TRITIUM LABELING OF 3 - TRIFLUOROMETHYL - 4 - NITROPHENOL *

Received on December 1, 1971.

3-trifluoromethyl-4-nitrophenol (TFM) is a selectively toxic agent which is currently being used in the Upper Great Lakes to control the population of the parasitic sea lamprey, *Petromyzon marinus (1,2)*. In order to facilitate investigations concerning the pharmacology and metabolism of this compound, tritium labelled TFM was prepared. This report is concerned with the preparation, purification and identification of tritiated 3-trifluoromethyl-4-nitrophenol.

EXPERIMENTAL

Reagents. Purified (98^{+}%) 3-trifluoromethyl-4-nitrophenol was obtained from Farbwerke Hoechst Co., Bridgewater, New Jersey and was recrystallized from benzene before use. Precoated silica gel thin layer chromatography plates (silplate - 254) were obtained from the Brinkman Instrument Co., Westbury, L. I., N. Y.. Prepacked silica gel columns (Type D-32) were obtained from Quantum Industries, Fairfield, N. J.. Scintillation counting materials were purchased from Packard Inst. Co., Chicago, Ill., 3-methyl-4-nitrophenol and 4-nitrophenol were obtained from Aldrich Chemical Co., Milwaukee, Wis.. Other chemicals and solvents were of analytical quality.

Procedure. TRITIATION. 0.5 gm of 3-trifluoromethyl-4-nitrophenol, mp 75-76°, was supplied to Amersham-Searle Co., Arlington Heights, Ill., for tritium labeling by Method TR-1 *(3)*. In brief, the TFM was mixed with 0.2 gm reduced platinum catalyst and 100 Ci of tritiated water. The mixture was heated to 100°C for five hours. After removal of labile tritium with ethanol the reaction product(s) was dissolved in benzene and sealed in glass ampules under nitrogen.

* This project was supported in part by a Grant in Aid by the Great Lakes Fishery Commission and by Sea Grant #1-35353 from the Department of Commerce.

700 mCi of stable tritium was associated with the reaction products.

PURIFICATION OF ^{3}H-TFM. Approximately 70 mCi of the tritiated material in a volume of 10 ml of benzene was centrifuged at 1000 x g for 5 minutes and the resulting particulate material in the tube was removed by aspiration and discarded. The benzene solution was extracted 3 times with 1 volume of 0.01 M sodium phosphate buffer pH 6.0, after which the aqueous phases were combined, acidified and extracted with 1 volume of benzene. The benzene fractions were combined and concentrated to a volume of 2 ml by air evaporation and placed on a prepacked silica gel column. The column was eluted with a mixture of benzene:diethyl ether:glacial acetic acid (50:25:0.5) and the yellow band corresponding to TFM was collected.

THIN LAYER CHROMATOGRAPHY. Thin layer chromatography was performed on 0.25 mm silica gel plates using benzene:diethyl ether:glacial acetic acid (50:25:0.5) (Solvent I) and benzene:acetone:NH_4OH (75:25:0.5) (Solvent II) as developing systems. Visualization was done under UV light and by exposure of the plate to ammonia vapors.

Radioactivity was determined on a Packard Tri-Carb Liquid Scintillation Spectrometer. The counting fluid was composed of 2L toluene, 1L Triton X-100, 8 gm PPO and 100 mg POPOP. The final specific activity of TFM was determined by the use of an internal ^{3}H standard and calculated using a mM extinction coefficient of 13.1 at 395 nm *(4)*.

VAPOR PHASE CHROMATOGRAPHY. Vapor phase chromatography was done on a Beckman GC-4 gas chromatograph with a nonradioactive electron capture detector. The column was a 6' x 4 mm glass column packed with 10% QF-1 on Gas Chrom Q, 80/100 mesh. The column temperature was 170°C and that of the detector was 200°C.

RESULTS

Figure 1 shows the results of thin layer radiochromatography of the reaction mixture before purification (Solvent I). It can be seen that the mix-

ture contained a large amount of unidentified tritiated material which was associated with the origin in addition to a radioactive peak which had a mobility comparable to that of authentic TFM. Figure 2 shows a radiochromatogram of the purified product. The only radioactivity on the chromatogram was associated with a spot which was yellow when exposed to ammonia vapors and had an rf identical to that of authentic TFM (rf =.52). The purified material also co-chromatographed with unlabelled carrier TFM under the above conditions.

Table 1 shows the results of thin layer chromatography of the purified product, 4-nitrophenol, 3-methyl-4-nitrophenol and authentic TFM in two solvent systems. It can be seen that the mobility of the tritiated material is identical to that of authentic TFM in both solvent systems and clearly dif-

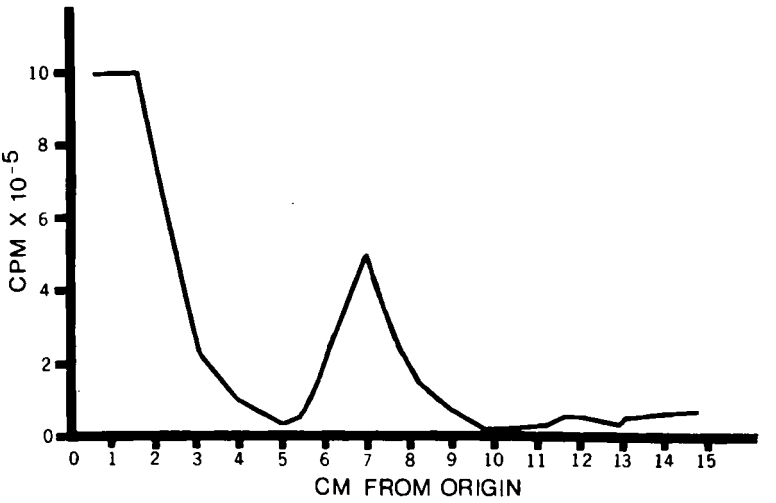

Fig. 1 Thin layer chromatogram of tritiation reaction products on silica gel in Solvent I. 5 µl of the original benzene solution was used.

TABLE I THIN LAYER CHROMATOGRAPHY OF ^{3}H-3-TRIFLUOROMETHYL-4-NITROPHENOL

Compound	rf	
	Solvent I	Solvent II
3-trifluoromethyl-4-nitrophenol (unlabelled)	.50	.11
3-methyl-4-nitrophenol	.62	.29
4-nitrophenol	.59	.23
tritiated product	.51	.11

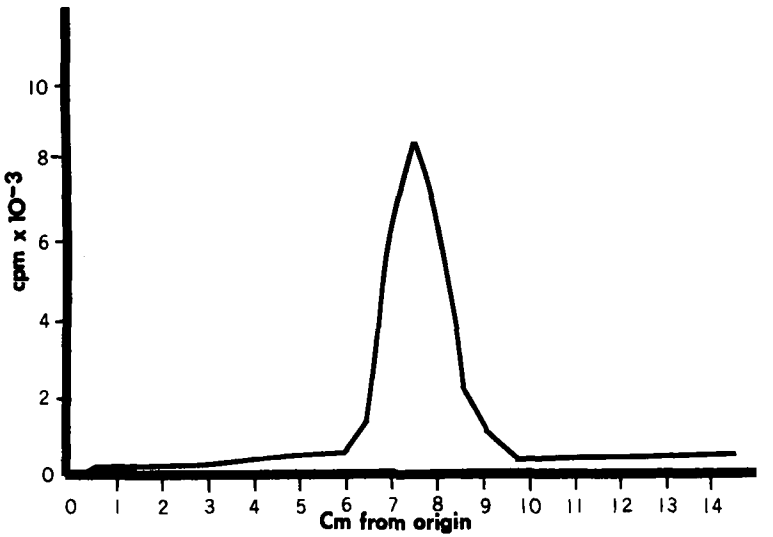

Fig. 2 Thin layer chromatogram of purified 3-trifluoromethyl-4-nitrophenol on silica gel in Solvent I. 5 µl of the final column fraction was used.

ferent from that of two compounds which were thought to possibly arise during the tritiation reaction.

Figure 3 shows the gas chromatographic pattern of the acetyl derivative of TFM (A) and that of the purified tritiated material (B). The retention time of each was found to be 2.91 min. Collection and analysis of the column effluent during elution of peak B revealed that 97% of the injected radioactivity was associated with this compound.

The tritiated material displayed an absorption maximum at 395 nm in 0.1 N NaOH which is identical to that of authentic TFM *(4)*.

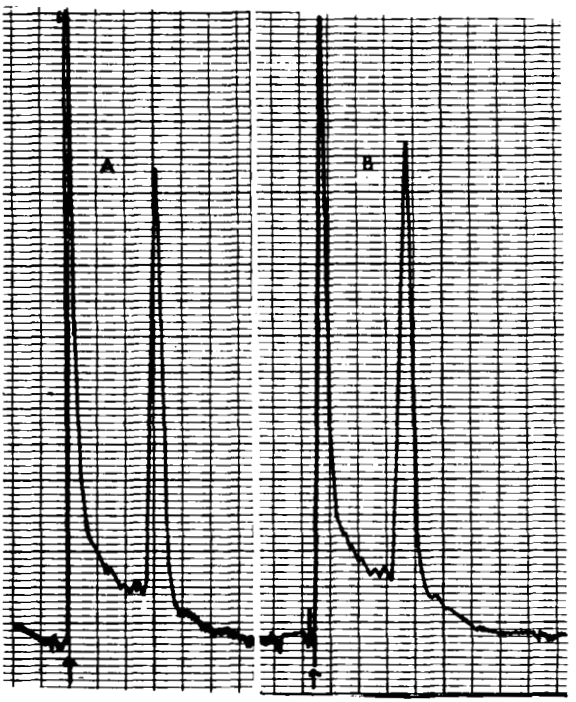

Fig. 3 Gas chromatographic elution pattern of : A. acetylated 3-trifluoromethyl-4-nitrophenol; B. acetylated tritium labelled 3-trifluoromethyl-4-nitrophenol. Conditions are described in Methods section.

A procedure for tritium labelling and purification of 3-trifluoromethyl-4-nitrophenol has been described. Analysis of the purified reaction product by thin layer chromatography and gas chromatography indicates that the tritiated material was identical to authentic 3-trifluoromethyl-4-nitrophenol. The specific activity of the purified material was found to be 20.5 mCi/mM and the recovery ranged from 3.1-3.3 mCi from each 70 mCi portion processed.

Acknowledgments

The authors wish to thank Miss Marilyn Miller for her technical assistance to Mr. John Allen and Mr. John Howell of the U.S. Bureau of Sport Fisheries & Wildlife, LaCrosse, Wisconsin, for their cooperation in this project.

REFERENCES

1. Applegate, V. C., Howell, J. H. and Moffett, J. W.. Great Lakes Fishery Commission Technical Report #1, 1961.

2. Applegate, V. C. and King, E. L.. Transactions of the American Fisheries Society 91: 342-345, 1962.

3. Evans, E. A. in "A Guide to Tritium Labelling Services", Radiochemical Center, Amersham, England, 1970.

4. Smith, M. A., Applegate, V. C. and Johnson, B. G. H.. J. Chemical and Engineering Data 6: 607, 1961.

John J. Lech

and

Phillip Moyer

Department of Pharmacology

The Medical College of Wisconsin

Milwaukee, Wisconsin 53233