INDOLEALKYLAMINE METABOLISM: SYNTHESIS OF DEUTERATED INDOLEALKYLAMINES AS METABOLIC PROBES

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SUMMARY

The synthesis of the deuterium labeled, endogenously occurring, indolealkylamine hallucinogens *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine *via* reduction of amide intermediates with lithium aluminum deuteride is described. The compounds were characterized with ^{1}H, ^{2}H and ^{13}C NMR. These compounds were synthesized for use as probes for investigating the metabolism of these compounds by MAO *via* the *in vivo* kinetic isotope effect.

KEY WORDS: Deuterium Label, Endogenous Hallucinogen, Indolealkylamine, Monoamine Oxidase, Metabolism, *In Vivo* Kinetic Isotope Effect.

INTRODUCTION

The indolealkylamine hallucinogens *N,N*-dimethyltryptamine (DMT, **4**) and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) have been identified as normal constituents of human blood,[1-6] urine,[7-10] cerebrospinal fluid[11,12] (CSF)as well as in a variety of plant species. DMT has also been identified as a putative neurotransmitter or neuromodulatory

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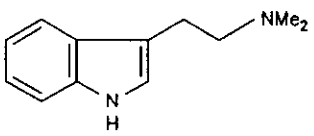
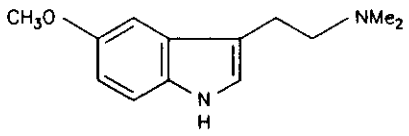
substance in rat brain.[13] The indole-*N*-methyltransferase enzymes capable of synthesizing DMT and 5-MeO-DMT from tryptamine derived from L-tryptophan and *S*-adenosyl-methionine have been described and characterized in human lung, brain, blood and CSF and in various mammalian species.[14]



DMT



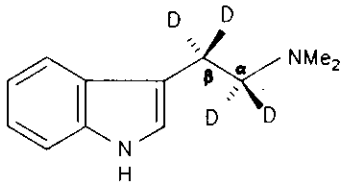
5–MeO–DMT

Numerous groups have attempted to relate mental disorders such as schizophrenia to high brain concentrations of these compounds resulting from perhaps a metabolic error, but a clear relationship between the two has not yet been delineated. However, the *in vivo* production of these interesting compounds strongly suggests that they serve some physiological role which is not yet understood.

For a number of years our group has been interested in studying the pharmacological properties of DMT and related indolealkylamines, in particular the *in vivo* metabolism. Indole-3-acetic acid (IAA) has been identified as the major *in vivo* metabolite of DMT. IAA is formed presumably *via* rapid oxidative deamination by monoamine oxidase (MAO) and is then excreted in urine.[15] These studies are in good agreement with more recent studies which have shown that DMT is essentially cleared from whole rat brain in *ca.* 30 minutes and that DMT is undetectable in the liver and blood plasma after 30 minutes.[16] The dominance of the deamination pathway makes it difficult to study the minor metabolites. Traditionally this difficulty has been surmounted by pre-treating animals with a MAO inhibitor such as pargyline. Inhibition of the major catabolic route leads to "shunting" to the minor metabolic routes, facilitating the study of the minor metabolites. Unfortunately pre-treatment of animals with pargyline can give experimental results which are difficult to interpret since pargyline also inhibits the *N*-oxidation and demethylation of

DMT by 90%.[17] This observation suggests that the reported potentiation of the behavior-disrupting effects and the reported tissue levels of DMT measured in animals pre-treated with pargyline may not have been solely due to MAO inhibition.

Mechanistically, the deamination step presumably involves abstraction of an α-proton in the rate determining step of the reaction followed by deamination. This assumption led our group to speculate that substitution of the α and β protons of the ethylamine side-chain with deuteriums would produce the same effect *via* an *in vivo* kinetic isotope effect as pre-treatment with an appropriate MAO inhibitor (3).



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Thus a ^{2}H-labeled compound would be an attractive alternative to pre-treatment with a MAO inhibitor for metabolic studies.

The results of the metabolism study using α,α,β,β-tetradeutero-*N*,*N*-dimethyltryptamine (α,α,β,β-[^{2}H]$_4$DMT, 3) have been published.[18] The labeled compound was metabolized at a significantly slower rate than proteo-DMT (4) and has indeed proved useful as a metabolic probe for studying the minor metabolic products. Although we concluded that the α-deuteriums were responsible for the observed isotope effect it is impossible to measure the contribution of the α-deuteriums alone, since the ethylamine side-chain was fully deuterated. Although the β-deuteriums are not involved mechanistically, it has been demonstrated in a similar system that the β-deuteriums of aromatic ethylamines change the rate of deamination and produce a small rate enhancement. We are now interested in demonstrating unambiguously that the α-position is responsible for the

observed effect and is the only position involved in the deamination step. For this reason, our present study requires compounds labeled only in the α-position.

In this paper the synthesis and spectral properties of α,α-[^{2}H]$_2$-*N,N*-dimethyltryptamine (**7**) and α,α-[^{2}H]$_2$-5-methoxy-*N,N*-dimethyltryptamine (**10**) and a convenient synthesis of α,α,β,β-[^{2}H]$_4$-*N,N*-dimethyltryptamine (**3**) is presented. Complete ^{1}H, ^{2}H and ^{13}C NMR assignments for α,α,β,β-[^{2}H]$_4$-5-methoxy-*N,N*-dimethyltryptamine (**11**, isotopic purity of 97.5%) are also described.[19]

RESULTS AND DISCUSSION

Benington and Morin previously synthesized **3** in four steps from indole for use as an internal standard, however, its synthesis was not reported.[20] In their synthesis indole was acylated with oxalyl chloride to give the 3-substituted indole which was immediately reacted with etherial dimethylamine to give the keto-amide (**2**). Reduction of the keto-amide with lithium aluminum deuteride (LAD) gave (**3**). Our group now utilizes commercially available indole-3-glyoxylic acid which affords the compound of interest in three steps. In a one-pot reaction the acid is converted to the acid chloride **1** with thionyl chloride which is not isolated but is immediately converted to the same keto-amide **2** by saturating the solution with dimethyl amine gas. In our hands, the preparation of **1** from the acid required low temperature and low concentration in order to prevent highly colored by-products. Attempts to isolate the acid chloride for analysis failed. The ^{1}H and ^{13}C spectra of **2** were rather complicated due to the formation of mesomeric forms and also due to the hindered rotation of the amide group. The ^{13}C spectrum of **2** has been reported but the resonances due to the mesomeric forms were not given.[21] We have tabulated the non-mesomeric assignments in Table 2 and the mesomeric resonances are reported in the Experimental. Reduction of the keto-amide **2** with LAD gave **3** which was readily purified by sublimation under diminished pressure. For spectral comparisons, we also reduced a small amount of **2** with

LAH to give proteo-DMT (**4**). Complete ^{1}H NMR assignments are given in Table 1 and the ^{13}C assignments are described in Table 2. In the ^{1}H NMR of **3**, the α and β-protons appear as apparent triplets centered at δ 2.95 and 2.64, respectfully, and the ^{13}C spectrum

TABLE 1. ^{1}H NMR DATA (δ) FOR DEUTERATED *N,N*-DIMETHYLTRYPTAMINE ANALOGS, INTERMEDIATES AND 5-METHOXY-*N,N*-DIMETHYLTRYPTAMINE ANALOGS AND INTERMEDIATES.

Compound	*H-1*	*H-2* ($J_{1,2}$)	*H-4*[1] ($J_{4,5}$)	*H-5*[2] ($J_{5,6}$)	*H-6* ($J_{6,7}$)	*H-7*	*H-α* ($J_{\alpha-\beta}$)	*H-β*	CH_3
2			7.84 (8.7)d			7.14			2.17
3	8.13	7.00 (2.1)d	7.61 (8.1)d	7.17 (7.8)▼t	7.11 (6.9)▼t	7.34	2.95 (8.1)▼t	2.64 (8.1)▼t	2.34
4	8.14	7.00 (1.5)d	7.69 (7.8)d	7.12 (7.8)▼t	7.11 (6.9)▼t	7.3	2.95 (8.1)▼t	2.64 (8.1)▼t	2.34
6	8.24	7.09 (0)bs	7.65 (7.8)d	7.20) (7.8)▼t	7.13 (7.8)▼t	7.40		3.83	3.03 2.98
7	8.22	7.02 (1.8)d	7.61 (7.8)d	7.18 (7.5)▼t	7.11 (6.9)▼t	7.35		2.34	2.17
9	8.20	7.04 (2.1)d	7.09 (2.1)d		6.88 (8.7)dd	7.23		3.79	3.03 2.98 3.88
10	7.88	7.00 (1.8)d	7.05 (1.8)d		6.86 (8.7)dd	7.25		2.90	2.34 3.86
11	7.04 (2.4)d	6.64 (3.6)d	7.40 (3.6)d		6.98 (9.0)d	8.32			2.34 3.86

[1] The values for H-4 and H-7 may be reversed.
[2] The values for H-5 and H-6 may be reversed.

showed two aliphatic resonances at δ 60.0 (α-carbon) and 23.0 (β-carbon). The remaining aromatic carbons were consistent with those previously reported. In the ^{1}H and ^{13}C NMR spectra of 3, there were no resonances at δ 2.95 and 2.64 and the resonances for the α and

TABLE 2.

^{13}C NMR DATA (δ) FOR DEUTERATED *N,N*-DIMETHYLTRYPTAMINE ANALOGS, INTERMEDIATES AND 5-METHOXY-*N,N*-DIMETHYLTRYPTAMINE ANALOGS AND INTERMEDIATES.

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-α	C-β	CH_2	OCH_3
2	139.0	112.1	126.5[b]	122.4[b]	121.6[b]	112.1	126.4	135.9			34.2	
3	122.0	111.1	119.2	119.2	121.9	112.8	127.5	136.3	[a]	[a]	45.5	
4	122.3	111.2	118.1	118.1	120.7	112.5	127.2	136.1	60.0	23.0	45.1	
6	123.4	111.2	118.7	120.9	118.2	108.1	127.2	136.0	170.5	37.2	34.9 34.6	
7	122.3	111.2	118.0	120.7	118.0	112.5	127.2	136.1	[a]	22.8	45.0	
9	123.5[c]	112.0[c]	100.3[c]	153.8	112.0[c]	108.3	127.4	131.1	171.7	35.6	31.3 37.8	55.8
10	122.3[c]	111.9[c]	100.6[c]	153.8	111.9[c]	113.9	127.7	131.4	[a]	23.5	45.4	55.9
11	122.2[c]	111.8[c]	100.7[c]	153.8[c]	112.0[c]	114.0	127.8	131.4	[a]	[a]	45.5	55.9

a. This peak was greatly attenuated and was barely discernable from the baseline.
b. These values are from Reference 20.
c. These values may be interchanged.

β-carbons were absent. The remaining ^{13}C signals were assigned based on 4 and the ^{2}H NMR showed two singlets at δ 2.92 (α) and 2.62 (β) in good agreement with structure 3.

Reaction of indole-3-acetic acid with thionyl chloride gave the acid chloride **5** which was reacted with dimethylamine to give the amide **6**. Reduction with LAD afforded **7** which was purified by sublimation. Characterization of **7** by ^{1}H NMR showed that the β-protons were shifted upfield to δ 2.34 and now appeared as a singlet. The ^{13}C spectrum lacked the signal at δ 60.0 and the β-C was shifted upfield 0.2 ppm to δ 22.8 consistent with adjacent ^{2}H. The ^{2}H spectrum showed one singlet at δ 2.61.

Using the same procedure, 5-methoxyindole-3-acetic acid was converted to the acid chloride **8** which was then converted to the amide **9**. Usual reduction and purification afforded **10**. The ^{1}H NMR showed a singlet at δ 2.90 and the ^{13}C NMR showed a greatly attenuated peak around δ 60.0. The ^{2}H NMR showed a singlet at δ 2.59. We also characterized **11** by NMR. The ^{13}C NMR lacked signals for the α and β-carbons and the ^{2}H NMR showed two singlets at δ 2.87 (α) and 2.59 (β).

EXPERIMENTAL

General Methods. All reagents and solvents were of the highest available purity and were used without further purification. Lithium aluminum deuteride (98%) was obtained from Aldrich Chemical Company. Thin layer chromatography (TLC) analyses were performed on Kieselgel aluminum backed silica gel 60 F_{254} plates (0.2 mm) obtained from E. Merck and were visualized using an ultraviolet light (254 nm) or I_2. Gas chromatography-mass spectrometry was achieved on a Hewlett-Packard 5985 spectrometer operating at 70 eV. Isotopic purity measurements were made by mass spectrometry and calculations are based on comparisons of the spectra with the corresponding proteo-compounds. Melting points were recorded in capillary tubes on a Mel-Temp apparatus and are uncorrected. All ^{1}H NMR spectra were recorded at 300 MHz with a Nicolet Fourier

Transform Spectrometer in $CDCl_3$ or DMSO-d_6. Resonances are reported downfield from internal tetramethylsilane. Multiplicities are reported as follows: s, singlet; d, doublet; ₸t, an apparent triplet, i.e. $J_{a,b} = J_{b,c}$; m, multiplet, b, broad. ^{13}C NMR spectra were recorded at 75.5 MHz. ^{2}H NMR spectra were recorded at 46.07 MHz using a one pulse experiment in $CHCl_3$ with 1-2% $CDCl_3$ serving as an internal standard. The ^{13}C NMR spectra were recorded at 75.5 MHz. Infrared spectra were recorded on a Nicolet IR\42 spectrometer. Microanalyses were performed by Atlantic Microlabs, P.O.Box 2288, Norcross, Ga. 30091-9990.

2-(3-Indolyl)-glyoxal Chloride (1). A solution of indole-3-glyoxylic acid (1.0 g, 5.3 mmol) in ether (100 mL) was stirred and cooled in a dry-ice bath for 15 min. $SOCl_2$ (2.0 mL, 17 mmol) was then gradually added to the solution. TLC analysis (10% MeOH-90% Tol) of the mixture showed complete disappearance of indole-3-glyoxylic acid after 1 h and formation of a higher running compound with R_f 0.39. The product was diluted with a large amount of dry ether and used directly without purification.

2-(3-Indolyl)-N,N-dimethylglyoxalamide (2). Dimethylamine gas was passed through the etherial solution of **1** for 3-5 min and the reaction mixture was stirred continuously for an additional 20-30 min. Excess solvent was removed to give **2** (0.89 g, 4.1 mmol) as a solid in 77% yield based on indole-3-glyoxylic acid. Mp = 184-185°C (lit. 184-185°C)[19]; ^{13}C NMR δ ($CDCl_3$-DMSO-d_6) 136.8, 136.5, 135.9, 126.4, 125.3, 124.0, 123.8, 123.2, 122.8, 122.2, 121.8, 112.64, 112.0, 40.2, 39.9, 39.6, 39.4, 37.9, 37.4, 35.1, 34.2; MS 216 [M+], 144 (100%), 116, 89, 72; IR 1618 cm^{-1} (CO).

α,α,β,β-[^{2}H]$_4$-N,N-Dimethyltryptamine (3). To a stirred suspension of LAD (0.1 g, 2.4 mmol, 98%) in dry ether (8 mL) was gradually added the amide **2** (0.1 g, 0.46 mmol) in CH_2Cl_2 (5 mL). The mixture was refluxed for 3-4 h in an oil bath, cooled in an ice bath, and treated with several drops of water to decompose excess LAD reagent. The reaction

was vacuum filtered to remove any remaining solids, dried ($MgSO_4$), and solvents removed. The yield was 67% (0.06 g, 0.31 mmol). Mp = 47-49°C; MS 192 [M+], 132, 60 (100%). Isotopic purity = 94%.

N,N-Dimethyltryptamine (4). LAH reduction of **2** gave **4** in 76% yield (0.066 g, 0.35 mmol). Mp = 44-46°C (lit. 45-46°C); MS 188 [M+], 130, 77, 58 (100%).

2-(3-Indolyl)-acetyl Chloride (5). Indole-3-acetic acid (2.0 g, 11.4 mmol) was converted to the acid chloride (ether, 200 mL; $SOCl_2$, 2.0 mL, 17 mmol) using the procedure described for the synthesis of **1**.

2-(3-Indolyl)-N,N-dimethylacetamide (6). Acid chloride **5** was converted to the amide **6** using dimethylamine as described for the synthesis of **2**. Sublimation under diminished pressure gave 1.6 g (7.9 mmol, 69%) based on indole-3-acetic acid. Mp = 117-119°C. MS 202 [M+], 130 (100%), 77, 72; IR 1634 cm^{-1} (CO).

Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found C, 71.13; H, 6.96; N, 13.75.

α,α-[^{2}H]$_2$-N,N-Dimethyltryptamine (7). LAD (0.1 g, 2.4 mmol, 98%) was suspended in dry ether (8 mL). The mixture was stirred and the amide **6** (0.25 g, 1.24 mmol) in CH_2Cl_2 (100 mL) was added over 5 min. The reaction was refluxed for 2-3 h in an oil bath at which time TLC analysis (MeOH) indicated disappearance of **6** and formation of a new spot at the origin. Workup as described for the synthesis of **3** and sublimation gave 0.16 g (0.84 mmol) of **7** in 68% yield. Mp = 44-46°C (lit. Mp = 44-46°C).[19] MS 190 [M+], 130, 60 (100%). Isotopic purity = 97%.

Anal. Calcd for $C_{12}H_{14}D_2N_2$: C, 75.76; H plus D as H, 9.52; N, 14.73. Found C, 75.09; H plus D as H, 8.56; N, 14.45.

2-(5-Methoxy-3-indolyl)-acetyl Chloride (8). 5-Methoxyindole-3-acetic acid (0.5 g, 2.44 mmol) was converted to the acid chloride (CH_2Cl_2, 100 mL; $SOCl_2$, 1.0 mL, 8.5 mmol) using the procedure described for the synthesis of **1**.

2-(5-Methoxy-3-indolyl)-N,N-dimethylacetamide (9). The acid chloride **8** was diluted with CH_2Cl_2 and immediately treated with dimethylamine gas. The excess solvent was removed and the crude product sublimed under diminished pressure. The yield was 74% (0.42 g, 1.8 mmol) based on 5-methoxyindole-3-acetic acid. Mp = 78-80°C. MS 232 [M+], 160 (100%), 145, 117, 72; IR 1629 cm^{-1} (CO).

Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found C, 67.00; H, 6.97; N, 12.00.

α,α-[^{2}H]$_2$-5-Methoxy-N,N-dimethyltryptamine (10). A suspension of LAD (0.1 g, 2.4 mmol, 98%) in dry ether (8 mL) was stirred and the amide **9** (0.25 g, 1.08 mmol) in CH_2Cl_2 (10 mL) gradually added. The mixture was refluxed for 2-3 h in an oil bath and then cooled to room temperature. Usual workup and purification gave **10** in 68% yield (0.16 g, 0.73 mmol) based on the amide **9**. Mp = 49-51°C. MS 220 [M+], 176, 160, 145, 132, 117, 60 (100%). Isotopic purity = 99.7%.

Anal. Calcd for $C_{13}H_{16}D_2N_2O$: C, 70.88; H plus D as H, 9.14; N, 12.72. Found C, 70.22; H plus D as H, 8.32; N, 12.09. Despite repeated sublimations and drying, we were unable to resolve the elemental analysis difference.

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