SYNTHESIS OF 3-[^{3}H]-5-(4'-AZOBENZENE ARSONIC ACID)-L-TYROSINE

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SUMMARY

The title compound (ABA-Tyr) was prepared by direct radiochemical synthesis from tritiated tert.-butoxycarbonyl-L-tyrosine and diazotised arsanilic acid followed by acidolytic deprotection and isolation by preparative high pressure liquid chromatography. The product had a specific activity of 9 Ci mmol^{-1} and a radiochemical purity of 90-95%.

Key words: Azobenzene arsonic acid tyrosine, ABA-Tyr, Catalytic tritiation.

INTRODUCTION AND DISCUSSION

(Azobenzene arsonic acid)-L-tyrosine (ABA-Tyr) is of fundamental interest in immunology as a low molecular weight antigen which induces specific delayed hypersensitivity in guinea pigs (1) and as a true immunogen which can serve as a carrier for a macromolecular hapten (2).

Synthesis of [^{14}C]-ABA-Tyr has been described in outline only (3) from diazotised arsanilic acid and tert.-butoxycarbonyl-L-[U-^{14}C]tyrosine (Boc-[^{14}C]-Tyr). We chose to investigate this reaction, which has been documented for non-radioactive materials (4), but using Boc-[^{3}H]-Tyr prepared from Boc-diiodo-L-tyrosine (5) by catalytic reduction using tritium gas. The

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diazonium salt was prepared as previously described (4), the only difference being that the reaction was carried out in carbonate-bicarbonate buffer pH 9.5, at which pH reaction is efficient (6). Carbonate-bicarbonate buffer was preferred to alternatives because less interference was expected from the salt generated by deprotection (sodium trifluoroacetate) in the subsequent handling. The crude product, which is slightly soluble in water at neutral pH, was applied to high pressure liquid chromatography (h.p.l.c.) columns after dissolution in aqueous sodium bicarbonate.

In model cold experiments which were assessed by analytical h.p.l.c. and which we do not detail here, reaction was seen to be practically instantaneous, as had been anticipated. Coupling times of 4-5 hours have been recommended (4,6). Analysis by h.p.l.c. indicated reaction was quantitative under the conditions we report. The conditions recommended provide only a convenient procedure suitable for radiochemical use and should not be regarded in any way as necessary for complete reaction to be achieved.

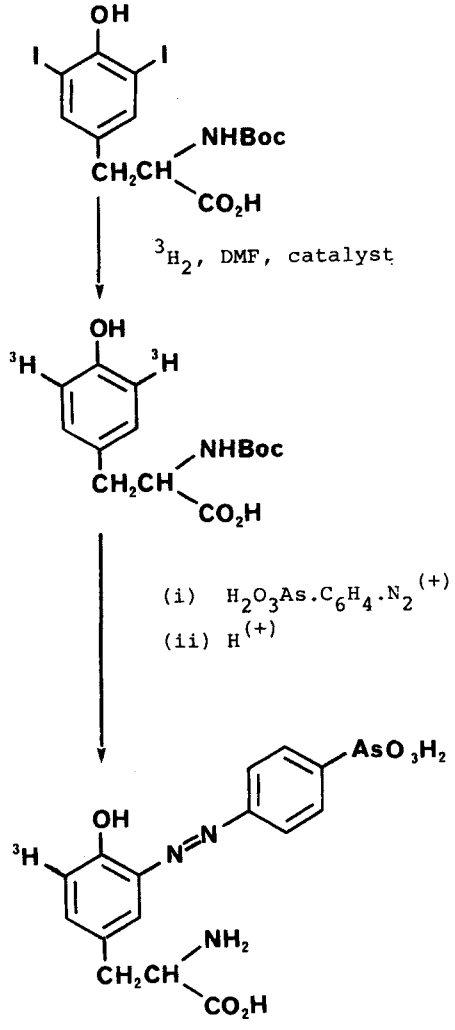
No attempt was made to optimise the specific activity of the product by varying catalyst or solvent in the tritiation step.

EXPERIMENTAL

A solution of diazotised arsanilic acid was prepared (4) from arsanilic acid (221 mg, 1 mmol) and stored in a total volume of 16.7 ml at $0^{\circ}C$ for use.

Concurrently with this preparation, Boc-Tyr(I_2) (16.3 mg, 30 µmol) (5) dissolved in N,N-dimethylformamide (0.5 ml) was hydrogenated for 100 min at room temperature using 8 Ci of $^3H_2$ gas in the presence of 10% Pd/C (20 mg) and 5% $Rh/CaCO_3$ (15 mg). The catalysts were removed by filtration through a pad of cellulose powder, the filtrate was evaporated and the residue was redissolved in ice-cold $Na_2CO_3/NaHCO_3$ buffer (0.4M, pH 9.5, 0.5 ml). To this solution were added with stirring, at intervals of 1 min, portions of the diazotised arsanilic acid solution (5 x 100 µl, total 30 µmoles) and NaOH (1.5M) solution (5 x 10 µl).

After a further 3 min stirring, the mixture was evaporated to dryness and the residue was kept for 30 min at room temperature with 90% trifluoroacetic acid (TFA:H_2O = 9:1, 5 ml). The mixture was evaporated to dryness and portions (5 ml) of methanol were evaporated from the residue until a solid was obtained. This was dissolved in water (12 ml) by the addition of saturated aqueous $NaHCO_3$ (0.5 ml). The mixture was passed through a Millex filter (0.5 µm) to remove insoluble material and a portion (2 ml) of the filtrate was applied, using a Rheodyne loop, to a column (50 x 0.7 cm) of Nucleosil $10C_{18}$ ODS-silica



SCHEME: Synthesis of [^{3}H]-ABA-Tyr

which was eluted at a flow rate of 4.6 ml min^{-1} with acetonitrile: water:acetic acid (125:875:1, by vol.). The column effluent was monitored at 280 nm (Cecil CE212) and fractions (30 sec) were collected automatically. The contents of fractions 15-23 were combined, evaporated to dryness and the residue was dissolved in water (1.0 ml). A portion (100 µl) of the solution was diluted to 5 ml (0.1M-NaOH) for quantitation at 325 nm. For estimation of tritium, a further portion (10 µl) of the concentrated solution was diluted to 25 ml (H_2O) and portions (10 µl) were counted in BBOT scintillator using [^{3}H] hexadecane as internal standard. The specific activity of the product was 9.3 $\pm$ 0.6 Ci $mmol^{-1}$ and the recovery corresponded to an overall yield of 15%.

The radiochemical purity of the product was estimated using a Panax E.0111/XPD-05 radiochromatogram scanner system after chromatography on thin layers of silica gel 60 using the solvent systems n-butanol:acetic acid:water (10:1:3, by vol.) and ethyl acetate:pyridine:acetic acid:water (5:5:1:3, by vol.). The purity was estimated as 93.7 $\pm$ 2.0 and 95.2 $\pm$ 0.1% respectively. Analytical h.p.l.c. using the same conditions of solvent and support as for the preparative h.p.l.c., followed by counting of the column effluent, indicated a radiochemical purity of 90.6 $\pm$ 3.6%. The product had identical chromatographic behaviour to unlabelled reference material in all the systems investigated.

The product was stored in water at a concentration of 6.7 mCi ml^{-1} at the temperature of liquid nitrogen (-196°C).

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