THE SYNTHESIS OF 5-HYDROXYINDOLE-3-(-2-D_2-ACETIC ACID) AND $\alpha,\alpha',\beta,\beta'$-$D_4$-5-HYDROXYTRYPTAMINE

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SUMMARY

Deuterium labelled 5-hydroxyindole-3-acetic acid and 5-hydroxy-tryptamine have been sythesized by a modification of the procedure previously described by Ek and Witkop (9) for the non-deuterated analogues. The deuterium is introduced by a solvent exchange reaction and by reduction with lithium aluminium deuteride. The isotopic yield was > 99%.

INTRODUCTION

5-Hydroxytryptamine (5-HT) occurs as a transmitter substance in neurons of the central nervous system of mammals (1, 2). The level of its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA) has been shown to reflect functional activity of such 5-HT neurons (3). Predominantly fluorimetric methods have been used previously for the estimation of these compounds in brain tissue (4, 5). The development of mass fragmentographic methods with very high specificity and sensitivity for the estimation of 5-hydroxyindoles in body fluids (6, 7, 8) necessitated the present synthesis of deuterium labelled 5-HIAA and 5-HT for use as internal standards.

DISCUSSION

When the solvents in the second step of the synthesis described by Ek and Witkop (9) are exchanged with their deuterated

analogues, dideuterated 5-benzyloxyindole-3-acetic acid and 5-benzyloxyindole-3-acetamide are formed (Fig. 1). The refluxing time used by Ek and Witkop (88 h) was prolonged to 170 h which increased the isotopic yield from 95% to > 99% (Fig. 2) and also increased the amount of acid formed. Recrystallization of 5-BIAA-d_2 (III) from H_2O did not affect the isotopic yield. The isotopic yield (> 99%) is substantially higher compared to a previous synthesis of dideutero-5-HIAA (90%) (6).

EXPERIMENTAL

5-Benzyloxygramine (II)

The compound was prepared as described by Ek and Witkop (9) from 5-benzyloxyindole (Fluka).

5-Benzyloxyindole-3-(2-d_2-acetamide) (V)

A solution of 2.60 g of 5-benzyloxygramine (II) and 2.32 g of sodium-cyanide in 30 ml of monodeuteroethanol (isotopic yield > 99%) and 7.50 ml deuterium-oxide (isotopic yield > 99.7%) was refluxed for 170 hours. The solution was diluted with water (200 ml). The precipitate was filtered off and washed with water. Drying gave 1.22 g of a colourless powder. Recrystallization from benzene-methanol gave (V) with mp. 156-157°, reported 158° (11).

α,α',β,β'-d_4-5-Benzyloxytryptamine hydrochloride (VI)

The crude amide (V) (0.31 g) was refluxed in ethyl ether (100 ml) for 48 hrs with lithium aluminium deuteride (0.6 g) (isotopic yield > 99%). The amine was isolated as the hydrochloride (VI) (0.32 g) mp. 242-247, reported 265° (12).

Fig 1

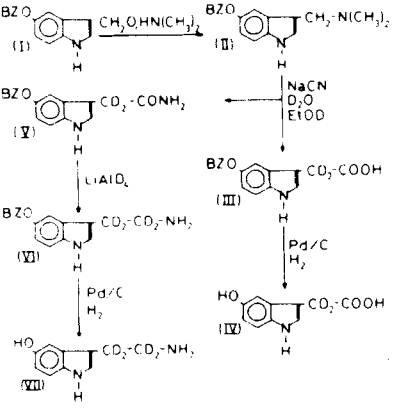


Fig 2a

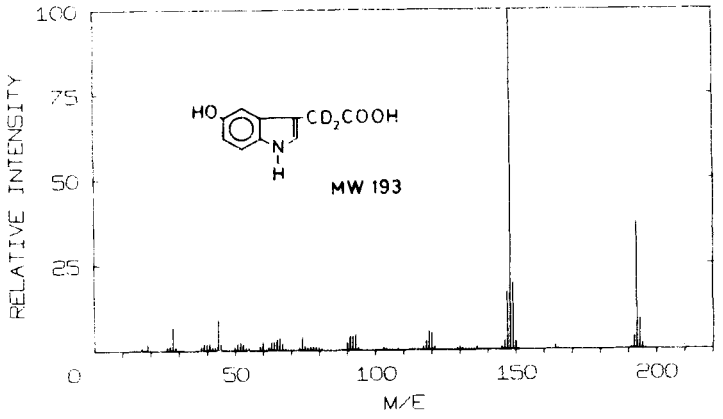
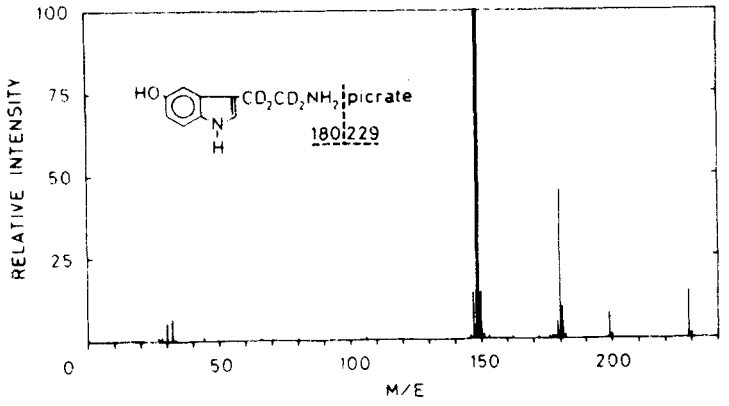


Fig 2b



α,α',β,β'-d_4-5-hydroxytryptamine picrate (VII)

The hydrochloride (VI) (100 mg) was dissolved in ethanol (50 ml) and hydrogenated with 0.1 g 5% Pd/C for 3 hrs at atmospheric pressure. The amine was isolated as the picrate (VII), it melted at 107-112°, resolidified at 130-140°, and finally melted at 181-185° (9, 10, 11).

5-Benzyloxyindole-3-(2-d_2-acetic acid) (III)

The filtrate from the synthesis of the amide (V) was distilled in vacuo to remove the ethanol and acidified with concentrated hydrochloric acid. The crystals were filtered off. Drying gave 1.13 g of a colourless powder. Recrystallization from water, using charcoal, gave (III) with mp. 143-145°, reported 149.0-150.5° (9).

The NMR spectrum showed no peak at δ 3.7 (α-acid position).

5-Hydroxyindole-3-(2-d_2-acetic acid) (IV)

The benzyloxyacid (III) (70 mg) was dissolved in ethylacetate (10 ml) and hydrogenated with 65-75 mg of 5% Pd/C for 1 hr at atmospheric pressure. The catalyst was filtered off and the ethylacetate was evaporated in vacuo. Mp. 158-160°, reported 166° (9).

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