PREPARATION OF DEUTERIUM LABELLED CATECHOLAMINES, CATECHOLAMINE PRECURSORS AND METABOLITES FOR USE AS INTERNAL STANDARDS IN MASS FRAGMENTOGRAPHIC DETERMINATION AND FOR TURNOVER STUDIES.

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SUMMARY

L-dihydroxyphenylalanine-$^2H_3$, L-tyrosine-$^2H_2$, dopamine-$^2H_3$, 3-methoxytyramine-$^2H_3$, homovanillic acid-$^2H_2$, homovanillic acid-$^2H_5$ and vanillylmandelic acid $^2H_2$ were prepared by hydrogen-deuterium exchange under acidic or basic conditions. 4-hydroxy-3-methoxy phenylethyleneglycol-$^2H_3$ was prepared from vanillylmandelic acid-^2H by reduction with $LiAl^2H_4$. The isotopic yield for L-dihydroxyphenylalanine was 90 % and for the other compounds >99 %. These labelled compounds could be used as internal standards in the mass fragmentographic analysis of the protium species in tissues and body fluids as well as in metabolic studies in vivo.

Recently mass spectrometric techniques have become available for the specific determination of endogenous and exogenous compounds with high degree of precision and sensitivity[1-3]. These methods require the availability of the isotopically labelled species as an internal standard. For many purposes incorporations of deuterium by simple exchange or reduction reactions provide compounds with the desired properties.

Most of our knowledge of the catecholamines in the brain has been obtained in experimental animals where it is possible to obtain

brain for analysis and where radioactive precursors can be employed for dynamic studies of transmitter function. In humans ethical and technical considerations point to the use of catecholamine precursors and metabolites labelled with stable isotopes. In the present paper we report the synthesis of a series of deuterium labelled compounds, which can be used for quantitative mass spectrometric analysis in tissues and body fluids, and also as tracers in turnover studies in humans.

The aromatic hydrogens in L-tyrosine, L-dopamine, 3-methoxy tyramine and homovanillic acid (HVA), which are activated for electrophilic substitution, were exchanged by heating the compounds in DCl in D_2O. The benzylic protons of homovanillic acid were also substituted under these conditions. This resulted in a total exchange of five hydrogens in the case of HVA and three in dopamine, 3-methoxytyramine and L-dihydroxyphenylalanine (L-Dopa). Tyrosine which is activated only in the 3- and 5-positions incorporated only two deuteriums. That an almost quantitative incorporation of deuterium was achieved in the assigned position is shown by the mass spectra of the methylester trifluoroacetyl derivatives of HVA, 4-hydroxy-3-methoxy phenylethyleneglycol (HMPG), dopamine and 3-methoxy tyramine (Fig 1a-d). The synthesis of deuterium labelled dopamine has recently been described by Perel et al[4].

The acidic hydrogen at the 2-position in 2-hydroxy-2 (4 hydroxy-3-methoxyphenyl) acetic acid (VMA) was exchanged by heating in NaOD: D_2O solution. The exchange of α-hydrogens with deuterium under these conditions have been described for some other carboxylic acids[4]. Thus homovanillic acid has been labelled with two deuteriums in this way[4,5]. When VMA was subjected to prolonged heating further hydrogens were exchanged. Comparison of the mass spectrometric fragmentation pattern with an unlabelled sample suggested that the hydrogens were the aromatic ones. The quantitative exchange of the aromatic hydrogens could, however, not be achieved even after one week of heating in NaOD/D_2O solution.

1. Mass spectra of the perfluoroacyl derivatives of deuterium labelled a) HVA, b) HMPG, c) dopamine and d) 3-MT.

Fig. 1a

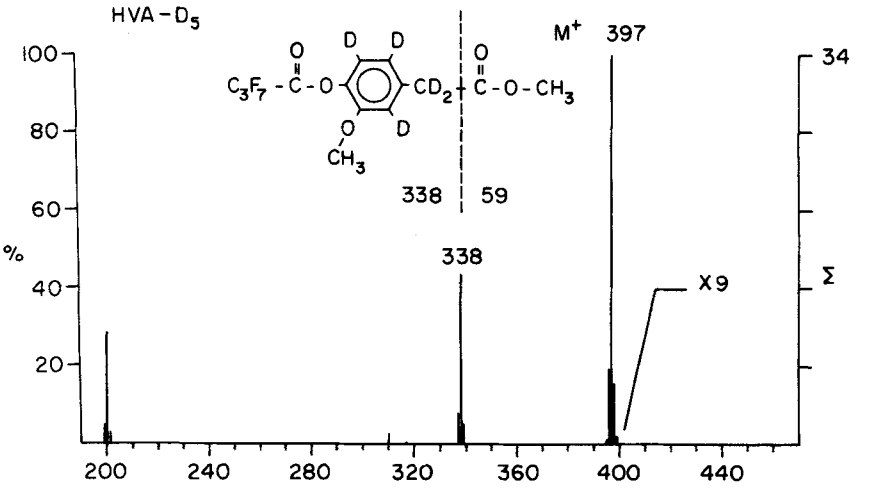


Fig. 1b

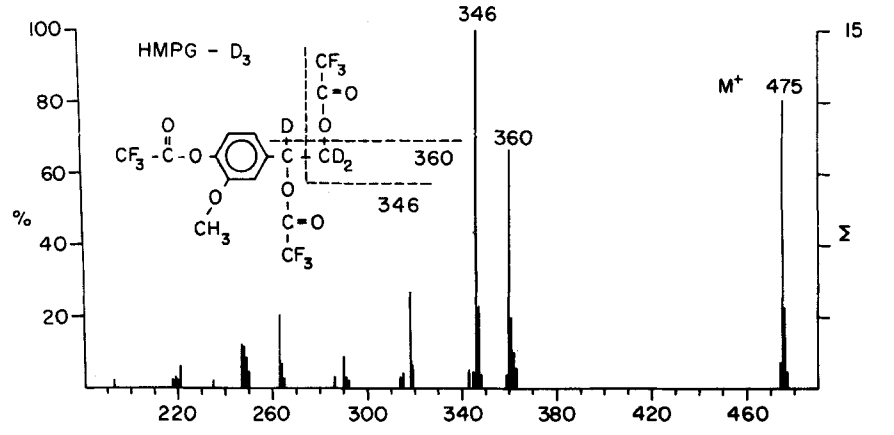


Fig. 1c

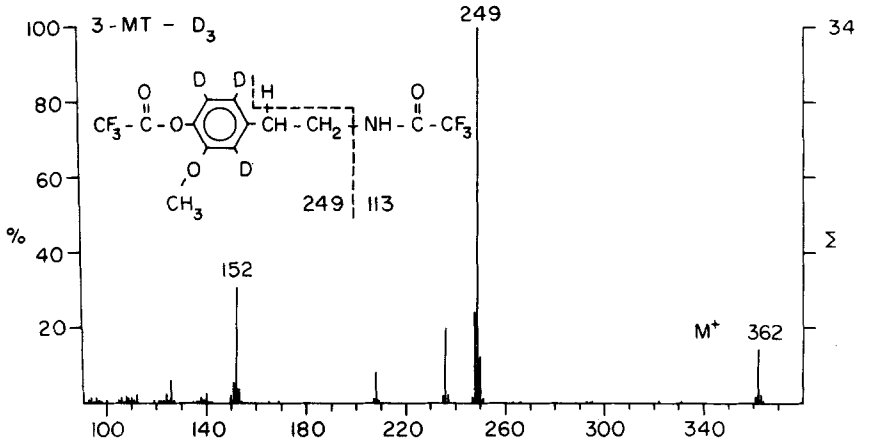
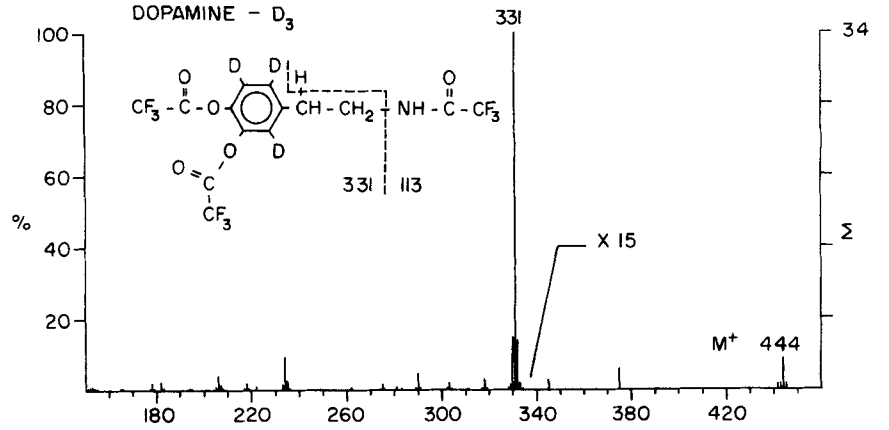

3-MT - D3
249
34
100
80
60
%
40
20
Σ
152
M+ 362
249 113
100 140 180 220 260 300 340

Fig. 1d


DOPAMINE - D3
331
34
100
80
60
%
40
20
Σ
X 15
M+ 444
331 113
180 220 260 300 340 380 420

(4-hydroxy-3-methoxyphenyl) ethane-1,2-diol-$^2H_3$ (HMPG-$^2H_3$) was prepared from methylesterified VMA-^2H by reduction with lithium aluminium deuteride in tetrahydrofurane.

When 3,4-dihydroxy benzaldehyde and 4-hydroxy-3-methoxy benzaldehyde (vanillin), precursors for the synthesis of noradrenaline and normetanephrine respectively, were subjected to the same conditions as for the preparation of labelled HVA (DCl in D_2O), the aromatic hydrogens were substituted only to about 70%, probably due to the deactivating effect of the aldehyde group.

Since these compounds were prepared to use as internal standards in mass fragmentography it is essential that no reexchange of deuterium occurred under conditions of isolation and preparation of derivatives and/or also in vivo. No exchange was found to exist for these compounds under neutral and mild acidic (pH=1) conditions at room temperature. Mass fragmentographic methods for the determination of HVA and HMPG with these standards have been reported elsewhere [6,7]. HVA-$^2H_5$, l-tyrosine-$^2H_2$ and L-DOPA$^3H_3$ have been introduced in animals and humans with no evidence of exchange of deuterium following isolation of metabolites from urine.

EXPERIMENTAL

Mass spectra were recorded on a LKB 9000 or a LKB 2091 instrument equipped with a 1% Se-30 columns. NMR-spectra were run on a Varian A60A instrument.

Preparation of derivatives

The methyl ester derivatives of the carboxylic acids were prepared in ethyl acetate by brief treatment with diazomethane in diethylether. Trifluoroacetic anhydride (heptafluorobutyric anhydride in the case of HVA) in ethyl acetate (1:1) was used to derivatize alcoholic, phenolic and amino groups[10,11].

l-L-amino-2 (3,4-dihydroxy-2,5,6 trideuterophenyl)propanoic acid (L-Dopa-$^2H_3$)

60 g (0.32 mol), L-Dopa was refluxed in 200 ml 10% DCl in

D_2O for 24 hours. The reaction mixture was cooled and pH adjusted to 5.5. The precipitated amino acid was filtered under N_2 atmosphere and washed with water and ethanol. The product was dried in a deccicator. Quantitative yield. Isotopic yield >90%, according to the NMR spectrum.

3,5 dideutero - L-tyrosine (L-tyrosine-$^2H_2$)

80 g (0.44 mol) L-tyrosine was refluxed in 320 ml 10% DCl in D_2O for 24 hours. After cooling pH was adjusted to 5.6. The precipitate was treated as described above for L-DOPA. Isotopic yield quantitative, according to the NMR spectrum.

1-Amino-2(3,4-dihydroxy-2,5,6 trideuterophenyl)-ethane (Dopamine-$^2H_3$)

100 mg (0.65 mmol) dopamine and 1.5 ml 10% DCl in D_2O were heated in a sealed tube for 40 hours at 130°. The solvent was evaporated and the hydrochloride was recrystallized from methanol-ether, yield 86%, mp. 235-240°, litt. 241°. Isotopic yield quantitative (Fig. 1c).

1-Amino-2(3,4-dihydroxy-2,5,6 trideuterophenyl)-ethane (3-methoxy-tyramine$^2H_3$)

100 mg (0.6 mmol) 3-methoxy tyramine was treated as described for dopamine above. The hydrochloride was recrystallized from ethanol, yield 40%, mp. 207-212°, litt. 210-211°.

2,2-dideutero-2-(4-hydroxy-3-methoxy-2,5,6 trideuterophenyl) acetic acid (homovanillic acid-$^2H_5$)

300 mg (1.7 mmol) homovanillic acid was heated in 2 ml 16% DCl in D_2O (sealed tube) for 30 hours at 120-130°. The solution was then cooled and extracted with ethyl acetate. The product obtained on evaporation of the solvent was dissolved in chloroform (3 ml) and put on a column of silocic acid. After washing with chloroform (10 ml) the product was eluted with 10% ether in chloroform[8]. Evaporation of the solvent yielded 186 mg, mp. 141-143°, litt. 142-143°. Isotopic yield quantitative (Fig. 1a).

2-deutero-2-hydroxy-2 (4-hydroxy-3-methoxyphenyl) acetic acid (Vänillyl-mandelic acid-^{2}H)

200 mg (1.1 mmol) vanillylmandelic acid was heated in 1.5 ml 4M NaOD in D_2O (sealed tube) for 20 hours at 120-130°. After cooling and acidification (2M HCl) the reaction mixture was extracted with ethyl acetate. 180 mg crude product was obtained after evaporation of the solvent. Isotopic yield quantitative according to the mass spectrum.

1,2,2 trideutero-1(4-hydroxy-3-methoxyphenyl)-ethane-1,2-diol (HMPG-$^2H_3$)

The vanillyl-mandelic acid-^{2}H was dissolved in tetrahydrofurane (2 ml) and methylated with diazomethane in ether. The solvents were removed and the methyl ester was dissolved in dry tetrahydrofurane (3 ml) and 150 mg of lithium aluminium deuteride was added. The reaction mixture was stirred for two hours at room temperature, 10 ml of water was added cautiously and the mixture was allowed to stand for 1 hour. The pH was then adjusted to about 4 and the precipitated hydroxides were filtered off. The aqueous solution was extracted with ethyl acetate (3x5 ml) and dried (Na_2SO_4). Evaporation of the solvent yielded 80 mg. Further purification was carried out on a Sephadex LH 20 column (1.5 m x 2.5 cm) with 30% methanol in 1.2-dichloroethane as eluent[9]. Over all yield (from VMA) was about 25%.

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