

PREPARATION OF [<sup>3</sup>H]N,N-DIMETHYLANILINE BY MICROWAVE DISCHARGE ACTIVATION  
OF TRITIUM GAS. ABSENCE OF RING SATURATION

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SUMMARY

[<sup>3</sup>H]N,N-Dimethylaniline was prepared from N,N-dimethylaniline by tritium labeling by microwave discharge activation of tritium gas; the product was formed without any ring saturated side products. High specific activity and high yield were achieved by input of high microwave power to excite the plasma and by dispersion of substrate on silica-alumina pellets to increase the contact surface with tritium gas. Circulation or no circulation of tritium gas during labeling did not effect the yield or the specific activity. Dispersion on supported Ni catalyst caused an increase in the specific activity of [<sup>3</sup>H]N,N-dimethylaniline than on plain silica-alumina. The lack of ring saturation in N,N-dimethylaniline in contrast to complete ring saturation in benzene and benzoic acid has been interpreted to indicate the involvement of tritium ions in addition to tritium atoms in tritium labeling.

Key Words: Tritium labeling, tritium ion, microwave discharge,  
N,N-dimethylaniline, substrate support, nickel catalyst

INTRODUCTION

Microwave discharge activation of tritium gas generates activated tritium species that can tritiate organic molecules [1,2] but also cause phenyl ring saturation, leading to the formation of large amounts of saturated analogs [3,4]. For example, benzene reacts with activated tritium giving rise predominantly to [<sup>3</sup>H]cyclohexane, toluene to [<sup>3</sup>H]methylcyclohexane, benzoic acid to [<sup>3</sup>H]cyclohexane carboxylic acid and phenylalanine to [<sup>3</sup>H]cyclohexylalanine. Ring saturation is a serious drawback of using the microwave discharge activation method for tritium labeling. Our recent studies [5] show that ring saturation is not universal and is dependent upon the nature of the substituents in the ring. N,N-Dimethylaniline is one of the compounds studied that can be

labeled with activated tritium giving excellent yield with no ring saturation. In this report we discuss the experimental conditions under which *N,N*-dimethylaniline can be tritiated to yield the labeled parent of high radiochemical purity and high specific activity. On account of its stability under these conditions, *N,N*-dimethylaniline is a suitable reagent for comparing the merits of different labeling methods.

#### RESULTS AND DISCUSSION

[<sup>3</sup>H]*N,N*-Dimethylaniline (*N,N*-DMA) was obtained by tritium labeling of *N,N*-dimethylaniline by microwave discharge activation of tritium gas, without formation of the saturated product [<sup>3</sup>H]*N,N*-dimethylcyclohexylamine. A minor labeled side product was identified as [<sup>3</sup>H]aniline, present in most cases in less than one percent but in a few cases, as much as five percent of the total radioactivity. The yield and specific activity of [<sup>3</sup>H]*N,N*-DMA prepared under different experimental conditions are given in Tables 1-4.

*N,N*-Dimethylaniline, when labeled as neat liquid at -196°C, gave only a low yield of [<sup>3</sup>H]*N,N*-DMA. Analysis by gas-liquid radiochromatography (GLRC) [3] showed that [<sup>3</sup>H]*N,N*-DMA was formed in 4.3% to 29.0% of the total incorporated tritium activity. Tritium distribution in the various solvent fractions generated in the process of purification is given in Table 1. At a tritium concentration of 3 torr and a microwave power of 20 W and 50 W, the major fraction of the labile tritium not incorporated in [<sup>3</sup>H]*N,N*-DMA, appeared mainly in the aqueous phase. Decreasing the tritium concentration from 3 torr to 1 or 2 torr and increasing the microwave power to 90 W caused a shift of the major fraction of the labile tritium activity to the *N*-hexane fraction, indicating that at higher microwave power and lower tritium pressure side products of a non-polar nature were formed. [<sup>3</sup>H]*N,N*-DMA labeled at higher microwave power inputs gave a higher yield than that labeled at lower microwave power.

Table 2 shows that the specific activity of [<sup>3</sup>H]*N,N*-DMA labeled on (980-25) and on Ni(980-25) increased from 9.75 to 21.83 Ci/M and from 21.96 to 42.17 Ci/M, respectively, when the microwave power input was increased from 20 W to 90 W. The peak percent of the labeled *N,N*-DMA as determined by GLRC ranged from 22.61 to 66.23% of the injected activity when crude and from 47.42 to 90.63%

after purification; these values were considerably higher than those of [<sup>3</sup>H]N,N-DMA labeled as neat liquid. The specific activity and yield increased with an increase in microwave power from 20 W to 90 W when the tritium concentration remained constant at about 3 torr. It may also be pointed out that [<sup>3</sup>H]N,N-DMA labeled on Ni(980-25) showed a consistently higher specific activity than that labeled on plain silica-alumina (980-25) support.

Table 3 shows the range of variations of the yield and specific activity of [<sup>3</sup>H]N,N-DMA labeled on supports and the effect of tritium gas circulation on labeling. Individual pellets of (980-25) and Ni(980-25) impregnated with the substrate were exposed to tritium plasma with and without tritium gas circulation. As a result of the heterogeneity of the reaction, the labeled pellets showed wide variations in activity. Circulation or no circulation of tritium gas during labeling has negligible effect on the yield and specific activity of the labeled product. Wessels et al. [6] also observed the same phenomenon in the labeling of peptides by the microwave discharge activation method. In the absence of gas circulation, the pellet(s) located near the tip of the cold finger, with more air space for tritium exchange and diffusion, gave higher yields of [<sup>3</sup>H]N,N-DMA than the pellets situated elsewhere on the cold finger. The spread in radiochemical yields among individual pellets was less pronounced with tritium gas circulation than without. This is apparent from the range and the standard deviations of the assay values, shown in Table 3. The support Ni(980-25) gave higher specific activity values of [<sup>3</sup>H]N,N-DMA than the support (80-25) under similar conditions.

Table 4 shows that labeling of N,N-DMA by adsorbed tritium produced [<sup>3</sup>H]N,N-DMA with a high yield and high specific activity. The labeled product contained fewer labile or exchangeable tritiums in the molecule as compared with samples labeled by other methods; this became evident from its liquid radiochromatogram in which no other radioactive peaks were detected. The recovery of the injected radioactivity from the HPLC column varied from 89 to 107%. On the other hand, HPLC analysis of [<sup>3</sup>H]N,N-DMA labeled on plain silica-alumina and on supported Ni catalyst showed a prominent radioactive peak in the region of tritiated water, which was probably caused by tritium back exchange of

the injected material on column or by the presence of labeled degradation products. Labeling of [ $^3\text{H}$ ]N,N-DMA as neat liquid or dispersed on glass fiber paper was less effective than on silica-alumina catalyst support and yielded [ $^3\text{H}$ ]N,N-DMA of low yield, with specific activities in the range of 1 to 2 Ci/M.

In contrast to benzene and benzoic acid [4] which form mostly the saturated analogs when labeled either in the neat form or dispersed on silica-alumina by microwave discharge activation of tritium gas, N,N-DMA yields only the labeled parent compound unaccompanied by the saturated analog N,N-dimethylcyclohexylamine. These results show that the phenyl ring saturation is determined by the nature of the substituent. The ability of the substituent groups such as N,N-dimethylamino and carboxyl groups to control the ring saturation by virtue of their electron donating and electron withdrawing properties is a clear indication of the involvement of tritium ions in addition to tritium atoms in this method of tritium labeling. According to the theoretical calculations of Borkman et al., [7] ionic species of tritium such as  $\text{T}_3^+$ ,  $\text{T}_2^+$  ions form reaction complexes with glycine and glycine zwitter ion, whereas neutral species of tritium and tritium atoms do not. The usefulness of  $\text{T}_3^+$  ion in labeling peptides has been confirmed by Bush et al. [8] and Richardson et al. [9]. The detailed mechanism of phenyl ring saturation by tritium is not known [10] but the characteristics of the low pressure plasma suggest, it may involve reactions in which the electrons may play an important role in ring saturation. Obviously, many factors such as the purity of tritium gas, nature of the supporting surface, and the substrate can influence tritium incorporation. For molecules such as N,N-dimethylaniline, labeling by microwave discharge activation of tritium gas, when carried out at high microwave power activation and with appropriate substrate dispersion, can be a very useful method of the preparation of marked molecules.

#### MATERIALS AND METHODS

N,N-Dimethylaniline (N,N-DMA) from Chem Service, Media, PA, was used without purification. It was labeled with tritium as neat liquid, in dispersed form on silica-alumina catalyst support (#980-25, Davison Chemical Co.,

Baltimore, MD) [11] and on supported nickel catalysts (5% Ni on #980-25) [12] or by using adsorbed tritium [13]. Detailed procedures are as follows:

(a) Labeled as neat liquid - About one-tenth of one milliliter of N,N-DMA was placed on the stage of the cold finger of the tritiation apparatus described elsewhere [3]. The system was evacuated to approximately 100-200 m Torr to remove all the volatiles before adding liquid nitrogen to the cold trap and further evacuated down to 10 m Torr before the system was isolated. About 1-5 torr of tritium gas was admitted to the isolated system and allowed to circulate for 5 minutes to condense out at -196°C any gaseous impurities that might be present prior to initiating the discharge with Tesla coil. The microwave discharge was maintained for 5 minutes at power levels varying from 20 W to 100 W. At the end of the discharge, the unused tritium was removed; the system was flushed with helium and the labeled product was rinsed off the cold finger with 10 ml of n-hexane.

(b) Labeled on support - N,N-Dimethylaniline was labeled in the dispersed form sorbed on 1/8" dia. pellets of supported Ni catalyst and plain silica-alumina catalyst support. Each of the five pellets was impregnated with 15 microliters of N,N-DMA approximately one hour prior to tritiation. The silica-alumina pellet (980-25) weighed approximately 25 mg each with a surface area of about 325 m<sup>2</sup>/g [11], and the molecule N,N-DMA has an area of 31.5 sq. Å measured from the Dreiding model. It may be calculated from these values that the pellets were covered with about three monolayers of N,N-DMA for reactions with tritium. These impregnated pellets were labeled with activated tritium according to the procedure described above. To determine the effects of circulation of tritium gas on labeling, plain silica-alumina (980-25) and supported Ni catalyst [Ni(980-25)] impregnated with N,N-DMA were placed together mixed on the cold finger of the tritiation apparatus and exposed to activated tritium. Labeled products were eluted off the individual pellets with 1 ml of benzene; an aliquot of it was purified and analyzed.

(c) Labeled with adsorbed tritium - Supported Ni catalysts were charged with tritium by exposure for 20 minutes to a tritium plasma of 5 torr pressure, generated at a microwave power of 20 W or 100 W [13]. The "tritium charged" pellets were retrieved from the apparatus and immersed in 0.1 ml of N,N-DMA. The labeled product [ $^3\text{H}$ ]N,N-dimethylaniline thus formed was radioassayed and analyzed.

#### Purification and Analysis

[ $^3\text{H}$ ]N,N-Dimethylaniline was purified by extraction of the benzene or n-hexane solution (fraction 1) containing the crude labeled product with an equal volume of 1N hydrochloric acid. The aqueous phase was washed with benzene (fraction 2) and made alkaline with solid sodium hydroxide. [ $^3\text{H}$ ]N,N-Dimethylaniline was back extracted from the aqueous solution (fraction 3) into benzene (fraction 4). All the fractions were radioassayed by liquid scintillation counting. Fraction 4 containing [ $^3\text{H}$ ]N,N-DMA was analyzed on SE-30 and Carbowax 20 M columns by GLRC, and the radioactive peaks were identified by their retention indexes according to the method of Buchman et al. [14]. The retention indexes of N,N-dimethylaniline and aniline have values of 1085 and 970 on SE-30 column and 1575 and 1767 on Carbowax 20 M column, respectively.

Labeled samples were prepared for determination of specific activity by high performance liquid chromatography (HPLC). In order to avoid interference with the detection of N,N-DMA, benzene was replaced with ethyl acetate in the extraction procedure. The tritiated N,N-DMA was purified by isocratic HPLC using methanol:water (70:30, v/v) as solvent on a 5  $\mu\text{m}$  Nova Radial Pak LC cartridge (Waters) or a 25 cm x C-18 Ultrasphere (Beckman-Altex) column. The effluent was collected in 0.25 ml fractions with Pharmacia FRAC 100 fraction collector into pico-vials (Packard) and were measured for radioactivity by liquid scintillation counting, after addition of 4 ml of Insta-Gel (Packard) to each vial. The mass peak was monitored at 254 nm and converted to micromoles using a standard curve of N,N-DMA. Peak areas were determined from the peak heights and in few cases by an electronic integrator (Hewlett-Packard Model 3392). The specific activity of [ $^3\text{H}$ ]N,N-DMA was obtained from its mass peak and the corresponding radioactivity peak.

TABLE I  
 THE EFFECT OF MICROWAVE POWER INPUT AND TRITIUM PRESSURE ON THE YIELD OF  $[^3\text{H}]N,N$ -DIMETHYLANILINE,  
 LABELED AS NEAT LIQUID

Sample No.	Microwave Power, W	Tritium Pressure, Torr	Activity of Crude Products, mCi	GLRC Peak, a %	Distribution of Radioactivity in Purification, b %	(Fr. 1) n-Hexane	(Fr. 2) Benzene	(Fr. 3) Aqueous Phase	(Fr. 4) $[^3\text{H}]N,N$ -DMA in Benzene
1008-56	20	3.14	9.90	6.66		15.57	3.28	72.13	9.02
1009-58	50	3.13	6.85	4.30		5.88	3.27	76.00	12.85
1022-54	90	1.03	9.61	7.44		42.70	5.40	25.70	26.20
1022-53	90	2.23	6.21	29.00		53.30	4.80	23.35	18.50
1009-57	90	3.20	5.05	10.14		16.43	4.72	63.66	15.20
1022-55	90	3.11	4.31	9.00		15.30	8.40	49.90	27.40

a % of the injected activity that appeared in the  $[^3\text{H}]N,N$ -dimethylaniline peak.

b See text.

TABLE 2  
DEPENDENCE OF SPECIFIC ACTIVITY AND YIELD OF [ $^3\text{H}$ ]N,N-DIMETHYLANILINE ON SUBSTRATE SUPPORT AND  
MICROWAVE POWER INPUT<sup>a</sup>

Sample No.	Microwave Power, W	Tritium Pressure, Torr	Support <sup>b</sup>	Yield, mCi		GLRC Peak <sup>c</sup> , %		Specific Activity, Ci/M
				Crude	Purified	Crude	Purified	
1015-54	90	3.17	A	4.50	2.13	49.35	68.57	21.83
			B	7.30	3.18	66.23	90.63	42.17
1015-55	60	3.18	A	4.50	1.55	44.67	47.42	19.31
			B	4.80	2.70	70.78	71.33	31.80
1015-56	20	3.22	A	2.80	0.60	22.61	55.91	9.75
			B	4.55	2.27	39.37	77.43	21.96

<sup>a</sup> All samples were exposed to tritium plasma for 5 minutes.

<sup>b</sup> Each pellet of support contained 15 microliters of N,N-dimethylaniline. A = plain silica-alumina (#980-25) catalyst support, and B = 5% Ni on silica-alumina (#980-25) catalyst support.

<sup>c</sup> % of injected radioactivity that appeared as [ $^3\text{H}$ ]N,N-dimethylaniline.

TABLE 3  
 VARIATION OF SPECIFIC ACTIVITY AND YIELD OF *N,N*-DIMETHYLANILINE LABELED ON SUPPORTS WITH OR WITHOUT  
 TRITIUM GAS CIRCULATION<sup>a</sup>

Sample No.	Support	T <sub>2</sub> Pressure, Torr	Circulation <sup>b</sup>	Mean Activity Per Pellet, mCi/c	Range of Specific Activity Ci/M	Specific Activity, (Mean $\pm$ S.D.) Ci/M
1017 - 52A(5) <sup>d</sup>	A	3.15	+	1.43 $\pm$ 0.47	11.25 - 45.18	29.89 $\pm$ 13.90
1017 - 52B(5)	B	3.15	+	1.34 $\pm$ 0.60	44.32 - 58.58	49.68 $\pm$ 5.39
1017 - 53A(5)	A	3.02	-	2.05 $\pm$ 0.41	20.45 - 34.79	30.85 $\pm$ 5.94
1017 - 53B(5)	B	3.02	-	1.29 $\pm$ 0.068	23.49 - 39.31	34.79 $\pm$ 7.33
1106 - 54B(8)	B	3.07	+	0.47 $\pm$ 0.055	20.0 - 40.0	26.87 $\pm$ 5.98
1106 - 55B(8)	B	3.09	-	0.57 $\pm$ 0.10	19.17 - 42.94	29.14 $\pm$ 7.83

<sup>a</sup> Microwave power input = 90 W.

<sup>b</sup> The sign + indicates circulation of T<sub>2</sub> gas in the apparatus during labelling, and the sign -, no circulation.

<sup>c</sup> Mean crude activity of each pellet in mCi.

<sup>d</sup> The number in parenthesis refers to the number of labeled pellets individually analyzed.

TABLE 4

EFFECTS OF SUPPORT, TRITIUM PRESSURE AND MICROWAVE POWER INPUT ON THE  
SPECIFIC ACTIVITY OF [<sup>3</sup>H]N,N-DIMETHYLANILINE

Sample No.	Support	Microwave Power, W	T <sub>2</sub> Pressure, Torr	Exposure Time, Min	Specific Activity, Ci/M
<u>(a) On Support</u>					
621 - 52	A <sup>a</sup>	100	2.05	5	72.70
213 - 64	A	80	3.88	5	22.70
1106 - 51	GFP <sup>b</sup>	90	2.01	5	1.21
<u>(b) With Adsorbed Tritium</u>					
1017 - 51	B <sup>c</sup>	20	5.17	20	64.77
1106 - 52	B	90	5.04	20	52.39
<u>(c) As Neat Liquid</u>					
1022 - 55	(neat)	90	3.15	5	1.78

<sup>a</sup> A = plain silica-alumina (#980-25) catalyst support.

<sup>b</sup> GFP = glass fiber paper (1 x 1 cm<sup>2</sup>).

<sup>c</sup> B = 5% Ni Silica-alumina (#980-25) catalyst support.

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