DEUTERATION OF CATECHOLAMINES, CATECHOLAMINE METABOLITES AND TRYPTOPHAN METABOLITES

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Summary

The preparation of some deuterium labelled catecholamines, catecholamine metabolites and tryptophan metabolites is described. Simple exchange reactions in DCl/D_2O solution or reductions with Li Al D_4 were used. The deuterium labelled compounds prepared are suitable for use as internal standards for quantitative mass-fragmentographic analysis of their unlabelled analogs in biological material. Mass spectra of trimethylsilyl and trimethylsilyl-N-trifluoroacetyl derivatives of 2,5,6-trideutero vanillactic acid, 1,1-dideutero-1-hydroxy-2(2,5,6-trideutero catechol)-ethane, 2,5,6-trideutero epinephrine, 2,5,6-trideutero normetanephrine and hexadeutero indole-3-acetic acid are given together with those of their natural occurring analogs.

The mass-fragmentographic determination of small amounts of biogenic amines and their metabolites in biological material has become of increasing interest in the past few years (1-7). The method combines both specificity and sensitivity and can achieve a high degree of precision with the use of analogs labelled with stable isotopes as internal standards. However, these compounds are not always commercially available, or are unsatisfactory because

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of the number of isotope atoms per molecule and/or the position of the label in the molecule. During the quantitative mass-fragmentographic analysis, the incorporation of too small a number of isotope atoms per molecule can give rise to a natural abundance contribution to the internal standard. Conversely, non-quantitative incorporation of isotope atoms in the internal standard will affect the compound to be measured. These phenomena result in non-linear calibration curves with an intercept (8) and may cause loss of precision, unless special arrangements are made.

We describe the synthesis of some deuterium labelled catecholamines, catecholamine metabolites and tryptophan metabolites, which can be used as internal standards for quantitative mass-fragmentographic analysis. The degree of incorporation of deuterium atoms and the quality of the products obtained were checked by mass fragmentography and gas chromatography. The methods described can easily be performed in the laboratory.

The preparations of homovanillic acid-d_2 and d_5 (9) and vanil ethanol-d_2 and 3,4-dihydroxyphenylacetic acid-d_5 (3) have already been described.

MATERIALS AND METHODS

Catecholamines, catecholamine metabolites and tryptophan metabolites were purchased from Sigma Chemical Co, St. Louis, U.S.A., 1-hydroxy-2(catechol)-ethane (CE) was prepared from 3,4-dihydroxyphenylacetic acid with Li Al H_4 as described for CE-d_5 (see Experimental), ethyl acetate (GC-spectroscopic quality) was obtained from Baker Chemicals, Deventer, The Netherlands. All other chemicals were "suprapur" or AR grade reagents from Merck, Darmstadt, Germany.

Gas chromatography/ mass spectrometry was performed on a Varian MAT 112 gas chromatograph/mass spectrometer unit equipped with a 2m 3% OV-1 on Supelcoport 80-100 mesh (Supelco Inc. Bellefonte, U.S.A.) glass column (i.d. 1.2 mm). Helium flow rate was 6^{ml}/min. Column temperatures from 150-220^{o}C were used without programming. Electron energy was 70eV, slit separator

temperature 260°C and source temperature 220°C.

EXPERIMENTAL

Deuteration

2,5,6-trideutero homovanillic acid (HVA-d_3), 2,5,6-trideutero vanillactic acid (VLA-d_3) and 2,5,6-trideutero-3,4-dihydroxyphenylacetic acid (DOPAC-d_3) were prepared from their unlabelled analogs by dissolving 50 mg of each of these compounds in 2 ml of 9% DCl in D_2O and heating for 6h at 80°C in sealed tubes in a heating block. The compounds were extracted into ethyl acetate after saturation with sodium chloride.

1,1-dideutero-1-hydroxy-2(2,5,6-trideutero vanil)-ethane (VE-d_5) and 1,1-dideutero-1-hydroxy-2(2,5,6-trideutero catechol)-ethane (CE-d_5) were prepared by dissolving 50 mg HVA-d_3 and DOPAC-d_3 in 5 ml of dry tetrahydrofuran and adding 50 mg Li Al D_4. The solutions were stirred for 6h at room temperature, 5 ml of water were added with care and the mixtures stirred for a further 2h. The pH was adjusted to 6.5, the solutions were saturated with sodium chloride and the compounds extracted into ethyl acetate.

2,5,6-trideutero epinephrine (E-d_3) and 2,5,6-trideutero norepinephrine (NE-d_3) were prepared from epinephrine and norepinephrine by heating 50 mg of each compound in 5 ml of 4.6% DCl in D_2O for 40h at 80°C in sealed tubes. The solutions became brownish-yellow and were centrifuged at 0°C. The supernatants were kept at -20°C .

2,5,6-trideutero metanephrine (M-d_3) and 2,5,6-trideutero normetanephrine (NM-d_3) were prepared as described for E-d_3 and NE-d_3, with the exception that the solutions were heated for 65h at 80°C.

Pentadeutero-5-hydroxyindole-3-acetic acid (5 HIAA-d_5), hexadeutero indole-3-acetic acid (IAA-d_6) and tetradeutero-5-methoxyindole-3-acetic acid (5-MIAA-d_4) were prepared by heating 50 mg 5-HIAA as well as saturated solutions of IAA and 5-MIAA in 2 ml of 9% DCl in D_2O for 20h at 80°C. After cooling

and filtration, the solutions were saturated with sodium chloride and the compounds extracted into ethyl acetate. The solution containing 5-HIAA-d_5 was kept at -20°C.

Derivatization

Aliquots of the solutions containing the deuterated compounds were dried under a stream of nitrogen at 40°C. Silylation was carried out by adding 100 µl bis (trimethylsilyl)-trifluoroacetamide and heating for 10 minutes at 80°C. E,NE,M and NM were then converted to their trimethylsilyl-N-trifluoroacetyl derivatives by the addition of 10 µl N-methyl-bis-trifluoroacetamide. This reaction takes place instantaneously.

RESULTS AND DISCUSSION

Mass spectra of trimethylsilyl (TMS) and trimethylsilyl-N-trifluoroacetyl (TMS-TFA) derivatives of VLA-d_3, CE-d_5, E-d_3, NM-d_3 and IAA-d_6, together with those of their natural occurring analogs are shown in fig 1-5.

Quantitative replacement of hydrogen, susceptible to exchange under the conditions described above, was achieved for HVA-d_3, DOPAC-d_3, VLA-d_3, VE-d_5 and CE-d_5, while attempts for quantitative incorporation of deuterium in E,NE,M,NM,IAA,5-HIAA and 5-MIAA resulted in unacceptable low recoveries of the desired products. For these compounds the conditions described (see Experimental) resulted in a reasonable compromise between degree of deuterium incorporation and recovery.

Although the deuterated compounds were not purified further, no interference of compounds formed in side reactions was observed when they were used as internal standards during mass-fragmentographic analysis.

Gas-chromatographic analysis did not show detectable amounts of DOPAC and HVA in the solutions containing VE an CE.

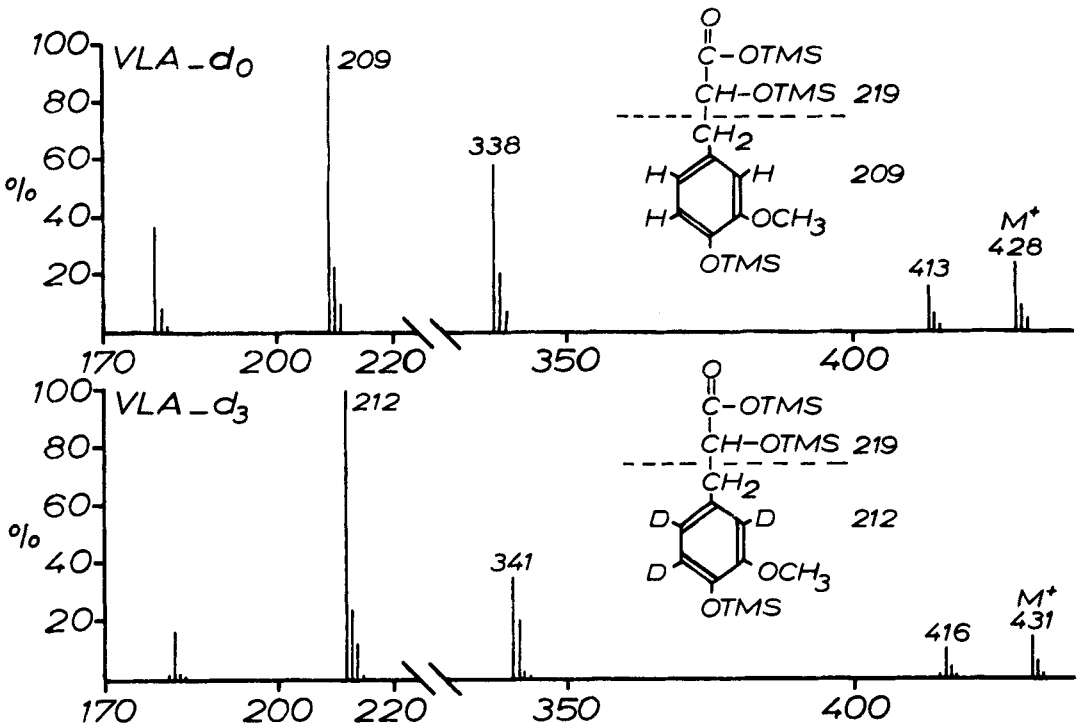
VLA_d0
209
338
413
M+
428
O
C-OTMS
CH-OTMS 219
CH2
H
H
H
OCH3
OTMS
209
%
100
80
60
40
20
170
200
220
350
400
VLA_d3
212
341
416
M+
431
D
212
219

FIG. 1

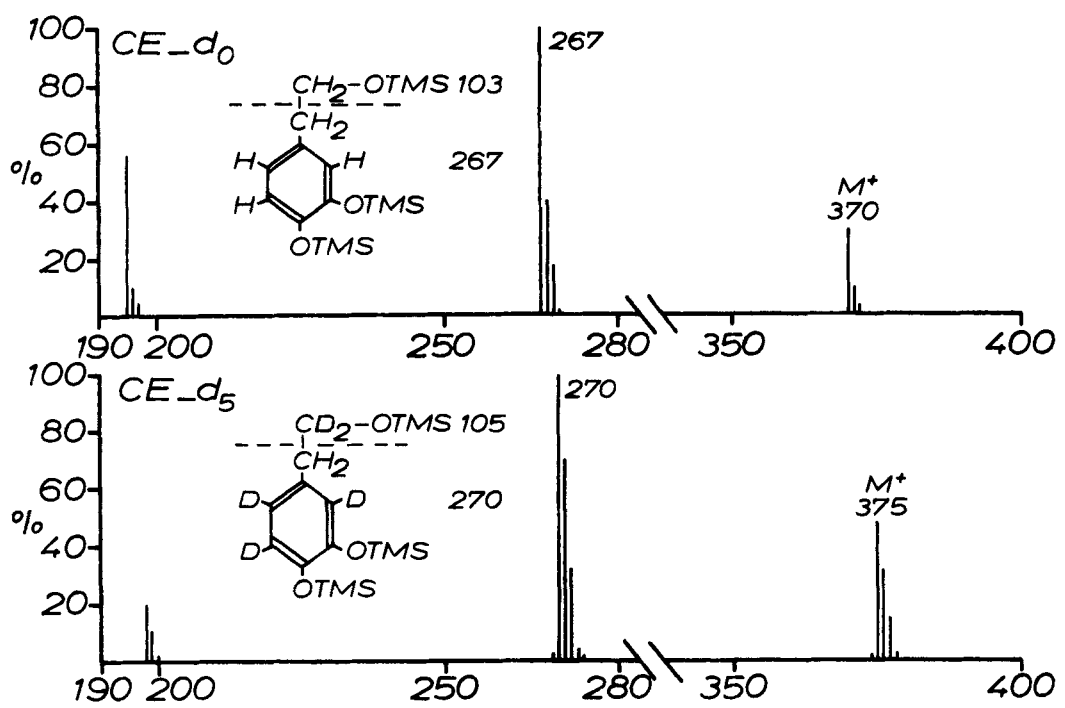
CE_d0
267
CH2-OTMS 103
CH2
H
H
H
OTMS
OTMS
267
M+
370
%
100
80
60
40
20
190
200
250
280
350
400
CE_d5
270
CD2-OTMS 105
D
D
D
OTMS
OTMS
270
M+
375

FIG. 2

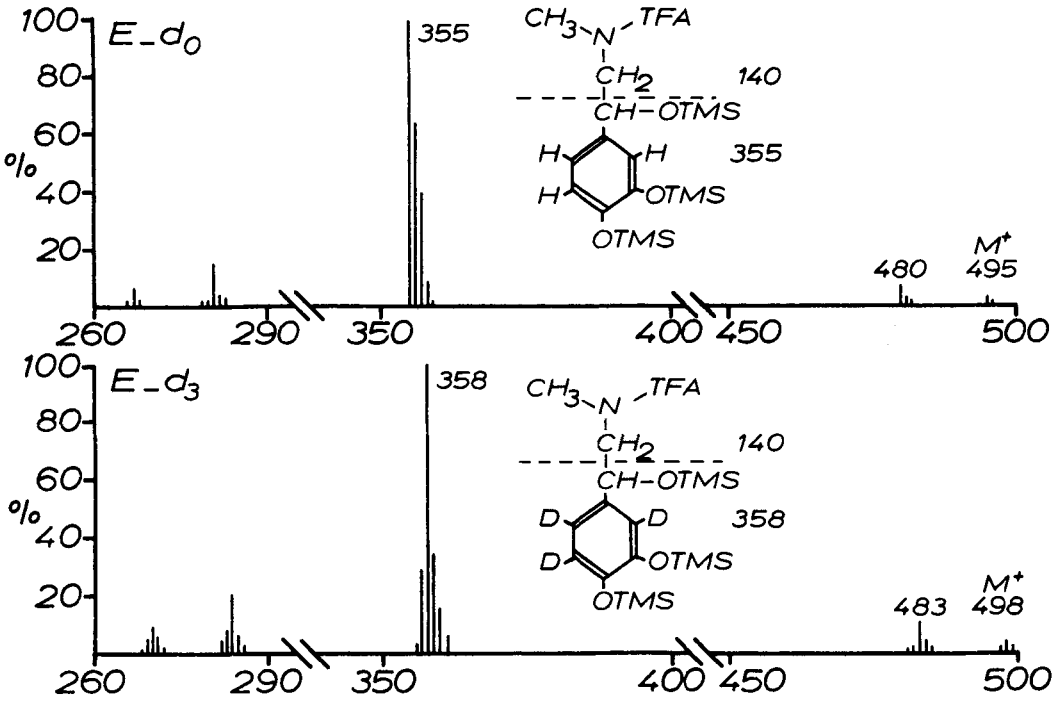
E_d0
355
CH3 N TFA
CH2
140
CH-OTMS
H H
H
355
OTMS
OTMS
M+
480
495
100
80
60
%
40
20
260
290
350
400
450
500
E_d3
358
CH3 N TFA
CH2
140
CH-OTMS
D D
D
358
OTMS
OTMS
M+
483
498

FIG.3

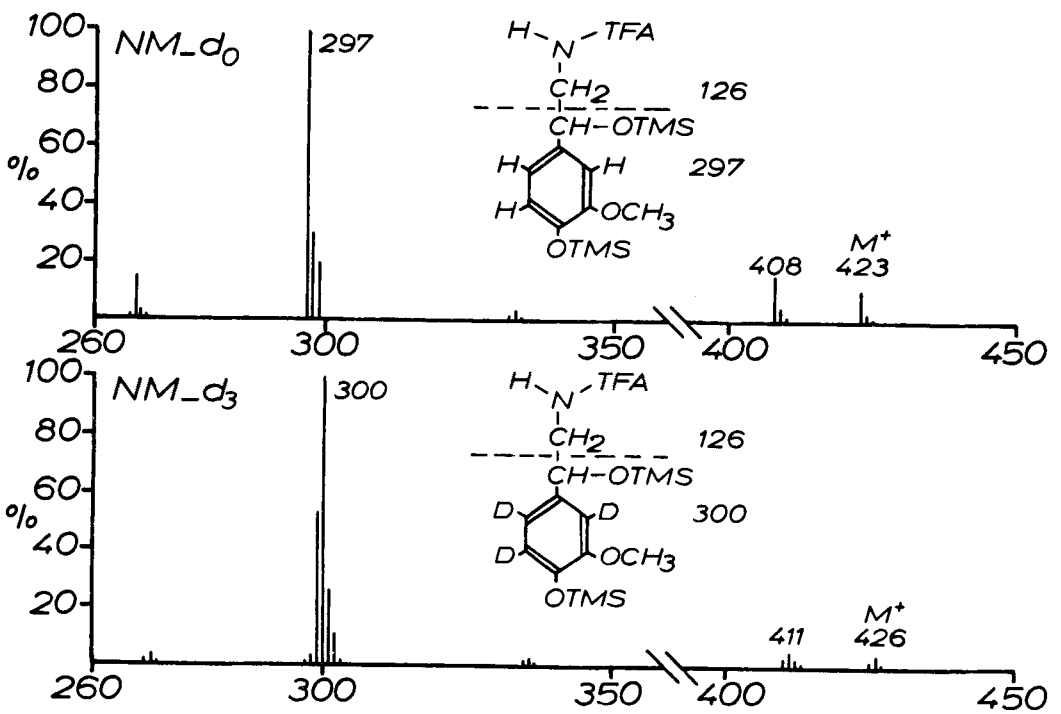
NM_d0
297
H N TFA
CH2
126
CH-OTMS
H H
H
297
OCH3
OTMS
M+
408
423
100
80
60
%
40
20
260
300
350
400
450
NM_d3
300
H N TFA
CH2
126
CH-OTMS
D D
D
300
OCH3
OTMS
M+
411
426

FIG.4

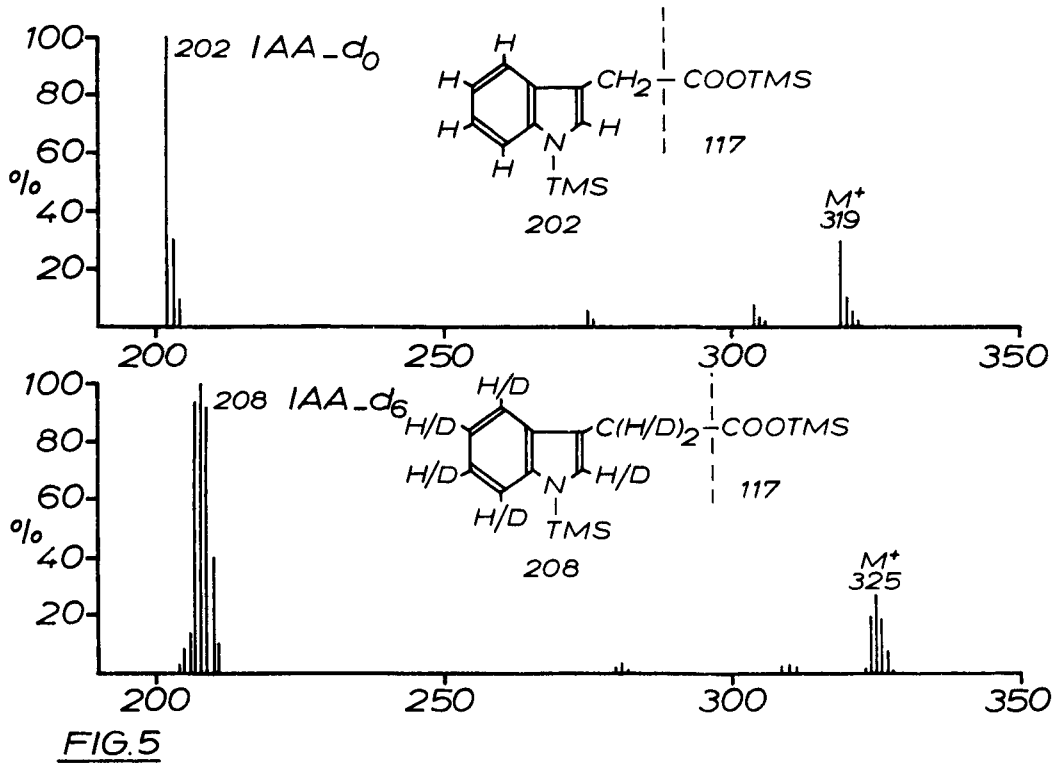

FIG.5

Table I shows the two parameters determining the shape of the calibration curve (peak height ratio $^{d}o/d_x$ versus amount of natural occurring compound added to a fixed amount of internal standard) for quantitative measurements of the unlabelled analogs (d_o) with the synthesized internal standards (d_x). The percentage $^{d}o/d_x$ (x = number of deuterium atoms incorporated per molecule) in the internal standard determines the magnitude of the intercept, while $^{d}x1/d_o$ ($d_{x1} = d_x$ in the natural occurring compound due to isotope abundance) in the natural occurring compound leads to deviation from linearity. Both ratios were determined by means of selective ion monitoring and were found to be less than 5%, which result made the internal standards suitable for use in the majority of applications (1-7). The use of derivatives that do not contain silicon, which has a high natural isotope abundance, might even improve linearity, by lowering the ratio $^{d}x1/d_o$ in the natural occurring compound.

No re-exchange of deuterium was noticed when the stock solutions of the internal standards were kept at 4°C (HVA,DOPAC,VLA,VE,CE,IAA,5-MIAA) and -20°C

(5-HIAA, E, NE, M, NM) nor during analytical procedures.

Table I, Contribution of the natural occurring compound (d_o) to the internal standard (d_x) and vice versa, due to isotope abundance in d_o and non-quantitative incorporation of isotopes in d_x.

compound		x	m/e (d_o) [a]	m/e (d_x,d_{x1}) [b]	$\%(\frac{d_{x1}}{d_o})$ in d_o [c]	$\%(\frac{d_o}{d_x})$ in d_x [e]
HVA	TMS_2	3	326	329	3.2	0.1
DOPAC	TMS_3	3	267	270	1.4	3.4
VLA	TMS_3	3	209	212	0.6	0.8
VE	TMS_2	5	209	212[f]	0.5	1.2
CE	TMS_3	5	267	270[f]	1.6	2.0
E	TMS_3TFA	3	355	358	3.8	0.6
NE	TMS_3TFA	3	355	358	3.8	1.3
M	TMS_2TFA	3	297	300	1.9	1.8
NM	TMS_2TFA	3	297	300	2.0	2.1
5-HIAA	TMS_3	5	290	295	0.2	4.5
IAA	TMS_2	6	202	208	0.9	1.0
5-MIAA	TMS_2	4	232	236	0.2	0.2

For abbreviations used, see text; x, number of deuterium atoms incorporated per molecule of internal standard; a, fragment used for monitoring the natural occurring compound; b, fragment used for monitoring the internal standard (d_x) and isotope abundance of the natural occurring compound (d_{x1}); c, percentage d_{x1} in natural occurring compound relative to d_o; e, percentage d_o in internal standard relative to d_x; f, fragments containing three deuterium atoms.

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