. cit.*).

This material was reduced (10 ml. of 5% lithium aluminium hydride in ether) and the crude benzyl alcohol obtained was treated overnight with a solution (2 ml.) of anhydrous zinc chloride (7 g.) in concentrated hydrochloric acid (5 ml.). The benzyl chloride was extracted with light petroleum (b. p. 80—100°), washed with sodium hydrogen carbonate solution, and filtered through anhydrous sodium sulphate. The solution was used directly for the next stage.

Diethyl Acetamido-([α-^{14}C]*benzyl*)*malonate.*—Thebenzyl chloride solution was added to diethyl acetamidomalonate (1·09 g.) dissolved in N-sodium ethoxide (5 ml.) and ethanol (10 ml.) and held for 6 hr. at 75—80°. The solvent was then evaporated and the residue extracted with chloroform. The filtered (charcoal) solution was evaporated to small bulk and crystallised by addition of light petroleum (yield, 827 mg., 67% from CO_2).

DL-[β-^{14}C]*Phenylalanine.*—Diethyl acetamido-([α-^{14}C]benzyl)malonate (216 mg.) was refluxed for 5 hr. with constant-boiling hydrochloric acid (1 ml.). Evaporation, dissolution in water, and precipitation with ammonia gave the crude phenylalanine, which was recrystallised from water. The yield was 81 mg. (70%), and the specific activity 1·96 mc/millimole. The compound showed the same R_F value as DL-phenylalanine when run on buffered paper with 1 : 1 benzyl–butyl alcohol (McFarren, *Analyt. Chem.*, 1951, **23**, 168).

Nitration of Diethyl Acetamidobenzylmalonate.—(*a*) To a solution of diethyl acetamidobenzylmalonate (1·54 g.) in acetic anhydride (4·0 ml.), cooled to 0°, fuming nitric acid (1·0 ml.) was added with stirring during 15 min. The mixture was set aside at room temperature for 2 hr. It deposited a few crystals. Ice and water (12 g.) were added; a precipitate was formed which when dry weighed 1·61 g. Two crystallisations from ethyl methyl ketone gave 0·78 g. (45%) of diethyl acetamido-*p*-nitrobenzylmalonate, m. p. and mixed m. p. 192—194° (cf. Elliott and Harington, *J.*, 1949, 1374) (Found: C, 54·6; H, 5·8; N, 7·9. Calc. for $C_{16}H_{20}O_7N_2$: C, 54·5; H, 5·7; N, 8·0%).

(*b*) To diethyl acetamido-([α-^{14}C]benzyl)malonate (611 mg.) in acetic anhydride (1·6 ml.) was added fuming nitric acid (0·40 ml.) at 0°, and the product was worked up as in (*a*), to give the ^{14}C-nitro-compound (231 mg.). Pure (unlabelled) nitro-compound (231 mg.) was recrystallised from the mother-liquors, giving a further amount of diluted ^{14}C-compound (227 mg.).

Diethyl Acetamido-p-*amino*([α-^{14}C]*benzyl*)*malonate.*—The ^{14}C-nitro-compound (458 mg.) obtained as in (*b*) above was suspended in ethyl acetate (10 ml.) and reduced in the presence of palladium–barium carbonate, and the product recrystallised from ethyl acetate–light petroleum (yield 368 mg., 87%).

p-*Di*-(2-*chloroethyl*)*amino*-DL-*phenyl*[β-^{14}C]*alanine.*—To the amino-compound (368 mg.) in glacial acetic acid (1 ml.) was added a 20% solution of ethylene oxide in water (3 ml.). After 24 hr., water (10 ml.) was added and the solution neutralised by solid sodium hydrogen carbonate (*ca.* 2 g.). The precipitate was extracted with chloroform (3 × 5 ml.), and the extract filtered through anhydrous sodium sulphate, and finally dried by boiling down to about half its volume. The solution was gently refluxed for 10 min. with thionyl chloride (0·5 ml.). Concentrated hydrochloric acid (5 ml.) was immediately added, and the mixture refluxed for 3 hr. under an air condenser, the chloroform being allowed to evaporate. After evaporation to

dryness *in vacuo*, the residue was dissolved in water (5 ml.) and decolorised with charcoal, and the product precipitated by the addition of sodium acetate solution. The product (315 mg., 89%) was obtained as a pale cream powder, and stored in this condition for recrystallisation before use. The specific activity was 1·28 mc/millimole, the radioactive yield from CO_2 22%, the m. p. 177—178°, and the mixed m. p. 179—180° (Found : Cl, 23·4. Calc. for $C_{13}H_{18}O_2N_2Cl_2$: Cl, 23·3%).

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