The synthesis of N-ε-2,4-Dinitrophenyl-3,5,6-^{3}H-L-Lysine

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Received July 29, 1970.

SUMMARY

The N-ε-2,4-dinitrophenyl-3,5,6-^{3}H-L-lysine has been prepared from the 1-fluoro-2,4-dinitrobenzene-3,5,6-^{3}H and from the N-α-carbobenzoxy-L-lysine. The specific activity obtained was 9,9 Ci/mmole and the radiochemical yield was of 34 %. The use of this product in biological experiments is discussed.

1. INTRODUCTION.

In order to investigate the cellular (lymphocyte) involvement in hypersensitive individuals, we have endeavoured to prepare a hapten labelled with a radioactive isotope of high specific activity. The dinitrophenyl group is a powerful immunogen. On the other hand, amino hydrogens easily lend themselves to substitution reactions [1]. Since liquid scintillation counting and autoradiography are extremely sensitive methods of, respectively, quantitation and localisation of cell-bound substances, we have prepared a radiolabelled ε-dinitrophenyl-L-lysine. Assuming that the cellular binding involves the intact molecule, the position of the label is mainly a matter of availability of starting products. The choice of tritium as a labelling isotope was governed by the very high specific activities attainable : 3 atoms of tritium per molecule would afford a theoretical specific activity of some 90 curies per millimole [2]. Such a product cannot be stored for any length of time, owing to very rapid autoradiolysis. However, lesser activities may be used and are still higher by at least one order of magnitude than are those of the corresponding ^{14}C compounds or tritiated compounds prepared by exchange [3].

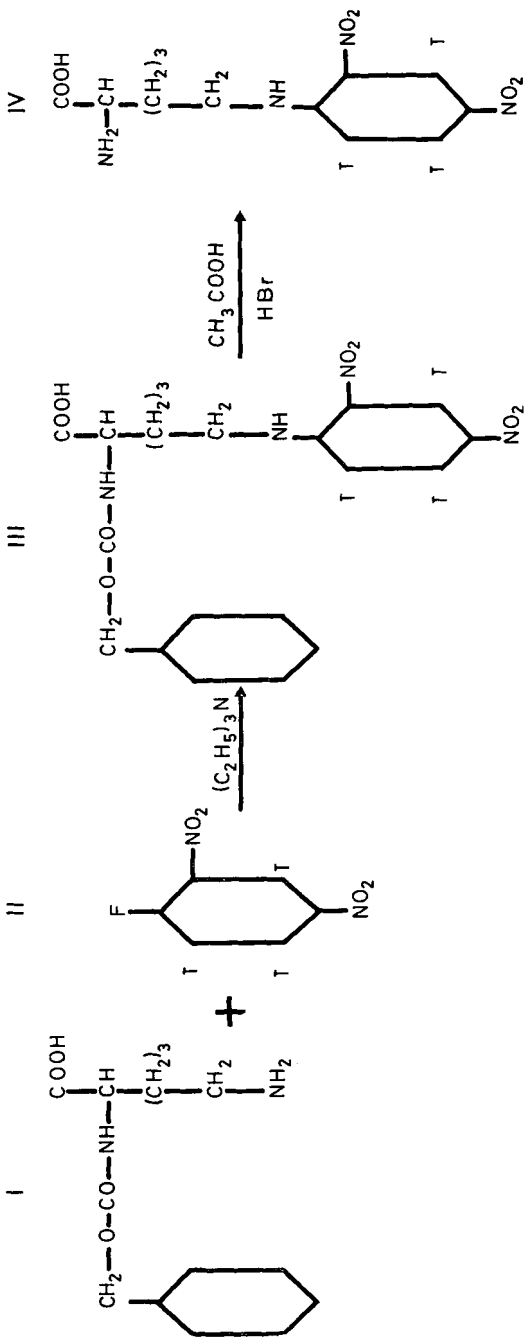

FIG. 1.

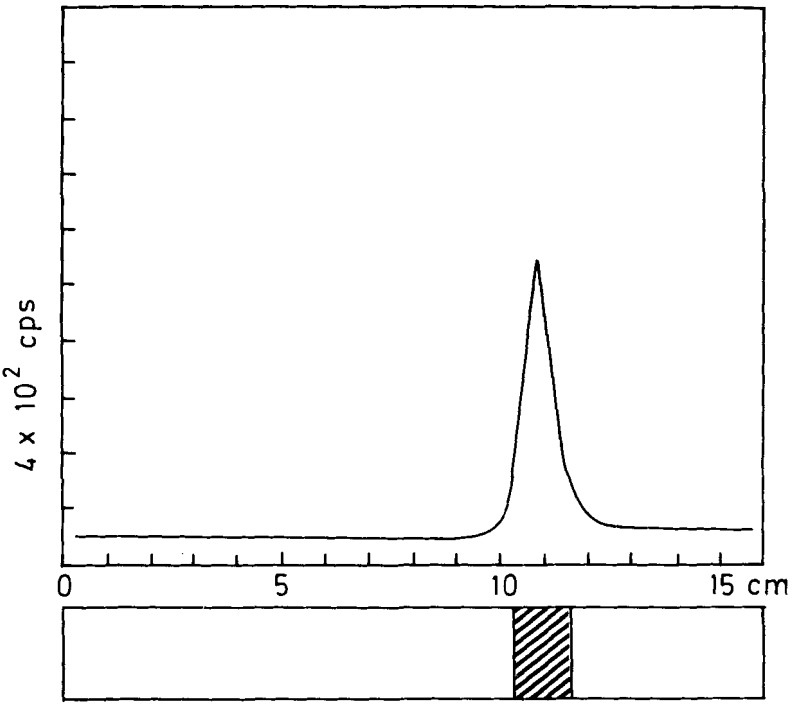

FIG. 2. Scanning of thin-layer chromatography. — Cellulose.
Solvent : propanol (1) : 70.
Aqueous ammonia 25 % : 30.
10 μC — ε-DNP-lysine-^{3}H.
10 γ — Carrier ε-DNP-lysine.

2. CHEMICAL SYNTHESIS.

As a starting product we used the *N*-α-carbobenzoxy-L-lysine * (I) [4]. The substitution of the ε-*N*-amino group with the radioactive DNP was performed with the 1-fluoro-2,4-dinitrobenzene-3,5,6-^{3}H (II) **. The specific activity of this product was 9,9 curies/millimole. No preliminary check on radiochemical purity was performed and the product was used immediately after delivery.

To 10 micromoles of I in 1 ml of *N*,*N*'-dimethylformamide (DMF) were added 5 mc i.e. 0.5 micromoles of II in 5 ml of benzene (Fig. 1) and an equimolar amount of triethylamine. The mixture was kept in the dark at room

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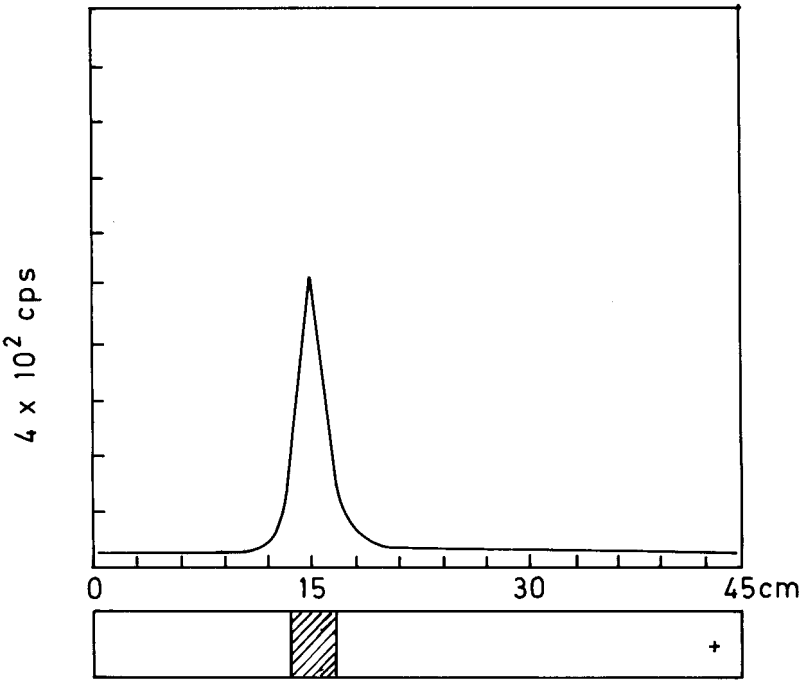

FIG. 3. Scanning of high voltage paper electrophoresis.
pH 1.9; 6,000 V, 30′.
Paper Whatman 3 MM.
10 μC — ε-DNP-lysine-^{3}H.
10 γ — Carrier-ε-DNP-lysine.

temperature for 2.5 hrs. The benzene was evaporated under vacuum in a rotatory evaporator and the residue was kept for 16 hrs under high vacuum in a dessicator. In all instances a trap filled with KOH pellets was connected between the evaporation device and the pump. The dry residue was taken up in 1 ml of glacial acetic acid, saturated with dry gaseous HBr, left at room temperature for 30 min and at 60° C for 15 min. This reaction splits of the carbobenzoxy group from I and from the *N*-α-carbobenzoxy-ε-*N*-dinitrophenyl-L-lysine (III) [5]. The product was dried under vacuum, taken up in 0.2 ml of 0.01 N HCl and extracted twice with 5 ml of ethyl ether. The ether was discarded and the aqueous solution was deposited on a 20 × 20 × 0.030 cm preparative thin layer plate. The cellulose layer had previously been washed with methanol. After a 4-5 hrs run of the solvent (*n*-propanol/25 % ammonia, 70/30) [6], the plate was dried and the yellow spot of the ε-*N*-DNP-lysine IV was eluted with methanol.

The purity of the product was confirmed by thin layer chromatography (Fig. 2) and high voltage paper electrophoresis (Fig. 3) of an aliquot in the presence of non-radioactive authentic carrier, followed by radioactivity

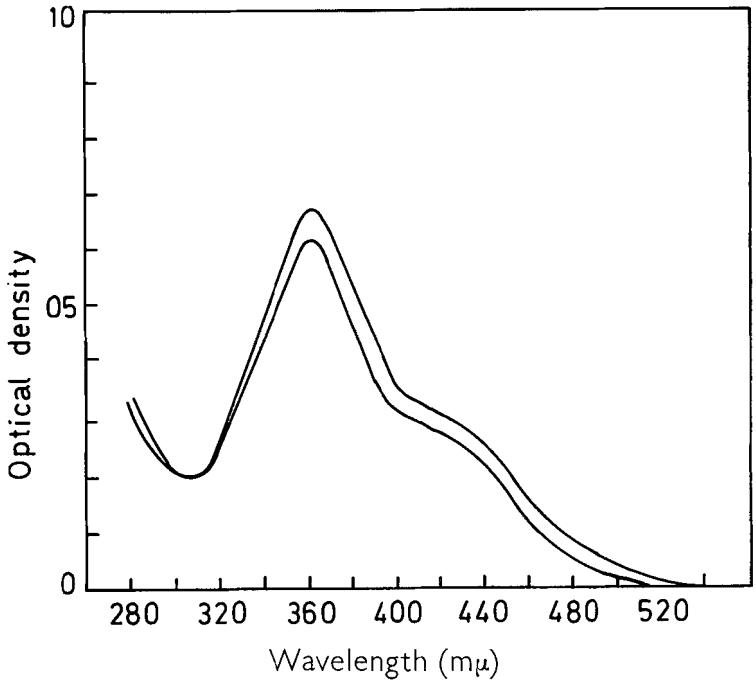

FIG. 4. Spectra of ε-DNP-lysine.
45×10^{-6} M/L in 0.1 molar $KHCO_3$.

scanning [7]. The ultraviolet spectrum of the product was identical with that of the authentic substance (Fig. 4). The yield was 34 % i.e. 0.15 micromole at 9.9 curies/millimole.

3. DISCUSSION.

The radiosynthesis reported above affords easily the adequate amount of highly active material. The same method can be applied to synthetic substrates of higher molecular weight, such as poly-L-lysine and copolymers of L-lysine and another amino-acid. Mono-α-*N*-DNP-derivatives may be obtained from ε-*N*-carbobenzoxy-compounds. Poly-ε-*N*-DNP substitution is achieved when the substrate has a protected α-amino group.

By adding a coupling reagent, the dicyclohexylcarbodiimide to the benzene solution of the tritiated ε-*N*-DNP-L-lysine, we have obtained a high molecular weight, water insoluble polymer. This product is almost certainly polydisperse and has not been investigated further.

At this specific activity the limits of detection are of the order of 2×10^{-4} μc for liquid scintillation counting and of 10^{-7} μC/cell for autoradiography [8].

This is equivalent, respectively, to some 12×10^9 molecules and 6×10^6 molecules of the ε-*N*-DNP-L-lysine. If it would be possible to achieve an adequate separation and concentration of DNP-sensitive cells, the liquid scintillation counting could easily measure the amount of the isotope in the cell population. The autoradiography however, although apparently much more sensitive would require at least some 10^7 binding sites per cell.

Higher specific activities would improve this figure, but only by the factor of 10 at 90 curies/millimole.

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