PREPARATION OF DEUTERIUM LABELLED CATECHOLAMINES

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SUMMARY

The preparation of deuterium labelled catecholamines and their metabolites is described. The procedures involve simple treatment of the compounds in DCl/D_2O solution. Mass spectra of these labelled compounds -- as their PFP or TFA derivative -- are presented. The relative intensity of ions suitable for selective ion monitoring are given for each compound. Results are presented concerning ease or difficulty of deuteration. The compounds studied include dopamine, tyramine, 3-methoxytyramine, norepinephrine, metanephrine, normetanephrine, homovanillic acid and dihydroxyphenylacetic acid.

KEYWORDS

Dopamine, 3-methoxytyramine, norepinephrine, homovanillic acid, 3,4-hydroxyphenylacetic acid, metanephrine.

INTRODUCTION

Catecholamines have been implicated in mental disorders and other related diseases (1) and have been intensely studied. We are currently interested in catecholamine levels in small (1 mg) subregions of rat striatum (2). Because of very low levels of these compounds in the samples, sensitivity and selectivity of the method of analysis is very important. Recently mass fragmentographic techniques have become available for the specific determination of these compounds with a high degree of precision and sensitivity. To quantitate these amines,

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isotopically labelled analogs are used as internal standards. The knowledge of the number of isotope atoms per molecule and the position of these atoms is important for using the molecule as an internal standard. Incorporation of at least three deuterium atoms per molecule is desired to shift the mass of the internal standard out of the mass range where carbon-13 and other naturally occurring isotopes make significant contributions to the measured intensity of the internal standard. It is also important to know accurately the percentages of different amounts of isotopic labelling in each compound. The structures of the compounds studied are given in Figures 1a and 1b and Table 1.

Past work on deuteration of catecholamines includes that of Lindstrom, et al (3) and Muskiet (4). In the present paper modifications of the methods in ref. (3) and (4) are described which result in high yield and extent of deuteration. Complete isotopic analyses of the products are given.

MATERIALS AND METHODS

Catecholamines and their metabolites were purchased from Sigma Chemical Co., deuterium chloride and deuterium oxide were obtained from Aldrich Chemical Co. and pentafluoropropionic and trifluoroacetic anhydride from Pierce Chemical Co.

Gas chromatography/mass spectrometry was performed on a Finnigan 4000 gas chromatograph/mass spectrometer. A glass column (1.8 x 2 mm i.d.) with 3% OV-17 on chromosorb Q, mesh size 100-120, was used for the analysis. Helium flow-rate was 20 ml/min. Electron energy was 70 eV. Source temperature was 250°C and injector and detector temperature 200°C. Samples were run at an isothermal column temperature of 160°C, except for metanephrine and normetanephrine for which the temperature was 180°C.

EXPERIMENTAL

Procedures modified from those described by Lindstrom (3), and Muskiet (4) were used for deuteration of these compounds. Various percentages of DCl in D_2O, temperatures and duration of deuteration were examined. The following procedures were found to be best suited for obtaining the deuterated compounds with least amount of side products and least amount of undeuterated compound.

Dopamine: 100 mg of dopamine hydrochloride and 2 ml of 20% DCl in D_2O were heated in a sealed vial at 130°C for 25 hrs. Solvent was evaporated and the product recrystallized with methanol-ether. This deuteration procedure was repeated for the product of the first reaction.

Tyramine: 200 mg of tyramine (free base) with 2 ml of 20% DCl in D_2O were heated in a sealed vial at 120-130°C for 30 hrs. Tyramine crystallized immediately after breaking the vial. Drying was completed in a vacuum desiccator.

3-Methoxytyramine: 100 mg of 3-methoxytyramine hydrochloride and 1 ml of 20% DCl in D_2O were heated in a sealed vial for 14-16 hrs at 90°C. The compound was obtained by drying in a vacuum desiccator.

Norepinephrine: 40 mg of norepinephrine were heated with 1 ml of 10% DCl in D_2O at 80°C for 11 hrs. The solution was transferred to a plastic vial (Eppendorf) and stored at 0-6°C.

Metanephrine: 25 mg of metanephrine hydrochloride were heated with 0.5 ml of 10% DCl in D_2O at 80°C for 18 hrs. The solution was stored in a plastic vial at 0-4°C.

Normetanephrine: 25 mg of normetanephrine hydrochloride were heated with 0.5 ml of 10% DCl in D_2O for 12 hrs. The solution was centrifuged and the supernatant stored at 0-4°C in a plastic vial.

Homovanillic and 3,4-dihydroxyphenylacetic acid: 200 mg of each compound were heated separately in 2 ml of 20% DCl in D_2O in a sealed vial at 110°C for 6-8 hrs. The compounds were extracted in ethyl acetate after saturation with NaCl. The products were obtained by drying the extract in a vacuum desiccator.

Derivatization: Microgram samples of dopamine, tyramine and 3-methoxy-tyramine were derivatized by the addition of 200 µl of ethyl acetate and 200 µl of PFPA or TFAA. After 15 minutes at room temperature, the samples were dried under nitrogen and reconstituted in 50 µl of 1:1 solution of ethyl acetate and anhydride.

In the case of norepinephrine, metanephrine and normetanephrine aliquots of the solution containing the deuterated compound were dried under a stream of nitrogen at 40°C. Derivatives were prepared in the same manner as described for other amines in the foregoing paragraph.

Small amounts of homovanillic acid and 3,4-dihydroxyphenylacetic acid were first esterified by treating with diazomethane. PFP derivatives were then prepared by adding 200 µl of ethyl acetate and 200 µl of the anhydride. After 15 min at room temperature, the reagents were evaporated and derivatives reconstituted in 50 µl of 1:1 ethyl acetate and PFPA.

RESULTS AND DISCUSSION

General structure of amines and acids are shown in figures 1a and 1b, respectively. The substituents, e.g., R_1, R_2 and R_3 are given in Table 1 for each compound. Degree of deuterium incorporation is given in Table 2, showing the per cent peak area after correction for carbon, nitrogen and oxygen isotope abundances for the major fragment in each compound. Mass spectra of all the deuterated compounds are shown in figures 2a-2h.

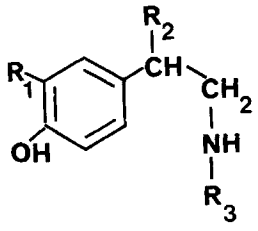


Fig. 1a

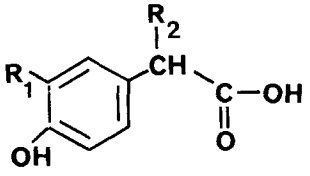


Fig. 1b

Table 1. Structures

Compound	Substituents		
	R_1	R_2	R_3
Dopamine	OH	H	H
Tyramine	H	H	H
3-Methoxytyramine	OCH_3	H	H
Norepinephrine	OH	OH	H
Metanephrine	OCH_3	OH	CH_3
Normetanephrine	OCH_3	OH	H
Homovanillic acid	OCH_3	H	-
3,4-Dihydroxyphenylacetic acid	OH	H	-

Table 2. Deuteration Results

Compound	d_o		d_1		d_2		d_3		d_4		d_5	
	m/e	%	m/e	%	m/e	%	m/e	%	m/e	%	m/e	%
DA-TFA_3	328	0.3	329	1.1	330	4.0	331	94.5	332	-	333	-
Tyra-TFA_2	216	0.2	217	0.6	218	7.0	219	32.7	220	59.5	221	-
3-MT-PFP_2	296	0.5	297	3.0	298	15.7	299	86.7	300	-	301	-
NE-TFA_4	440	2.4	441	20.5	442	43.8	443	35.7	444	3.8	445	1.4
MN-PFP_3	458	2.8	459	18.4	460	52.1	461	32.4	462	5.3	463	0.3
NMN-PFP_3	458	8.9	459	32.3	460	45.0	461	18.1	462	3.1	463	1.2
HVA-PFP_1	283	0.3	284	1.4	285	1.9	286	12.6	287	44.3	288	39.5
DOPAC-PFP_2	415	1.5	416	2.3	417	5.3	418	22.0	419	42.7	420	26.1

Dopamine shows the incorporation of three deuterium atoms on the ring which resulted from electrophilic substitution. The procedure for deuteration was repeated once to obtain 94% d_3-dopamine. The stability of d_3-dopamine against deuterium loss was studied as a function of time and pH. Under very acidic conditions (6M HCl) after 8 hrs only 29.4% d_3-dopamine was left whereas in 0.02 M HCl, after 8 hrs, 69.3% d_3-dopamine was present. There was no significant change when d_3-dopamine was left at pH 7 for the same period of time.

All four aromatic hydrogen atoms on tyramine are displaced by deuterium atoms. The procedure described above gives primarily d_2-tyramine with a significant amount of undeuterated compound. Therefore, the procedure was repeated two times to obtain 59.5% d_4-tyramine. The yield after each deuteration procedure was 99%. There was some back exchange when d_4-tyramine was left in pH 2 solution for 24 hrs (59.5% to 53.8%).

3-Methoxytyramine also shows displacement of three aromatic hydrogen atoms. The procedure for the deuteration of this compound described by Lindstrom (3) converted it largely to dopamine (86%). The procedure here gives about half dopamine and half 3-methoxytyramine. There was no significant change in the extent of deuteration when the compound was left at pH 2 for 24 hrs. However, conversion of 3-MT to dopamine was very significant (dopamine goes from 47% to 44%) at this pH.

All five hydrogen atoms -- three aromatic and two benzylic -- are replaced by deuterium atoms in homovanillic acid. Under the conditions described by Lindstrom (3), almost all (97%) of the compound was converted to DOPAC with 82% of the DOPAC as d_5DOPAC. The procedure given here for HVA gave less than 5% DOPAC, but deuteration was not as complete (d_o-HVA 48%; d_5-HVA 5.4%). Therefore, the procedure was repeated once for about 3 hrs. This resulted in 82% HVA and 18% DOPAC with only 0.3% d_oHVA (44.3% d_4HVA and 39.5% d_5-HVA). The overall yield after two deuteration procedures was 73%. Back exchange studies did not show any significant loss of deuterium when the compound was left in pH 2 solution for 24 hrs.

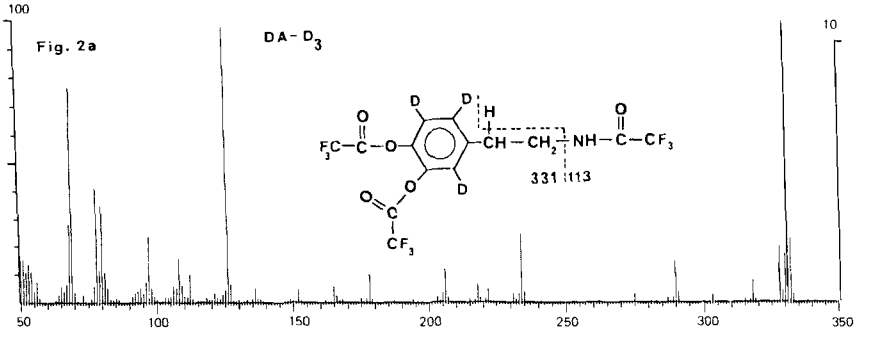
DOPAC also shows displacement of five hydrogen atoms at the positions described for HVA. The yield was approximately 80%. Back exchange of deuterated DOPAC was also not very significant under the conditions described for HVA.

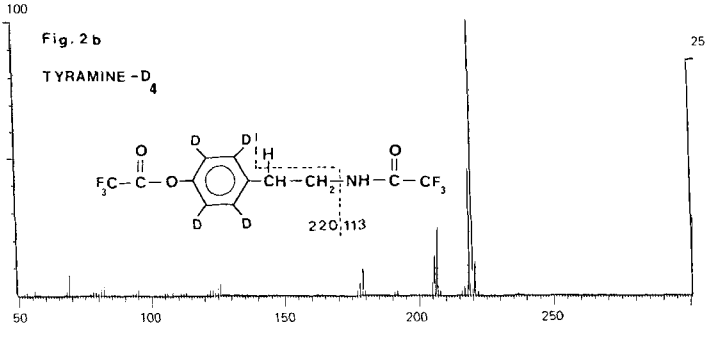
In the case of norepinephrine, metanephrine and normetanephrine only two hydrogen atoms on the ring were displaced by deuterium atoms. These compounds were less stable than those discussed above, however the solutions (compound in DCl/D_2O) were stable for 2-3 weeks when stored at 0-4^oC.

Deaeration of DCl with nitrogen, before its addition to the compound, gave better yields by preventing the oxidation of the compound. It was especially useful in the case of amines. Prolonged heating caused decomposition or oxidation of the compounds. Side products increased to significant amounts in the case of

norepinephrine, metanephrine and normetanephrine when these compounds were heated for longer time periods. Considerably less exchange of deuterium atoms was accomplished when the compounds were heated for shorter durations. Temperature effects were very significant in all cases. Higher temperatures caused decomposition of the compounds in some cases and changed the compound totally in others. For example, HVA was converted to DOPAC and 3-MT to dopamine.

Formation of deuterated DOPAC during the deuteration of HVA is not necessarily a problem and can, in fact, be useful in analysis of these metabolites in biological samples. Levels of DOPAC and HVA, and their ratios in biological samples have been determined (5,6). By adjusting the variables, e.g., temperature, duration of deuteration and percentage of DCl in D_2O, similar ratios of deuterated DOPAC and HVA can be obtained. Thus the combined internal standard can be directly used for the analysis and quantitation of HVA and DOPAC in the same sample.





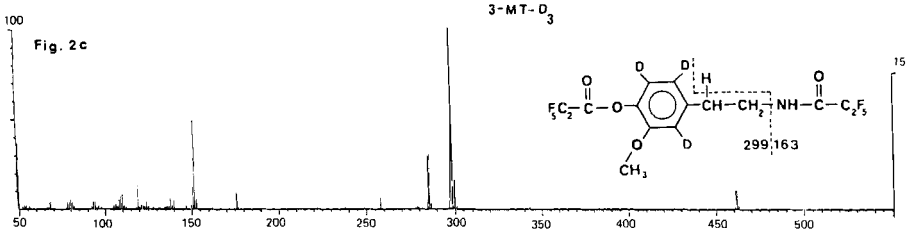
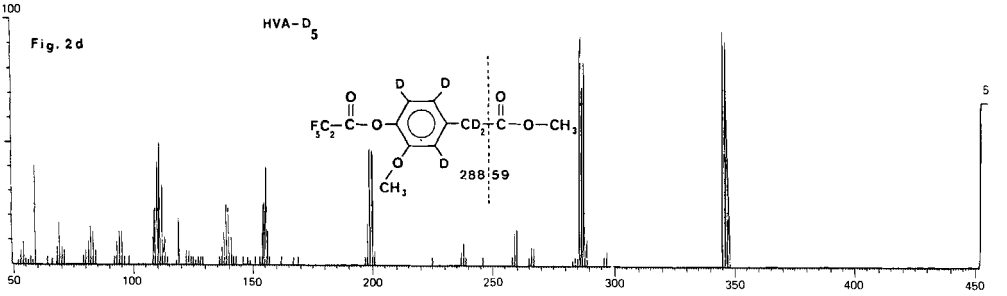


Fig. 2c
3-MT-D3
299.163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HVA-D5
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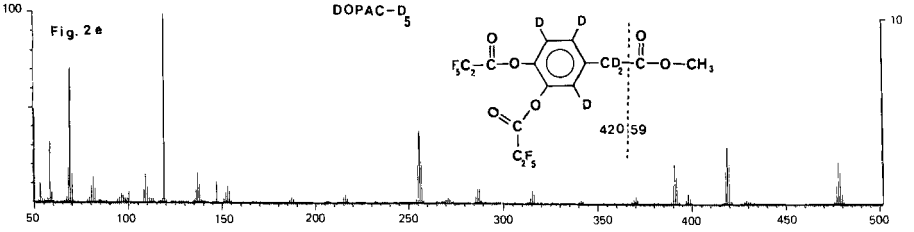


Fig. 2e
DOPAC-D5
420.59

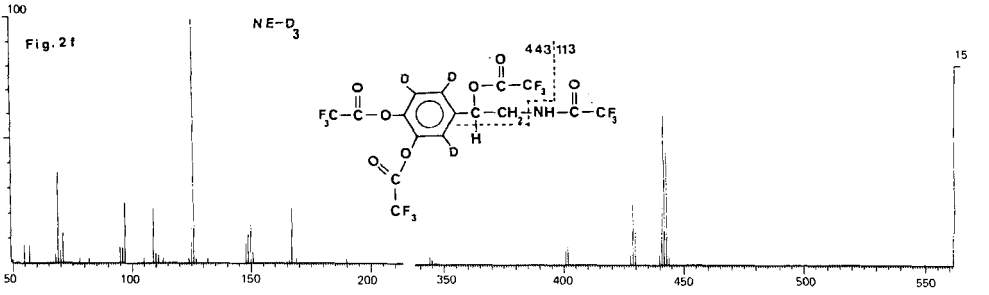
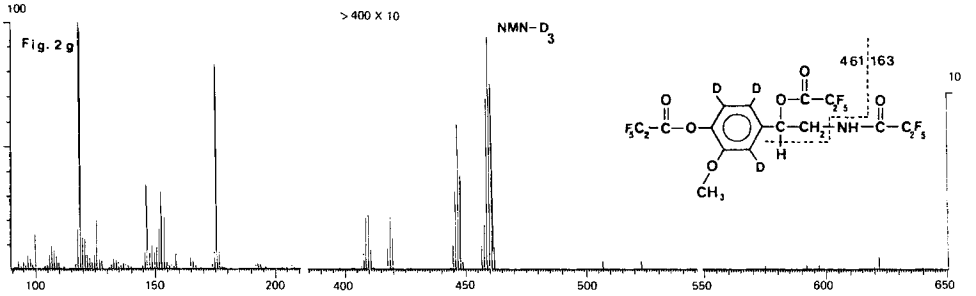
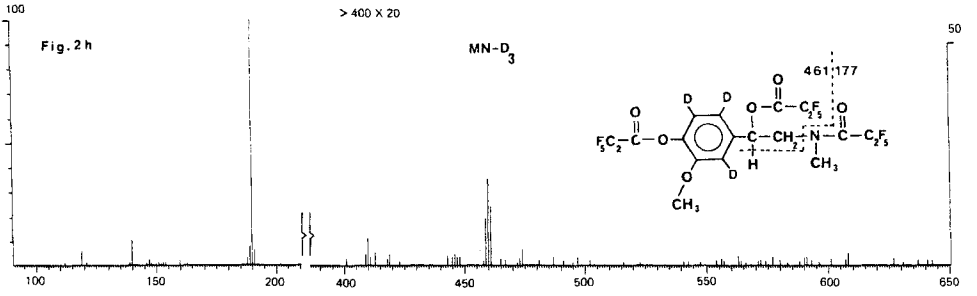


Fig. 2f
NE-D3
443.113





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