N-^{14}C-MONOMETHYLATION OF THIONIN

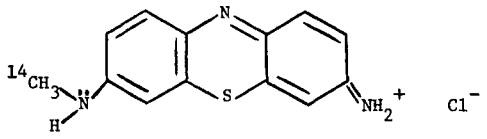
Received on December 16, 1974.

Since the report by Klopper and Moe (1) that Toluidine Blue O caused a blue coloration of the pancreas and parathyroid glands, there has been much interest in preparing a radioactive derivative of Toluidine Blue O for use as a pancreas and parathyroid scanning agent (2-4). Results in this and other laboratories using ^{35}S-Toluidine Blue O indicated that the phenothiazine ring of the dye molecule was not concentrated in the pancreas of experimental animals (5, 6).

In order to study the possibility that demethylation of the dye molecule by tissues other than the pancreas was responsible for the blue coloration of the pancreas, we wished to study the distribution and fate of the N-methyl groups on Toluidine Blue O. However, this dye is difficult to prepare with labeled N-methyl groups since the molecule has two primary amino groups but is not symmetrical. We chose, therefore, to prepare 3-imino-7-methyl-^{14}C-amino-3H-phenothiazine hydrochloride (N-methylthionin, Azure C) (I), a diaminophenothiazine dye which has similar chemical properties to Toluidine Blue O.

We sought a method which would allow the introduction of a N-methyl group on only one of the two primary amino groups. By using mild hydrogenation conditions, 1.6 atmospheres of pressure at room temperature, and a platinum oxide catalyst and by carefully manipulating the concentration of the reactants, we were able to obtain N-methyl-^{14}C-thionin hydrochloride in good yield (Table I.). The desired product, N-methyl-^{14}C-thionin hydrochloride, having a specific activity of 28.0 mCi/mmole, was obtained with an overall radiochemical yield of 28.7%. More importantly, N-methyl-^{14}C-thionin hydrochloride accounted for 84% of the radioactivity recovered. In addition to the major product, 3-imino-7-

dimethyl-$^{14}C_2$-amino-3-H-phenothiazine hydrochloride (Azure A) accounted for 13.5% of the radioactivity and 3-methyl-^{14}C-imino-7-methyl-^{14}C-amino-3-H-phenothiazine hydrochloride accounted for 2% of the radioactivity. The small amount of radioactive contaminants were removed by column chromatography to yield N-methyl-^{14}C-thionin hydrochloride for use in distribution and metabolism studies.



I

N-Methyl-^{14}C-thionin Hydrochloride

TABLE 1

Thin Layer Chromatography of Products from Reductive Methylation of Thionin

Compound	R_f	Activity (μCi)
N-Methyl-^{14}C-thionin Hydrochloride	0.48	285.5
N,N-Dimethyl-$^{14}C_2$-thionin Hydrochloride	0.40	46.8
N,N'-Dimethyl-$^{14}C_2$-thionin Hydrochloride	0.33	6.71
N,N,N'-Trimethyl-$^{14}C_3$-thionin Hydrochloride	0.27	1.85

EXPERIMENTAL

One milligram of formaldehyde-^{14}C (0.017 mmole, 59.2 mCi/mmole) was added to 0.034 mmole of thionin[a] in ethanol. One and one-half milligrams of platinum oxide was added, and the mixture was stirred at room temperature for 3 hours at 1.6 atmospheres of H_2 in a microhydrogenator.

The reaction mixture was placed on a dry chromatography column of silica gel (70-325 mesh) and eluted with ethanol, conc. hydrochloric acid (99:1). The 50 ml fractions collected from the column (4x50 cm) were evapor-

a Allied Chemical Corp., 40 Rector St., New York 6, N.Y.

ated to dryness. The identity and purity of each fraction were determined using a thin-layer chromatography system previously reported to separate the Azure dyes on the basis of the number of N-methyl groups (7). The fractions were spotted on aluminum backed silica gel plates[b] and developed in ethanol-conc. hydrochloric acid (99:1).

Each thin-layer chromatogram was then scanned with a Berthold Radio-scanner[c] to determine the amount and the R_f of each radioactive substance on the chromatogram. The total amount of radioactivity in each fraction was determined by counting an aliquot in a liquid scintillation counter[d] and correcting the count by internal standardization.

The concentration of the N-methyl-^{14}C-thionin hydrochloride was obtained by measuring the optical density of a solution of N-methyl-^{14}C-thionin hydrochloride ($\sim 10^{-7}$M in H_2O at pH 3.5 with a path length of 1 cm) and comparing to the optical density of known concentrations of Azure C prepared in a similar manner. Knowing the concentration of radioactive dye present in the solution, one could easily calculate the specific activity.

ACKNOWLEDGEMENT

Supported in part by National Institute of General Medical Sciences, Grant GM 17271.

Robert R. Eng and Larry A. Spitznagle
Pharmaceutical Sciences Department
School of Pharmacy, BG-20
University of Washington
Seattle, Washington USA

REFERENCES

1. Klopper, P. J. and Moe, R. E., Surgery, 59:1101 (1966).

2. Mortenson, R. A. and McRae, J., Arch. Surgery, 100:710 (1970).

3. Kang, G. C. and DiGuilio, W., J. Nucl. Med., 9:643 (1968).

b EM Laboratories Inc., 500 Exec. Blvd., Elmsford, N.Y. 10523
c Berthold Radioscanner 6000 series LB 2722 (Varian Aerograph 2 pi Thin Layer Scanner), 2700 Mitchell Drive, Walnut Creek, California 94598
d Beckman LS-230 Liquid Scintillation Counter, Beckman Instruments, Inc. Fullerton, California 92634

4. Whitake, R. H., Calc. Tiss. Res., 8:133 (1971).

5. M. R. McKamey, "An Analysis of the Purity and Stability of Toluidine Blue O, an N-methylated Aminophenothiazine Dye." Ph.D. Thesis, University of Washington, Seattle, Washington, 1973.

6. Archer, E. G., Potchen, E. J., Studerand, R. and Siegel, B., J. Nucl. Med., 13:85 (1973).

7. M. R. McKamey and L. A. Spitznagle, submitted to J. Pharm. Sci. (1974).