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Deuterium Labelling of Tryptamine, Serotonin and their *N*-Methylated Metabolites Using Solvent Exchange Reactions *

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Technically uncomplicated methods based on catalytic isotope exchange in deuterated solvents are described for the deuteration of tryptamine, serotonin and their *N*-methylated metabolites. Heterogeneous platinum catalysis, homogeneous acid catalysis and their combination have been employed. The properties of the labelled derivatives prepared with each technique as well as their use in mass spectrometric work are discussed.

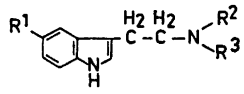
Stable isotopes are widely used as internal standards in quantitative mass spectrometry. Although labelled chemicals have been made commercially available for this purpose, they still often have to be synthesized in the laboratory.

Problems may be encountered when trying to find technically simple and economical means for labelling the compound(s) of interest. In our studies on the *N*-methylating pathway of indoleamine metabolism, deuterated internal standards for tryptamine, *N*-methyltryptamine (NMT), *N*,*N*-dimethyltryptamine (DMT), serotonin, *N*-methylserotonin (NMS) and *N*,*N*-dimethylserotonin (bufotenin) were required.

In this paper we describe the deuteration of these amines using solvent exchange reactions with heterogeneous platinum catalysis and homogeneous acid catalysis.

RESULTS AND DISCUSSION

Acid catalyzed exchange. With acid catalysis using D_2SO_4 in heavy water, complete deuteration of the indole ring sites was achieved (see Table 1). Increasing the strength of the acid (up to 10 M), increasing the reaction temperature from 110 to 140 °C or lengthening the reaction time from 24 to 48 h did not make the labelling proceed further. In the mass spectra of the trimethylsilyl (TMS) derivatives of the labelled products the molecular ions were shifted 4 or 5 mass units in the serotonin and tryptamine derivatives, respectively. The same shifts were observed in the fragments containing the indole nucleus produced by cleavage between the α and β carbons of the ethylamine side chain, whereas the lighter fragments contained no label. Differentiation between the deuteriums of the indole nucleus and those attached to the side chain carbon closest to the indole nucleus is difficult on the basis of the mass spectra. However, based on previous work on the acid catalyzed labelling of tryptophan, it is evident that the deuteriums are attached to the indole nucleus only, and not to the β carbon of the side chain.[1] Practically no decomposition of any of the amines was observed during labelling as indicated by GLC.



Tryptamine,	$R^1 = R^2 = R^3 = H$
NMT	$R^1 = R^2 = H$, $R^3 = CH_3$
DMT	$R^1 = H$, $R^2 = R^3 = CH_3$
Serotonin	$R^1 = OH$, $R^2 = R^3 = H$
NMS	$R^1 = OH$, $R^2 = H$, $R^3 = CH_3$
Bufotenin	$R^1 = OH$, $R^2 = R^3 = CH_3$

Scheme 1.

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Table 1. The relative isotope distribution (%) of the deuterated tryptamine, serotonin and their *N*-methylated metabolites prepared with acid and platinum catalyzed exchange reactions and their combination. The *m/e* value of the molecular ion of the unlabelled amine is given after each compound.

Compound		M	M+1	M+2	M+3	M+4	M+5	M+6	M+7	M+8	M+9	M+10	M+11	M+12	M+13	M+14	M+15
Acid catalyzed exchange																	
Tryptamine-TMS	(232)	–	–	–	1.0	7.0	73.2	14.8	3.3	0.4	–	–	–	–	–	–	–
NMT-TMS	(246)	–	0.9	1.4	4.9	13.0	61.2	18.2	0.4	–	–	–	–	–	–	–	–
DMT-TMS	(260)	–	0.5	1.0	2.1	3.9	73.4	15.9	3.0	0.2	–	–	–	–	–	–	–
Serotonin-di-TMS	(320)	–	–	1.4	7.0	65.7	15.8	8.0	1.8	0.3	–	–	–	–	–	–	–
NMS-di-TMS	(334)	–	0.5	4.3	10.1	16.8	16.0	4.8	0.5	–	–	–	–	–	–	–	–
Bufotenin-di-TMS	(348)	–	0.7	3.4	8.1	57.7	18.1	7.4	4.0	0.6	–	–	–	–	–	–	–
Platinum catalyzed exchange																	
Tryptamine-TMS		–	–	–	–	1.9	2.5	7.3	24.6	27.3	27.7	6.8	1.6	0.3	–	–	–
NMT-TMS		–	–	–	–	0.9	3.3	5.1	9.3	14.4	19.5	15.8	15.8	12.6	2.9	0.4	–
DMT-TMS		–	–	–	0.5	2.2	4.1	7.5	9.8	12.5	15.3	14.5	12.0	9.3	7.1	4.6	0.6
Serotonin-di-TMS		–	–	0.6	2.0	12.2	18.4	23.5	21.1	15.3	4.1	2.4	0.3	–	–	–	–
NMS-di-TMS		–	–	–	1.4	9.7	18.6	26.2	21.4	13.8	5.3	2.1	1.3	0.2	–	–	–
Bufotenin-di-TMS		–	–	–	0.8	7.6	11.0	14.0	17.8	16.9	12.7	7.4	5.1	2.9	2.7	0.9	0.2
Platinum catalyzed exchange after back exchange of the indole ring deuteriums																	
Tryptamine-TMS		1.1	8.5	21.7	26.6	28.7	9.9	2.8	0.8	–	–	–	–	–	–	–	–
NMT-TMS		1.3	7.7	12.8	19.2	24.4	16.7	11.5	3.8	2.1	0.5	–	–	–	–	–	–
DMT-TMS		1.0	6.1	10.6	14.1	18.2	16.6	13.9	9.7	7.5	1.7	0.6	–	–	–	–	–
Serotonin-di-TMS		2.9	11.7	21.3	24.7	30.0	8.2	1.0	0.2	–	–	–	–	–	–	–	–
NMS-di-TMS		1.9	9.3	18.5	24.3	25.6	14.1	3.7	1.6	0.3	–	–	–	–	–	–	–
Bufotenin-di-TMS		2.3	15.3	17.2	23.1	26.7	8.5	3.1	2.2	1.0	0.8	–	–	–	–	–	–

Platinum catalyzed exchange. With platinum catalysis, the indole ring sites were also readily deuterated, the exchange occurring within a reaction period of 3 – 4 h. The deuteration of the side chain was slower and to obtain the derivatives reported in Table 1, a reaction period of 48 h and one back reduction of the catalyst were required. The mass spectra of the labelled products characteristically possessed wide isotope clusters. The most intense fragment in the molecular ion cluster was shifted by 6 – 9 a.m.u. compared to the spectra of the unlabelled amines. The istope shift was also observed in the lighter fragment after side chain cleavage indicating the deuteration of the side chain.

The derivatives presented in Table 1 were obtained in a yield of 25 – 30 %. The yield was slightly better for the tryptamine than for the 5-hydroxytryptamine derivatives. Temperature programmed GLC runs (150 – 270 °C, SE-30 column) of the end products revealed no extra peaks compared to the starting material indicating that the labelled analogues did not contain any decomposition products interfering with the GLC/MS analysis.

Selective partial labelling of the ethylamine side chain. By first deuterating the amines with the platinum-catalyzed exchange and then removing the label from the indole ring sites, analogues deuterated on the side chain only could be obtained. In the spectra of the TMS derivatives of the labelled end products the intensity maxima of the molecular ion clusters were found 4 a.m.u. above those in the spectra of the unlabelled compounds. The lighter fragments produced by side chain cleavage were found to retain the deuteriums introduced by the platinum catalyzed exchange.

For successful use in biochemical work, the labelled derivative must be isotopically stable during storage and under the analytical or experimental conditions. The deuterated amines were stable when stored in ethyl acetate or water solution at neutral pH at – 18 °C for at least 6 months. In acidic water solution back exchange involving the indole ring deuteriums gradually took place, the rate of exchange depending upon the strength of the acid and on temperature. The deuteriums on the side chain were, however, inert to the acid induced back exchange and the side chain retained its isotope composition even at low pH. This is of practical importance since the isolation procedures of the amines often include extractions with acidic water solutions.

The acid catalyzed procedure is the easiest way of labelling the amines discussed and the derivatives obtained are useful as internal standards when not brought under acidic conditions during isolation and analysis.[2] If such conditions are employed, the analogues labelled on the side chain should be used. The wide isotope clusters produced by the platinum catalyzed reaction are an advantage when the analogue is used as a tracer. Previously such easily recognizable isotope clusters have been created by mixing specifically labelled isotopes.[3]

An alternative technique for isotope labelling is the specific chemical synthesis, which recently has been described for a number of tryptamine derivatives.[4] This, however, is sometimes laborious and requires a separate procedure for each compound to be labelled. When catalytic solvent exchange is employed the same procedure can be applied to the deuteration of several different compounds. In addition to the indole derivatives presented here, steroids have also been successfully labelled in our laboratory and by other workers.[5]

EXPERIMENTAL

The amines were purchased from Sigma Chem. Co. (St. Louis, Mo., USA), the platinum oxide (PtO_2) was obtained from Fluka AG (Buchs, Switzerland), CH_3COOD, D_2SO_4 and D_2O (99.75 % isotopic abundancy) from E. Merck AG (Darmstadt, Germany). The silylation reagents were products of Pierce Chem. Co. (Rockford, Ill., USA).

Deuteration with platinum catalysis. Platinum oxide (100 mg) was suspended in 1 ml of heavy water in a test tube and reduced to metallic platinum by introducing by a Pasteur pipette a hydrogen flow through the suspension at a rate of 30 ml per min for 30 min. The amine to be labelled (10 – 20 mg), dissolved in 4 ml of 30 % CH_3COOD in D_2O (v/v), was added. The tube was shaken thoroughly and closed tightly under nitrogen flow and kept at 100 °C for 24 h. The liquid phase was removed, the catalyst washed with D_2O, back reduced as described above, and the reaction was continued for another 24 h. After centrifugation, the liquid phase was removed and adjusted to pH 10 with 20 % aqueous ammonia and extracted several times with 3 ml portions of ethyl acetate. The extracts were pooled and evaporated under nitrogen. The residue was dis-

solved in ethyl acetate to form a stock solution containing about 1 mg per ml of the labelled amine. For use as internal standard small aliquots were evaporated under nitrogen and redissolved in water. The catalyst could be used several times after washing and back reducing it.

Deuteration with acid catalysis. For labelling with acid catalysis the amine was dissolved in 2 M D_2SO_4 in D_2O and kept at 110 °C for 24 h. The isolation of the labelled product was carried out as described above.

Selective labelling of the ethylamine side chain. To obtain analogues labelled on the side chain only, deuterium exchange with platinum catalysis was first carried out. The indole ring deuteriums were removed by incubating the labelled amine in 2 M H_2SO_4 in H_2O at 110 °C for 24 h. The end product was isolated as described above.

Gas chromatography – mass spectrometry. The amines were converted into trimethylsilyl derivatives for GC/MS. The silylation mixture contained pyridine, bis[trimethylsilyl]trifluoroacetamide, *N*-trimethylsilyldiethylamine and trimethylchlorosilane (100:100:30:1, v/v/v/v).[6] The reaction mixture was kept at 85 °C for 30 min. After silylation the reagent was evaporated under nitrogen and the derivative was redissolved in ethyl acetate for injection into the instrument. A Varian Aerograph model 1 700 gas chromatograph coupled to a Varian MAT CH-7 mass spectrometer was used. The GC column was 2 % SE-30 on 80 – 100 mesh Gas-Chrom Q operated at 170 – 190 °C. The 70 eV electron impact spectra were recorded using a Varian Spectro System 100 MS data system. The isotope composition of the labelled derivatives was determined by repetitive scanning over a short mass range (10 – 20 ions) employing a Statos 1® analogue recorder. The contribution of naturally occurring heavy isotopes to the intensities of the MS peaks was not subtracted.

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