NOTES

Tritium Labeling of 3,2'-Dimethyl-4-Aminobiphenyl by Catalytic Exchange*

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Tumor induction of various types in man and other animals has been associated with a variety of aromatic amines. Among these compounds, 3,2'-dimethyl-4-aminobiphenyl** has been utilized as a model substance. Subcutaneous injection of this compound into rats has given rise to neoplasms of the colon and at other locations [1, 5] but not at the site of injection. Some endemic and morphologic similarities have been noted between the occurrence of colonic neoplasms in man and DMAB-induced colonic neoplasms in the rat [4]. Induction of colonic tumors by this agent has been associated with the fecal stream [2]. DMAB has been labeled with tritium by catalytic exchange to facilitate the study of its metabolism.

All samples were counted in a toluene scintillation solution containing 4 g of PPO** and 100 mg of dimethyl POPOP** per liter. Quantitative tritium analyses were accomplished by counting samples along with a graded quenched series of standards and calculating the results with a FORTRAN tracer calculation program written in this laboratory.

Vacuum distilled DMAB, 50 mg, in 0.30 ml of acetic acid was treated by stirring overnight at 80 °C with 10 Ci of tritium oxide and 25 mg of reduced platinum catalyst [3]. The product was obtained from the reaction mixture and excess tritium removed by treatment with 10 ml of dilute hydrochloric acid followed by removal of the solvent *in vacuo*. The crude hydrochloride had a specific activity of 16 mCi/mg***.

The hydrochloride, suspended in 10 ml of 0.1 N hydrochloric acid, was treated with 1.5 ml of 1 N NaOH and allowed to equilibrate for several hours. DMAB as the free base was extracted with dichloromethane and washed neutral with water. The alkaline extracts with the alkali labile tritium contained 200 mCi and the recovered free base 510 mCi of ^3H.

DMAB-^3H was chromatographed over a 1 × 5 cm column of neutral aluminum oxide (Woelm) deactivated with 6.5 g of water per 100 g of dry alumina. Elutions were with 15 ml aliquots of hexane, hexane-benzene mixtures, and benzene. In the initial chromatogram, each eluate contained significant activity. The 20 and 30 percent benzene fractions contained the DMAB-^3H

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** The abbreviations used are : 3,2'-dimethyl-4-aminobiphenyl, DMAB; 2,5-diphenyloxazole, PPO; 1,4-bis-2(5-methyl-5-phenyloxazolyi)-benzene, dimethyl POPOP.

*** The initial catalytic exchange was performed by New England Nuclear Corporation, 575 Albany Street, Boston, Massachusetts.

and were combined. Carrier DMAB, 26.5 mg, was added and the diluted preparation rechromatographed. The elution pattern was typical for DMAB in this system. Chromatography of other fractions showed that no significant DMAB-^{3}H activity was present.

Celite, 6 g, was coated with 3 ml of the polar phase of the system cyclohexane 374 ml, methanol 375 ml, and water 12.7 ml and an 8.5 × 240 mm column was formed. The DMAB-^{3}H from the second alumina chromatography was applied to the column in mobile phase and 2 ml fractions collected. The main peak of activity and DMAB was eluted between 16 and 24 ml. A small amount of yellow pigment, only partially resolved, preceeded the elution of the main peak.

The DMAB-^{3}H from the above chromatograph was rechromatographed in a similar way in the partition system cyclohexane 300 ml, nitromethane 75 ml, methanol 15 ml. The DMAB-^{3}H was eluted between 28 and 38 ml. The residue of the yellow pigment was eluted between 14 and 18 ml.

An aliquot of the DMAB-^{3}H was evaporated and redissolved in absolute alcohol. Concentration was determined by ultraviolet spectrophotometry (log $\varepsilon = 4.158$, 2,630 Å), and ^{3}H by scintillation counting. The product had a specific activity of 4.55 mCi/mg and 111 mCi were finally recovered.

Carrier DMAB hydrochloride was recrystallized several times from methanol-acetone and yielded white plates, m.p. 192-204° C (Kofler block). An aliquot of DMAB-^{3}H from the second partition chromatograph, 1.10×10^{-3} mCi was mixed with 16.91 mg of carrier DMAB hydrochloride to yield a preparation with a calculated specific activity of 6.53×10^{-5} mCi/mg. The preparation was then crystallized twice from methanol. The assays before and after crystallization are shown in Table 1.

TABLE 1. Crystallization of DMAB-^{3}H hydrochloride.

Sample	mCi/mg × 10^{-5}
Carrier diluted DMAB-HCl	6.58
First Crystallization	6.62
Second Crystallization	6.44

DMAB-^{3}H, 1.5 mCi, in 1 ml of benzene was diluted with 9 ml of hexane. The solution was extracted five times with 1 N sodium hydroxyde and three times with water. Approximately 20 minutes were allowed for each alkaline wash to equilibrate with the DMAB-^{3}H. Each aqueous wash was 1 ml. The aqueous washes were assayed for tritium and contained a total of 9.3×10^{-4} percent of the starting activity. Of this activity, 4.8×10^{-4} percent was in the first alkaline extract.

DMAB is slightly volatile and is also oxidized rather easily by atmospheric oxygen. Carrier DMAB was added to the preparation so as to lower its specific activity to the level desired for its use in biological experiments and to minimize loss of activity by volatilization. It was found sufficient to simply exclude oxygen during solvent evaporations by using a nitrogen stream. The labeled compound has been stored in dilute benzene solution at 5° C and has shown good stability.

The final preparation of DMAB-^{3}H was homogeneous by the criteria of chromatography of the free base and crystallization as the hydrochloride. The ultraviolet spectrum of the material used for the determination of the specific activity was identical with that of the pure compound. Extraction with 1 N sodium hydroxide removed an insignificant amount of activity. The DMAB-^{3}H preparation satisfies the usual requirements for purity and stability for biological experimentation.

Although tritium has been used to label DMAB, the objective is to follow the carbon skeleton of the compound. Ring metabolism of DMAB-^{3}H would be expected to release tritium activity. Preliminary experiments in rats have shown that relatively small amounts of tritium activity are released to form ^3H_2O *in vivo*. The retention of tritium in the molecule allows the tracing of DMAB in biological experiments.

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