242. *Preparation of Optically Active Lysine labelled with* ^{14}C *and* ^{15}N.

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Syntheses of [*carboxy*-^{14}C], [α-^{14}C], and [α-^{15}N]lysine are described. The attempted separation of the [α-^{14}C]-labelled enantiomorphs by addition of optically active carrier lysine was not completely successful owing to extensive coprecipitation, which was demonstrated by an enzymic assay of the products. The L-[α-^{15}N]lysine was prepared in 90% yield from $^{15}NH_3$ by utilizing the inversion of configuration which occurs when the D-bromo-acid is treated with ammonia.

For metabolic studies to be reported elsewhere, we required L-lysine labelled with ^{14}C at the α- and the carboxyl-carbon atom severally and with ^{15}N as one of the two nitrogen atoms. Various syntheses of labelled lysine have been reported in the American literature. Thus, Weissman and Schoenheimer (*J. Biol. Chem.*, 1941, **140**, 779) utilizing the method of Eck and Marvel (*ibid.*, 1934, **106**, 387) obtained this amino-acid labelled with deuterium and ^{15}N in the α-position. DL-Lysine containing ^{15}N in the ε-position was prepared by condensation of labelled potassium phthalimide with ethyl 6-bromo-2-carbethoxy-2-phthalimidohexanoate * (Fink, Enns, Kimball, Silberstein, Bale, Madden, and Whipple, *J. Exp. Med.*, 1944, **80**, 455). Recently two groups of workers utilized the method of Fischer and Weigert (*Ber.*, 1902, **35**, 3772) for the preparation of lysine labelled with ^{14}C in the ε-position (Olynyk, Camp, Griffith, Woislowski, and Helmkamp, *J. Org. Chem.*, 1948, **13**, 465; Borsook, Deasy, Haagen-Smit, Keighley, and Lowy, *J. Biol. Chem.*, 1948, **176**, 1383).

In isotope work it is desirable to introduce the labelled atom at the latest possible stage of the synthesis. None of the methods described above was therefore thought suitable for our purpose and this also applies to various synthesis of lysine recently reported (Warner and Moe, *J. Amer. Chem. Soc.*, 1948, **70**, 3918; Degering and Boatright, *ibid.*, 1950, **72**, 5137; Gagnon and Boivin, *Canad. J. Res.*, 1948, **26**, *B*, 503). In the present work DL-[*carboxy*-^{14}C]lysine was obtained by an adaptation of Adamson's synthesis (*J.*, 1939, 1564). Methyl 2-keto*cyclo*hexane-1-[^{14}C]carboxylate was prepared by carboxylation of the sodium derivative of *cyclo*hexanone with [^{14}C]carbon dioxide, the procedure being a modification of that used by Levine and Hauser (*J. Amer. Chem. Soc.*, 1944, **66**, 1768). It was then treated with hydrazoic acid in chloroform in the presence of dry hydrogen chloride under the conditions used by Adamson (*loc. cit.*) for the corresponding ethyl ester. Hydrolysis with hydrochloric acid gave α-aminopimelic acid from which was obtained, under the usual conditions of the Schmidt reaction, lysine which was isolated as the dipicrate. This was converted into DL-lysine monohydrochloride on which all radioactive assays were carried out. The net yield of [*carboxy*-^{14}C]lysine was 12·2%, based upon the barium [^{14}C]carbonate used. An adaptation of Gaudry's synthesis (*Canad. J. Res.*, 1948, **26**, *B*, 387) to small-scale work for the synthesis of [*carboxy*-^{14}C]lysine has been reported by Borsook, Deasy, Haagen-Smit, Keighley, and Lowy (*J. Biol. Chem.*, 1950, **184**, 529): the yield stated was similar (10—15%), but the method is based on the more expensive sodium [^{14}C]cyanide.

DL-Lysine labelled with ^{14}C at the α-carbon atom was synthesized as follows: 4-Chlorobutan-1-ol, obtained from tetrahydrofuran (Starr and Hixon, *J. Amer. Chem. Soc.*, 1934, **56**, 1595), was converted into the chloro-bromide by an adaptation of the method of Cloke, Anderson, Lachmann, and Smith (*ibid.*, 1931, **53**, 2791). The chloro-bromide in slight excess was then caused to react with potassium phthalimide to give, apart from the desired *n*-chlorobutylphthalimide, some 1 : 4-diphthalimidobutane. The chloro-compound on treatment with sodium iodide in acetone yielded 4-iodobutylphthalimide. The latter was condensed with ethyl phthalimido[α-^{14}C]malonate, prepared from ethyl [α-^{14}C]malonate (*Org. Synth.*, 1927, **7**, 34, 78). The condensation product which was not isolated was heated under reflux with a mixture of hydrochloric and acetic acids. A somewhat similar

* Geneva nomenclature, $CO_2H = 1$.

synthesis of α-labelled DL-lysine using ethyl acetamidocyanoacetate has recently been described by Fields, Walz, and Rothchild (*J. Amer. Chem. Soc.*, 1951, **73**, 1000).

A dilution or carrier technique has been used with apparent success in the " resolution " of labelled cystine and methionine by Wood and Gutmann (*J. Biol. Chem.*, 1949, **179**, 535) and also in that of labelled glutamic acid by Kögl, Halberstadt, and Barendregt (*Rec. Trav. chim.*, 1949, **68**, 387). This method consists in adding an excess of unlabelled optically active amino-acid to the synthetic racemic labelled compound and then recrystallizing the optically active substance or a suitable derivative to constant radioactivity. In principle such a method should be particularly applicable to all cases in which the solubility of the optically active amino-acid or that of its chosen derivative is not markedly greater than that of the corresponding racemic compound. Preliminary experiments had shown that the solubilities of L-lysine picrate and DL-lysine picrate in water at 26° were approximately identical (about 0·65 g. per 100 c.c.). It was thus to be expected that, in absence of co-precipitation, addition of L-lysine in considerable excess to the labelled DL-lysine and crystallization of the picrate from a sufficiently large volume of solvent should yield a preparation of L-lysine substantially free from the labelled D-enantiomorph. An excess of carrier L-lysine (5 mmole) was therefore added to the solution of DL-[α-^{14}C]lysine (1 mmole) obtained from the hydrolysis of the phthalimido-compound. The mixture was then converted into the picrate which was crystallized from a volume of water calculated to be more than sufficient to retain the D-[α-^{14}C]lysine picrate together with a somewhat larger amount of L-lysine picrate of much lower specific radioactivity. D-[α-^{14}C]Lysine picrate was isolated from the mother-liquor in a similar manner after addition of a ten-fold excess of unlabelled D-lysine. The two optically active picrate fractions were recrystallized until the radioactivity remained essentially constant. The mother-liquors of all the above fractions were combined and the lysine picrate converted into the free amino-acid which was racemized by 20% hydrochloric acid at 170°.

This method of " resolution " rests on the assumption that the solubility of DL-lysine picrate in water is not affected by the presence of a large excess of the picrate of the optically active lysine. However, this may not be justified since it has been shown, with the aid of radioactive tracers, that extensive co-precipitation can take place between derivatives of different amino-acids (Keston, Udenfriend, and Cannan, *J. Amer. Chem. Soc.*, 1949, **71**, 249). Such a co-precipitation, if it occurred to a moderate extent, could not be readily demonstrated by measuring optical rotation, *e.g.*, the solution which is obtained on addition of 5 parts of unlabelled L-lysine to one part of labelled DL-lysine contains L- and D-lysine in the proportion of 11 : 1, whilst the total radioactivities of the two fractions are equal. The specific radioactivity of the D-lysine is therefore 11 times greater than that of the L-lysine. Contamination of the L-lysine picrate with 1% of D-lysine picrate, which would be difficult to demonstrate by classical methods or simple radioactivity measurements, would thus mean that about 10% of the radioactivity of the L-lysine fraction is due to D-lysine. It was therefore decided to examine the purity of these fractions by more specific techniques. Gale and Epps (*Biochem. J.*, 1944, **38**, 232) have shown that the purified cell-free preparation of lysine decarboxylase obtained from *Esch. coli* or *Bact. cadaveris* is stereochemically specific for L-lysine, and the reaction goes almost to completion and is not inhibited by the D-isomer. In separate experiments, radioactive L-lysine and radioactive D-lysine obtained from the picrates described above were therefore mixed with a known excess of unlabelled DL-lysine and incubated with a relatively large amount of enzyme; the cadaverine resulting from the L-lysine and also the unchanged D-lysine were isolated as the dibenzamido-derivatives (cf. Neuberger and Sanger, *Biochem. J.*, 1944, **38**, 125), and their radioactivities were determined and compared with that of the original lysine mixture. Results showed that about 10—12% of the radioactivity of the L-lysine fraction was due to D-lysine. This agrees with the observed decrease in the radioactivity of the L-lysine picrate during the first recrystallization. With the D-lysine fraction, for which conditions for " resolution " were somewhat less favourable, contamination (in terms of radioactivity) with the L-enantiomorph was about 20%. It is probable that more extensive recrystallization with large volumes of solvents might have eliminated the contaminating enantiomorphs. Such a procedure was, however, not practicable in view of the large losses of radioactive material

which it entails. It thus appears preferable to use methods of resolution not involving addition of carriers.

The method which was employed to introduce ^{15}N into the α-position of lysine yields without any resolution L-lysine which is almost optically pure. Reaction of an α-bromo-acid with ammonia is generally a nucleophilic bimolecular substitution and thus inversion of configuration occurs (Neuberger, *Adv. Protein Chem.*, 1948, **4**, 297; Brewster, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 178). ε-Benzoyl-D-lysine (Neuberger and Sanger, *loc. cit.*) was therefore converted with nitrosyl bromide into the optically active 6-benzamido-2-bromohexanoic acid, a reaction in which configuration is fully retained. The D-bromo-acid was then treated with 2·4 mols. of ^{15}N-labelled ammonia. The resulting ε-benzoyl-L-[α-^{15}N]lysine was hydrolysed to the free amino-acid which contained about 5% of the D-enantiomorph. Since the excess of $^{15}NH_3$ was fully recovered, the yield in terms of isotope was about 90%. This method of synthesizing amino-acids which contain ^{15}N and are optically almost pure can be extended to most other α-amino-acids.

Experimental

Radioactivity Determinations.—All radioactivity determinations were carried out on samples of " infinite thickness " (Popják, *Biochem. J.*, 1950, **46**, 560). Background of the instrument was 8—10 counts/min., and a sample containing 10^{-3} μc of ^{14}C per mg. of substance gave approx. 1100 counts/min. when counted as described. Sufficient counts to give a standard error of ±1—2% were taken, but slight variations in disc size introduced an additional error of ±3%.

^{15}N *Determinations.*—These were done with the mass spectrometer after combustion of the samples by the Kjeldahl procedure.

Preparation of Methyl 2-Ketocyclohexane-1-[^{14}C]*carboxylate.*—In a 100-c.c. three-necked round-bottomed flask with ground-glass joints equipped with a mercury-sealed stirrer, separating funnel, and reflux condenser was placed commercial anhydrous liquid ammonia (50 c.c.). To the stirred solution were added the minimum amount of sodium necessary to produce a blue colour, and a few small crystals of ferric nitrate, followed by 1·4 g. of sodium. When the sodium was all converted into sodamide, *cyclo*hexanone (5·0 g.) in absolute ether (30 c.c.) was added during 5 minutes. Ammonia was removed as rapidly as possible by warming and ether added to keep the volume to about 60 c.c. After the ether had been refluxing for a few minutes, the bath was removed, and the mercury-sealed stirrer replaced as rapidly as possible by a magnetic-induction stirrer. The reflux condenser and separating funnel were then removed and the flask was fitted on to a manifold of a high-vacuum system. After cooling of the ethereal suspension in liquid air, the vacuum system was evacuated to 0·001 mm., and then isolated from the vacuum pump.

The ethereal suspension was then allowed to warm to −15°, and carboxylation was carried out with stirring (Calvin, Heidelberger, Tolbert, Reid, and Yankwich, " Isotopic Carbon," Wiley, New York, 1949, p. 177) and use of [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (11·0 g., 7·21 mc.). After 1 hour, excess of [^{14}C]carbon dioxide was removed and trapped in barium hydroxide solution.

After the reaction mixture had come to room temperature, the minimum amount of water was added to enable a satisfactory separation from the ethereal layer to be carried out. The aqueous phase was cooled to 0° in a three-necked flask fitted with a dropping funnel and gas inlet and outlet tubes. 50% Excess of ice-cold 20% sulphuric acid was added drop by drop, while a slow stream of nitrogen was passed through the flask. The [^{14}C]carbon dioxide evolved was trapped in a chain of absorbers containing N-sodium hydroxide and saturated aqueous barium hydroxide.

The liberated β-keto-acid was extracted into ether, and converted into methyl 2-keto*cyclo*hexane-1-[^{14}C]carboxylate as described by Levine and Hauser (*loc. cit.*). Distillation *in vacuo* yielded 3·0 g. of pure ester. 4·52 G. of barium [^{14}C]carbonate was recovered, containing a total activity of 1·80 mc.

DL-[α-carboxy-^{14}C]*Lysine.*—The conversion of the methyl 2-keto*cyclo*hexane-1-[^{14}C]carboxylate into DL-lysine dipicrate followed the experimental procedure outlined by Adamson (*loc. cit.*) for the corresponding ethyl ester. The crude dipicrate was converted into DL-lysine monohydrochloride which, after purification, had a total activity of 0·66 mc (12·2%) in 1·1 g. of the dihydrated salt.

4-*Chlorobutylphthalimide.*—4-Chloro-*n*-butyl bromide (75 g.) and potassium phthalimide, ground together, were heated under reflux for 6 hours and then cooled. Water (250 c.c.) was

added and the mixture shaken until the solid was granular. The precipitate was filtered off, washed with water, and dried. The material was then dissolved in hot ethanol (500 c.c.) and filtered hot. On concentration to 250 c.c. a further precipitate was obtained. The *monophthalimide* crystallized on further concentration and cooling to 0°. It was recrystallized from a small amount of ethanol and had m. p. 76·5° (yield, 50% in terms of potassium phthalimide) (Found: C, 59·7; H, 5·1; N, 5·9; Cl, 14·9. $C_{12}H_{12}O_2NCl$ requires C, 60·6; H, 5·1; N, 5·9; Cl, 14·9%). The materials which were relatively insoluble in warm ethanol were combined, washed again with ethanol, and, after drying, recrystallized from glacial acetic acid. 1 : 4-Diphthalimidobutane, recrystallized again from a large amount of ethanol, had m. p. 224° (Found: C, 69·1; H, 4·5; N, 7·9. Calc. for $C_{20}H_{16}O_4N_2$: C, 69·4; H, 4·6; N, 8·0%). Langenbeck, Woltersdorf, and Blachnitzki (*Ber.*, 1939, **72**, 671) give m. p. 219°.

4-*Iodobutylphthalimide*.—A solution of the above chloro-compound (35·5 g.) in acetone (400 c.c.) containing sodium iodide (37 g.) was boiled for 6 hours. Removal of sodium chloride and evaporation to dryness gave the crude iodo-compound which was recrystallized from ethanol and then had m. p. 88—89°. Gabriel (*Ber.*, 1909, **42**, 1249) gives m. p. 88—89·5°.

Ethyl Phthalimido[α-^{14}C]*malonate*.—To a solution of ethyl [α-^{14}C]malonate (0·206 g.; 1·44 mc; obtained from the Radiochemical Centre, Amersham) in dry, freshly distilled carbon tetrachloride (20 ml.) was added a small excess of bromine (0·212 g). in carbon tetrachloride (3·6 c.c.). After 1 hour, when the bromination appeared to be complete, carrier bromomalonate (1·31 g.) was added. The solution was kept at room temperature for 16 hours. The solvent was then removed in a stream of dry air at room temperature. The residue was dissolved in dry toluene (20 ml.) and heated with potassium phthalimide (1·146 g., 10% excess) in a sealed tube at 120—130° for 2 hours and then at 140° for 2 hours. The solution was filtered and the residue extracted with benzene. The solid (1·1 g., 7·0 μc) was discarded and the filtrate evaporated to dryness in a stream of dry air. After addition of carrier ethyl phthalimidomalonate (1·06 g.) the product was recrystallized from ether–light petroleum (b. p. 60—80°), yielding ethyl phthalimido-[α-^{14}C]malonate (2·35 g.).

DL-[α-^{14}C]*Lysine*.—Ethyl phthalimido[^{14}C]malonate (2·087 g., 6·85 mmoles) in dry ethanol (15 ml.) was cooled quickly by immersion in liquid air and simultaneously a solution of sodium (0·186 g.) in dry ethanol was added. The solvent was removed *in vacuo*, giving ethyl sodiophthalimido[α-^{14}C]malonate. 4-Iodobutylphthalimide (2·57 g.) was added and the mixture heated at 150—155° for 3 hours. After addition of dry toluene (5 c.c.) heating was continued for further 20 minutes. The toluene was then removed and the residue refluxed for 20 hours with a mixture of concentrated hydrochloric acid (30 c.c.) and acetic acid (15 c.c.) and then evaporated to dryness *in vacuo*; water (40 c.c.) was added and the mixture continuously extracted with ether for 48 hours. The ethereal extract, which was only feebly radioactive, was discarded. The aqueous layer was concentrated to dryness *in vacuo*, giving crude DL-[α-^{14}C]lysine dihydrochloride. The product was dissolved in water, and the solution filtered and used directly for the following operations.

Isolation of L-[α-^{14}C]*Lysine Picrate*.—The L-lysine which was used as carrier had been obtained by hydrolysis of casein with hydrochloric acid. It had $[\alpha]_D^{22}$ +24·9° (*c*, 5·0 in 5N-hydrochloric acid). The recorded values vary somewhat; thus Dunn and Rockland (*Adv. Protein Chem.*, 1947, **3**, 354) quote the unpublished value of $[\alpha]_D^{25}$ +25·72° (*c*, 1·64 in 6·08N-hydrochloric acid), but Greenstein, Gilbert, and Fodor (*J. Biol. Chem.*, 1950, **182**, 451) give $[\alpha]_D$ +23·0°. All values for specific rotation are stated in terms of the free amino-acid. It is unlikely that our L-lysine carrier contained more than 2—3% of the D-enantiomorph. The carrier L-lysine dihydrochloride (7·74 g., 35 mmoles) was added to the above solution of DL-[α-^{14}C]lysine. After adjustment of the pH to 6·0 with 1·94N-sodium hydroxide (27·5 c.c.), picric acid (9·58 g., 41·85 mmoles) and 1·94N-sodium hydroxide (21·6 c.c., 41·85 mmoles) were added. Water was added to a final volume of 400 c.c. and the mixture heated until clear. After 24 hours at 0° the L-lysine picrate (10 g., 127 μc) was filtered off. Recrystallization from water (200 c.c.) afforded 9·0 g. of material having a specific radioactivity of 9·76 μc/g. After two further recrystallizations an apparently constant value was obtained; thus with successive recrystallizations of a small portion the specific radioactivities were 9·33, 9·00, 8·97 μc/g. respectively.

Isolation of D-[α-^{14}C]*Lysine Picrate*.—To the mother-liquors from the above isolation of L-[α-^{14}C]lysine picrate, D-lysine monohydrochloride {6·4 g., 35 mmoles; $[\alpha]_D^{22}$ −24·1° (*c*, 2·5 in 6N-hydrochloric acid)}, 1·94N-sodium hydroxide (18·0 c.c., 35 mmoles), and picric acid (8·02 g., 35 mmoles) were added; the picrate crystallized from the solution (800 c.c.). There were obtained 9·8 g. of material with specific radioactivity of 9·27 μc/g. Recrystallization of a portion

(0·709 g.) from water (25 c.c.) afforded a sample with essentially unchanged radioactivity (9·33 μc/g.).

Isolation of D- *and* L-[α-^{14}C]*Lysine from the Picrates.*—To a solution of L-[α-^{14}C]lysine picrate (9·0 g.) in hot water (100 c.c.) was added concentrated hydrochloric acid (5 c.c.), and the solution continuously extracted with hot benzene until the aqueous layer was no longer yellow. The aqueous solution was then evaporated to dryness *in vacuo* and the residue crystallized from aqueous ethanol by addition of pyridine. Recrystallization from aqueous ethanol afforded L-[α-^{14}C]lysine monohydrochloride monohydrate (3·531 g.; specific radioactivity 15·3 μc/g.). From the mother-liquors a further crop (0·55 g.) was obtained. The total yield of L-[α-^{14}C]lysine was 62·5 μc. D-[α-^{14}C]Lysine picrate was similarly converted into the monohydrochloride monohydrate (4·348 g., specific radioactivity 16·5 μc/g.). The mother-liquor yielded a further 0·35 g., the total yield being 77·6 μc.

Isolation of DL-[α-^{14}C]*Lysine.*—The picrate from the combined mother-liquors was converted into lysine and racemization completed by heating this in 20% hydrochloric acid (60 c.c.) at 170° for 18 hours. The DL-lysine was precipitated as phosphotungstate which was converted into the monohydrochloride monohydrate (3·2 g.). Purification *via* the benzylidene copper complex (Turba, *Z. physiol. Chem.*, 1948, **283**, 19; 3·3 g.; 57 μc) yielded DL-[α-^{14}C]lysine dihydrochloride (2·56 g.; 52·5 μc). The specific radioactivities of the three fractions of radioactive lysine are given in the following Table which also shows the calculated radioactivities based on those of the barium carbonate obtained by wet combustion (Van Slyke and Folch, *J. Biol. Chem.*, 1940, **136**, 509) of the lysine hydrochlorides.

Specific radioactivities of [α-^{14}C]*lysine fractions.*

Compound	Radioactivity (μc/mmole)	Compound	Radioactivity (μc/mmole)
L-Lysine monohydrochloride monohydrate :		DL-Lysine dihydrochloride :	
(*a*) directly	3·1	(*a*) directly	4·5
(*b*) calc. from $BaCO_3$	3·1	(*b*) calc. from $BaCO_3$	4·4
(*c*) from picrate	3·2	(*c*) from benzylidene Cu complex	4·6
D-Lysine monohydrochloride monohydrate :		(*d*) from recryst. picrate	4·8
(*a*) directly	3·3		
(*b*) calc. from $BaCO_3$	3·4		
(*c*) from picrate	3·3		

Although the relatively high specific radioactivity of the racemic lysine compared with the two optically active fractions is not easy to explain, this discrepancy is probably not due to an impurity, as the radioactivity measurements of the various derivatives are in good agreement. The total radioactivity in all three fractions was 192 μc, corresponding to a yield of 18·9% based on ethyl[α-^{14}C]malonate.

Enzyme Experiments.—The crude decarboxylase was obtained as described by Gale and Epps (*loc. cit.*). The organism *Bact. cadaveris* (National Collection of Type Cultures, no. 6578) was grown by Dr. M. R. Pollock, to whom we express our gratitude. The cells, after incubation, were thoroughly washed and the suspension was poured into acetone (5 vols.) cooled to $-10°$. On repeated washing with cold acetone, centrifugation, and drying, a powder was obtained which gave Q_{CO_2} values for L-lysine at 30° and pH 6 of approx. 250. In several experiments the enzyme was further purified as described by Gale and Epps.

A warm solution of L-[α-^{14}C]lysine picrate (78·9 mg.; specific radioactivity, $9·4 \times 10^{-3}$ μc/mg.) and DL-lysine hydrochloride monohydrate (0·5005 g.) in concentrated hydrochloric acid (10 c.c.) was extracted continuously with warm benzene until colourless. It was then concentrated to dryness, and the residue taken up in water and again concentrated to dryness *in vacuo*. The dihydrochloride was dissolved in water, and the solution brought to pH 6·0 by addition of N-sodium hydroxide and made up to 35 ml. Enzymic decarboxylation was carried out by incubation at 30° for 2 hours of a mixture containing 15 ml. of phosphate buffer (pH 6·0), 15 ml. of the lysine solution, and usually about 7·0 ml. of the enzyme solution. The amount of enzyme chosen was three times that required to decarboxylate the L-lysine present in 20 minutes under the conditions used. The activity of the enzyme preparation was estimated manometrically immediately before the experiment. At the end of the incubation the protein was coagulated by heat; the solution was filtered and made alkaline. Benzoylation (benzoyl chloride, 0·72 g.; N-sodium hydroxide, 6·5 c.c.) gave dibenzoylcadaverine which, recrystallized several times from small volumes of ethanol, had m. p. 134—135° (von Braun, *Ber.*, 1904, **37**, 3583, gives m. p.

135°). The dibenzoyl-D-lysine could not be obtained crystalline in the experiment. The specific radioactivity of the dibenzoylcadaverine was 800 × 10^{-6} μc/mg.; the value calculated from that of the picrate and the amount of non-radioactive DL-lysine added on the assumption that all radioactive lysine in the mixture had the L-configuration was 920 × 10^{-6} μc/mg. The difference between the observed and the calculated radioactivity indicates that about 15% of the radioactivity in the original lysine used was due to the D-enantiomorph. Another enzyme experiment gave a similar value of 12·8%.

The optical purity of the D-[α-^{14}C]lysine picrate was also investigated by the decarboxylase method. In this experiment the dibenzoyl-D-lysine was obtained crystalline by acidification of the mother-liquor left from the crystallization of the dibenzoylcadaverine; recrystallized several times from acetone it had m. p. 145—146° (Neuberger and Sanger, *loc. cit.*, give m. p. 145°). The specific radioactivity of the dibenzoylcadaverine was 310 × 10^{-6} μc/mg. whilst that of the dibenzoyl-D-lysine was 1220 × 10^{-6} μc/mg. This and a parallel experiment indicated that the D-lysine preparation contained 19—23% of the L-enantiomorph in terms of radioactivity.

ε-Benzoyl-L-[α-^{15}N]*lysine*.—D-6-Benzamido-2-bromohexanoic acid (7·3 g.), from D-lysine (Neuberger and Sanger, *loc. cit.*), was added to a solution of [^{15}N]ammonia (62·5% isotope excess) containing 0·95 g. (2·4 equivs.) of $^{15}NH_3$. The container was tightly corked and kept for 4 days at 40°. The solution was then acidified to pH 6·0 with hydrochloric acid, and the solid (4·24 g.) collected and dried (73%) The material was recrystallized by dissolution in acid and addition of sodium acetate. The excess of [^{15}N]ammonia was recovered by addition of alkali to the original mother-liquor and distillation into dilute sulphuric acid.

L-[α-^{15}N]*Lysine Dihydrochloride*.—The ε-benzoyl compound (3·0 g.) was heated under reflux with concentrated hydrochloric acid for 20 hours. Excess of acid was removed by two evaporations to dryness. The residue was dissolved in water and brought to pH 5. Sodium hydroxide (1 equiv.) and picric acid (3·0 g.) were then added. The mixture was heated until clear. The crystalline picrate obtained on cooling to 0° was recrystallized from water and decomposed as described above; the lysine crystallized first as monohydrochloride monohydrate and then as dihydrochloride, $[\alpha]_D^{20}$ (in terms of lysine) +22·4° (*c*, 4·00 in 5% hydrochloric acid). Isotope analysis showed 31·1% excess ^{15}N.

We thank Dr. R. Bentley and Mr. G. Dickinson for the ^{15}N analyses.

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[*Received, November 22nd*, 1951.]