NOTES

CHEMICAL SYNTHESIS OF N-(METHYL-^{14}C OR -^{3}H)-ETHYL-N-METHYL-N-NITROSOCARBAMATE.

Received on August 14, 1973

INTRODUCTION

The nitrosamide ethyl-N-methyl-N-nitrosocarbamate (NMUT) is a versatile carcinogen (1). There appears to be no method in the literature for the chemical synthesis of labeled ^{3}H- and ^{14}C-NMUT. Schoental reported (2) the synthesis of ^{14}C-NMUT by nitrosation of the then commercially available ethyl-N-methyl-carbamate. However, no data on the conditions of the reaction, purity, yield and chemical properties of the product were published. We report here a simple and rapid procedure for such a synthesis.

DISCUSSION AND RESULTS

Labeled methylamine hydrochloride was mixed with unlabeled methylamine hydrochloride, and then reacted with ethylchloroformate in a heterogenous reaction media to give methyl labeled ethyl-N-methyl carbamate. This compound was then nitrosated directly with nitrous acid generated *in situ* from 35% nitric acid and sodium nitrite. The labeled product was isolated and stored at -20°C in the dark. The yield was 34%. A serious problem was encountered with ethanol residues remaining from the labeled methylamine hydrochloride, which is supplied in ethanol solution. Small traces of ethanol were found to produce impurities in the final product. A simple method was devised to remove the ethanol by co-evaporating the

ethanol with ether followed by heating for one hour at 100°C under high vacuum. This procedure removed ethanol residues.

The infra-red spectra of the labeled compounds were identical to that of an authentic sample. The U.V. and visible spectra of both compounds when dissolved in 0.15 M sodium acetate (pH 6.5) were similar to that of the authentic sample, (λmax. 240 nm; Emax. 6700). The visible spectrum showed three poorly resolved peaks in the 390-410 nm region (λmax. 400 nm; Emax. 92). Thin layer chromatography on silica gel plates showed a single spot as detected by three different methods; the radioactivity was found only in that spot. Thus the compounds are radiochemically and chemically pure as indicated by I.R., U.V., and thin layer chromatography.

EXPERIMENTAL

Authentic unlabeled NMUT was obtained from the National Cancer Institute, Bethesda, Maryland. Unlabeled methylamine hydrochloride (Fisher Scientific) was recrystallized from ethanol and thoroughly dried overnight in a vacuum dessicator. Ethylchloroformate was obtained from Eastman. ^{14}C- and ^{3}H-methylamine hydrochloride were from New England Nuclear (the specific activities were 8.4 mc/mM and 34 mc/mM respectively). Infra-red spectra were determined on a Perkin-Elmer 337 instrument. Aluminum silica gel thin layer plates, with no fluorescent indicator were purchased from Brinkman Instrument Co. Thin layer chromatography was carried out using the following two solvent systems:

A. Hexane-ethyl ether-methylene chloride (100-15-10)

B. Hexane-ethyl ether-methylene chloride (110-15-5)

The Rf values of NMUT in the systems A and B were 0.7 and 0.35, respectively. The nitrosamide was detected on the plates by the following three methods: short wave U.V. radiation alone; spraying with the Griess reagent (5); and, spraying with the diphenylamine reagent (4). Once visualized by these methods, the plates were cut into 1 cm strips and the radioactivity was measured by liquid scintillation spectrometry. Visible and ultraviolet spectra were determined on a Perkin-

Elmer model 202 recording spectrophotometer.

The procedure for the chemical synthesis was a modification of the previously published method for the unlabeled compound (3). Unlabeled solid methylamine hydrochloride (65.5 mg) was weighed into a round bottom flask to which 0.25 mc of ^{14}C-methylamine hydrochloride (2.0 mg) in ethanol was added. The ethanol was evaporated under nitrogen with a hot air gun. To the flask was added 3.0 ml. of anhydrous ethyl ether and the ether was also evaporated; this procedure was repeated twice. The round bottom flask was put in an oven at 110°C for one hour, under high vacuum. The flask was removed from the oven and cooled for 15 minutes; 1.0 ml. of H_2O and 0.5 ml. of ether was added with mixing. The flask was then placed in an ice water bath and 0.5 ml. of 2 N NaOH was added. To this mixture was added 20 µl of ethyl chloroformate followed by 0.25 ml. of 1 N NaOH. Addition of the chloroformate and 1 N NaOH was continued until a total of 80 µl of chloroformate and 1.0 ml. of 1 N NaOH were added within five minutes. The reaction was stirred vigorously for 15 more minutes; subsequently, 0.5 ml. of ethyl ether and 0.2 ml. of 35% HNO_3 were added, followed by 350 mg. of sodium nitrite in 0.5 ml. of water. A total of 0.7 ml. of 35% HNO_3 was added with vigorous stirring during a fifteen minute period. The reaction was allowed to stir vigorously for another 15 minutes, after which the contents were transferred to a centrifuge tube. The layers were separated and the aqueous layer was washed three times with 1 ml. volumes of ether. The ether layers were combined and washed once with 2 ml. of water. The layers were separated and the ether layer was dried over anhydrous magnesium sulfate for 15 minutes. The dried layer was filtered, and the ether evaporated under nitrogen. The yellowish-orange product was then stored dessicated in the freezer. Similar procedures were used to synthesize the tritium labeled compound, except that ^{3}H-labeled methylamine hydrochloride was used in the synthesis.

The specific activity of the ^{14}C-compound was 0.25 mc/mM and the tritium

labeled compound was 1.0 mc/mM. This procedure is adaptable for the synthesis of higher specific activities of these compounds. The yield for both compounds was 34%.

Adhid Alarif, Suzanne Kimball, and Samuel S. Epstein*

* Environmental Health Programs and the Department of Pharmacology, School of Medicine. Case Western Reserve University, Cleveland, Ohio 44106
Work supported by NIH (NCI) Contract No. E-72-3286.

REFERENCES

1. Druckrey, H., Preussman, K., Ivankovic, S., Schmal, O., Afkham, J., Blum, G., Mennel, H. O., Muller, M., Petropolos, P., und Schneider, H. - Z. Krebsforsch. 69: 103 (1967).

2. Schoental, R. - Biochem. J. 102: 5c (1967).

3. Blatt, A. H. - "Organic Synthesis", Wiley, New York, Vol. 2, 1948, p. 464.

4. Sen, N. P., Smith, D. C., Schwinghamer, L., and Marleau, J. J. - Assoc. Analyt. Chem. 52: no. 1, 47 (1969).

5. Preussman, R., Daiber, D., and Hengy, H. - Nature, 201: 502 (1964).