DEUTERATED BIOGENIC AMINE METABOLITES: PREPARATION OF RING-DEUTERATED 4-HYDROXY-3-METHOXYMANDELIC ACID

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Selected ion monitoring (SIM) assays are now widely used for measuring the concentrations of monoamine neurotransmitters and their metabolites in urine, blood, cerebrospinal fluid, and brain extracts (1-5). Although these assays have a high degree of sensitivity and specificity, the successful application of them to routine work requires the availability of suitable internal standards. For precise and specific quantitative SIM assays isotopically labelled analogues are generally preferred as internal standards. 4-Hydroxy-3-methoxymandelic acid (commonly referred to as vanillylmandelic acid (VMA)) labelled with deuterium in the methoxy group has been prepared both enzymatically, from S-adenosyl-L-methionine-methyl-d_3 and dihydroxymandelic acid using catechol-O-methyl transferase (4), and chemically from catechol, deuterated iodomethane, and glyoxylic acid (6). Ring-deuterated VMA can be prepared by a hydrogen-deuterium exchange reaction under acid conditions (2), but because of rapid polymerization of VMA under these conditions, the yield is very low and it is difficult to purify the final product (5). We wish to report that in one step using the platinum catalyzed reaction between aromatic ring protons and deuterium oxide (7), we have been able to prepare, in reasonable yield, crystalline ring-deuterated VMA suitable for use as an internal standard in SIM assays.

Platinum oxide (132 mg, 0.6 mmol) was suspended in water (12 ml) and, with care taken to ensure that the temperature did not go above 50°C, reduced by the cautious and stepwise addition of sodium borohydride (920 mg, 24 mmol). The platinum was then washed several times by decantation with water and, after

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warming the slurry to 70°C, the metal was pelleted by centrifugation (3,000 x g 15 min). The pellet was resuspended in D_2O (1 ml) and pelleted again by centrifugation and then blotted dry and placed in equal proportions in four glass ampoules (2 in x 0.75 in) each containing 1.1 ml of D_2O (616 mmol, 99.7 atom percent deuterium) and 10 mg of VMA (0.06 mmol). After connecting the ampoules to a mechanical pump, with a liquid nitrogen trap and an isolation valve in the line, the mixtures were thoroughly degassed. This was achieved by freezing the contents of each ampoule and evacuating the headspaces. With the vacuum line closed the contents of the ampoules were then allowed to thaw. This process was repeated five times, after which, while the D_2O was still frozen and under vacuum, the neck of each ampoule was sealed in a flame. Following incubation for 3 weeks at room temperature with continual agitation the contents of the ampoules were removed and quickly filtered. The filtrates were combined and first acidified (4N HCOOH, 200 µl) and then extracted with ethyl acetate (3 x 4 ml). The pooled organic layers were shaken with activated charcoal and filtered again. The solvent was then removed from the filtrate in a stream of nitrogen, and the remaining yellow oil immediately crystallized when scratched with a spatula after the addition of benzene (2.0 ml). The resulting white needles were collected by filtration and dried under vacuum.

For analysis, the corresponding pentafluoropropionyl (PFP) derivative was prepared (8). Residual reagents were removed in a stream of nitrogen and the derivatized product was redissolved in ethyl acetate for gas chromatography which was carried out on a silanized glass column (6 ft x 2 mm i.d.) packed with 3 percent OV-17. Mass spectra were recorded with a Finnigan 3200 Quadrupole mass spectrometer in which the parameters of the source were manipulated to maximize the signal of the ion at m/e 464 obtained from standard calibration gas (perfluorotributylamine).

The PFP derivative of deuterated VMA showed only one GC peak which had a retention time identical to that of the PFP derivative of authentic VMA (3.1 min when the column was programmed at a rate of 4°C/min from an initial starting temperature of 110°C). The presence of two and predominantly three deuterium atoms in the labelled sample is indicated from a comparison of the

mass spectra (Figure 1). The proposed fragmentation pattern suggests two of the deuterium atoms are located on the aromatic ring with the third deuterium atom on the α-carbon atom of the side chain. NMR analysis (60 MH_z) in d_6-acetone confirmed the complete loss of the aromatic protons on the carbon atoms designated 5 and 6 in Figure 1, with approximately 75 percent loss of the α-proton.

Using reaction mixtures of the same composition as already described, we have studied the effects of both temperature and time on the rate of production of d_3-VMA and the recovery of VMA . At the completion of each incubation, 100 μg of 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA) in 100 μl of H_2O was added as an internal standard to 10 μl aliquots of each reaction mixture. PFP derivatives were then prepared of the lyophilized aliquoted samples. The production of d_3-VMA was estimated from the relative intensities of the molecular ions of the d_0, d_1, d_2 and d_3 species (corresponding to the ions at m/e 622, 623, 624 and 625 respectively). The recovery of VMA was estimated from the combined intensities of the molecular ions of the d_0, d_1, d_2, and d_3 species relative to the intensity of the molecular ion of the PFP derivative of HVA (m/e 460) which eluted immediately after VMA from the GC column.

Production of d_3-VMA was maximal after 3 weeks incubation at room temperature when the d_3-species accounted for 70 percent of the VMA present. At this point the recovery of VMA was estimated to be 79 percent. Incubation of the reaction ampoules at either 35°C or 55°C did not appear to significantly decrease the time necessary for maximal production of the d_3-species, and after 3 weeks at these temperatures the recovery of VMA was estimated to be 32 percent and 21 percent respectively.

Production of deuterated VMA on a larger scale, using 100 mg of VMA, 330 mg of platinum, 2.3 g of sodium borohydride and 7 ml of D_2O, yielded 30 mg of dried crystalline product (30 percent yield). GC/MS analysis of the PFP derivative suggested the product consisted of a mixture of the d_2- and d_3-species in the approximate ratios of 1.3:1 respectively.

As another example of the utility of this method for preparing deuterated

aromatic compounds we have also been able to prepare, using the same approach, ring deuterated 3-methoxy-4-hydroxyphenylethylene glycol, metanephrine and normetanephrine, for use as an internal standard in SIM assays. This method of preparing isotopically labelled internal standards is worthy of merit for the preparation of deuterated water soluble aromatic compounds on a small scale because it avoids complex synthetic chemistry, is simple, and can afford the deuterated products in relatively high yield.

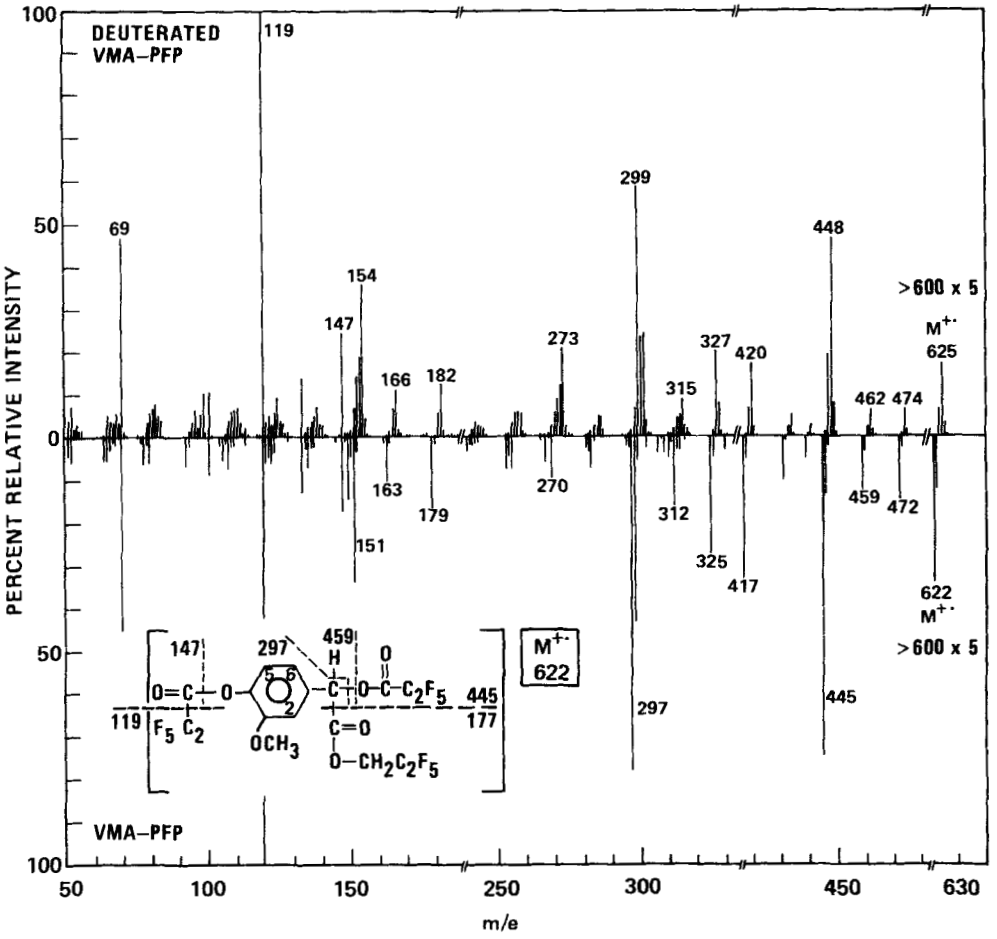

Figure 1. EI mass spectra of the PFP derivatives of a sample of deuterated (top) and undeuterated VMA (bottom).

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