PREPARATION OF TRITIATED 2,4-DIAMINO-5-ADAMANTYL-6-METHYLPYRIMIDINE

Sigmund F. Zakrzewski

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263 USA

E. Anthony Evans

The Radiochemical Centre, Amersham, Buckinghamshire, England

All correspondence addressed to:

S.F. Zakrzewski, Ph.D.

Clinical Pharmacology and Therapeutics

Roswell Park Memorial Institute

666 Elm Street

Buffalo, New York, 14263

Key words: diamipyrimidines, antifolates, adamantane derivatives, tritium, catalytic hydrogen-tritium exchange, specificity of labeling

SUMMARY

Tritium labeled 2,4-diamino-5-adamantyl-6-methylpyrimidine was prepared by a platinum metal catalyzed tritium exchange for 4 hr at 70°. The crude material was purified on a Sephadex G-25 column and converted to its water soluble ethanesulfonate salt. The analysis of the purified material revealed that 43% of the tritium was located in the 6-methyl group and the remainder in the adamantane moiety of the molecule.

0362-4803/81/050683-11$01.00 Received February 1, 1980

INTRODUCTION

2,4-diamino-5-adamantyl-6-methylpyrimidine (DAMP) (1) is a new antitumor agent (2) whose mode of action is related to the inhibition of dihydrofolate reductase (3). The tissue distribution, and disposition of DAMP in rats has been studied previously using ^{14}C-labeled material (4). Although it was possible to prepare enough of (^{14}C)-DAMP of sufficient specific activity to conduct pharmacokinetic study in small animals (4), preparation of sufficient quantities of this material to conduct pharmacokinetics in dogs proven not to be practicable. Because of the likelihood that ^{3}H-DAMP could be prepared readily in larger quantities than the ^{14}C-labeled material, the synthesis of this compound was undertaken. This report described the preparation and purification of (^{3}H)-DAMP.

MATERIALS AND METHODS

DAMP was custom prepared by Starks Associates, Inc., Buffalo, N.Y., according to the published procedure (1). Sephadex G-25 (fine) was purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N.J., and ethanesulfonic acid from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. The pre-coated TLC plates of silica gel on aluminum (20x20 cm) were from E Merck Laboratories, Inc., Elmsford, N.Y.

Preparation of chromatographic columns

For purification of (^{3}H)-DAMP a K 26/40 column (Pharmacia Fine Chemicals, Inc.) was used. The column was fitted at the bottom with a plunger adjusted so as to give a total useful length of the column of 37 cm. The inlet of the column (bottom) was connected through peristaltic pump, and the outlet (top) through a Gilson UV monitor (coupled with a Gilson recorder)

to a 4-way valve so as to allow either passage of the eluting solvent from a reservoir, and collection of the effluent, or passage of the effluent back to the column. The column was filled with a slurry of 85 g of Sephadex G-25 in water. Before use the column was washed with 0.1 M HCl.

For final purification of ^{3}H-DAMP ethanesulfonate (DAMP-ES) the K 16/40 column filled with Sephadex G-25 to a height of 30 cm was used. This column was also equilibrated with 0.1 M HCl before use but it was eluted in the conventional manner by passing the solvent from the top and collecting fractions of the effluent emerging from the bottom of the column.

Estimation of radiopurity

To determine the radiopurity of ^{3}H-DAMP, aliquots of DAMP or DAMP-ES solution were applied to TLC strips 2x20 cm. The strips were developed with an organic layer of a mixture of ethoxyethanol-ethylacetate-4% formic acid (EEF, 1:3:2). After drying, the strips were cut in 0.5 cm segments and the radioactivity of each segment was determined in a Packard liquid scintillation spectrometer, Tricarb model 2450, using hydromix (Yorktown Research, Hackensack, N.J.) as a scintillation fluid.

RESULTS

Tritiation of DAMP

Platinum oxide (Adams' catalyst; 200 mg) was reduced with hydrogen in ethanol solution (ca. 1 ml). After removal of the ethanol *in vacuo* tritiated water (1 ml containing approximately 200 Ci) was condensed into the reaction tube containing the reduced platinum oxide. 2,4-Diamino-5-adamantyl-6-methyl-

pyrimidine (200 mg) was added to the tube which was sealed under vacuo. The tube and contents were then heated in a rocking furnace for 4 hours at 70°C. After cooling, the contents were frozen, the tube was opened and the tritiated water recovered by distillation on a vacuum line. The residue was dissolved in ethanol/benzene (3:2 v/v) (50 ml), filtered

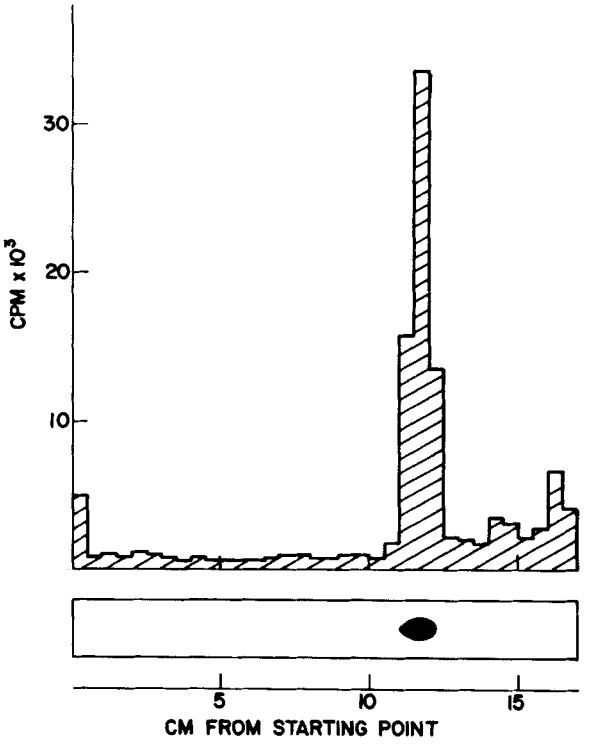

Figure 1 - TLC chromatogram of (^{3}H)-DAMP. 1 µl of the solution of crude (^{3}H)-DAMP was diluted with 100 ul of a solution of non-radioactive DAMP in ethanol-benzens. 10 µl of this solution were applied to a TLC-silica gel (with fluorescing indicator) and the strip was developed as described in Materials and Methods. DAMP could be located on the strip as a UV absorbing spot. The radioactivity was determined as described in Materials and Methods.

through a Whatman No. 1 filter paper and evaporated to dryness in vacuo to remove any readily labile tritium. The crude ^{3}H-DAMP (45 mCi) was immediately redissolved in ethanol/benzene (3:2 v/v) (25 ml), and purified by the method below.

Figure 1 represents the radioactive profile of a TLC chromatogram of the crude ^{3}H-DAMP. By comparing the total radioactivity under the main peak with that in other areas of the strip the radiochemical purity of this material was estimated to be 64%.

Purification of ^{3}H-DAMP

It was established previously that DAMP is retained on the Sephadex G-25 column beyond the total elution volume, whereas products of its chemical degradation and biotransformation are not (4,6). A procedure for purification of (^{3}H)-DAMP was developed on the basis of these observations.

Purification of (^{3}H)-DAMP by chromatography on Sephadex G-25 columns was conducted in small batches. In each case an aliquot of ethanol-benzene solution containing 3 to 4 mg of DAMP was evaporated to dryness, the residue was redissolved in 2 ml of 0.1 M HCl and this solution was pumped into the bottom of the column (K 26/40). The elution of the column was then carried out with 0.1 M HCl. The UV absorbance (at 254 nm) of the effluent emerging from the top of the column was continually recorded. The effluent was discarded until the appearance of a major peak was indicated on the recorder. At this point the four-way valve was switched so as to pass the effluent back to the column. When the UV absorbance of the effluent descended to the background level the valve was switched back to its

original position and the elution was continued. This recycling was repeated three times. The final effluent that contained DAMP was collected and saved.

The effluents collected after processing five batches (total of 15-20 mg of DAMP) were combined, the pH was adjusted to 11 and the solution was placed in a refrigerator overnight. The precipitate which formed was separated by centrifugation and washed with water.

Preparation of ethanesulfonate salt of DAMP

The washed precipitate was suspended in 2 ml of water. Upon addition of 5 µl of ethanesulfonic acid the precipitate dissolved. Occasionally there was light turbidity which could be disregarded. This solution was applied to a K 16/40 column which was eluted with 0.1 M HCl in a conventional manner. The

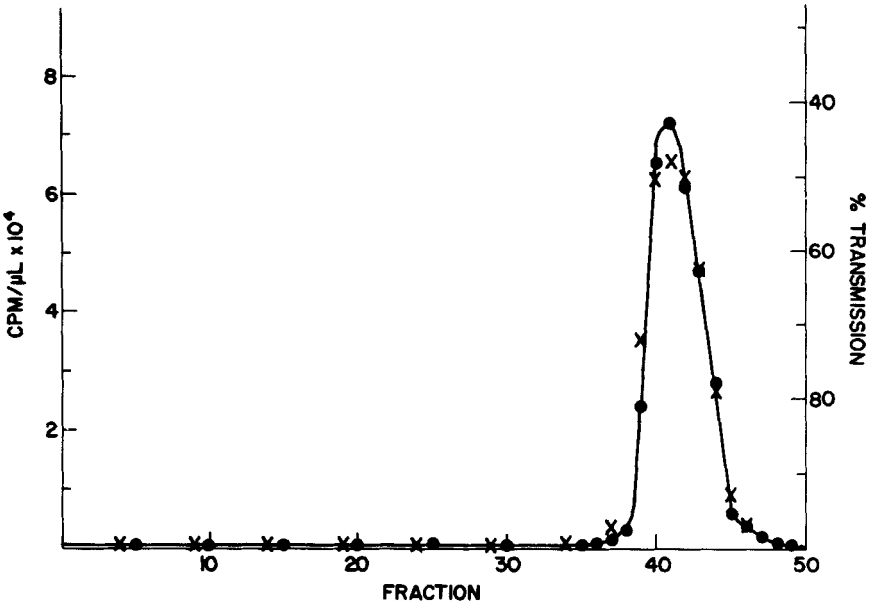

Figure 2 - Chromatographic profile of (^{3}H)-DAMP-ES on a column of Sephadex G-25. Eluting solvent 0.1 M HCl; fractions of 3 ml were collected. O Radioactivity X Optical transmission

UV absorbance of the effluent was recorded continuously and the effluent was collected in 3 ml fractions. Aliquots of 1 µl of each fraction were withdrawn for counting of the radioactivity (Figure 2). The fractions containing DAMP were combined, lyophilized, and the residue was redissolved in 2-3 ml water. The concentration of this material was determined from its UV spectrum (E_{280}=6.6x10^3) (Figure 3). The yield was 70-90% of the starting preparation. The specific radioactivity varied for different preparations between 27 and 70 µCi/µmole.

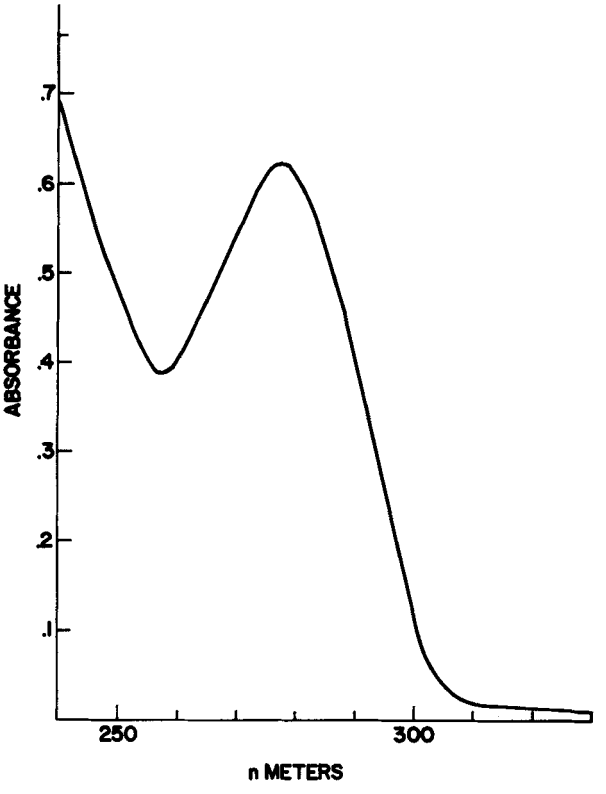

Figure 3 - Ultraviolet spectrum of (^{3}H)-DAMP-ES in 1 mM ethanesulfonic acid. Concentration = 96.3 uM.

Specificity of labeling

In order to establish the location of tritium in ^{3}H-DAMP a 20 mg sample containing about 34 μCi of the radioactive material was heated in 5 ml of 70% acetic acid at 120° for 3 hours. It was previously reported that this treatment leads to the complete loss of DAMP's biological activity (5). Figure 4 shows a TLC chromatogram of DAMP before and after this treatment. The UV absorbing spot with Rf 0.13, which is also radioactive is undoubtedly the pyrimidine portion of DAMP, since it strongly

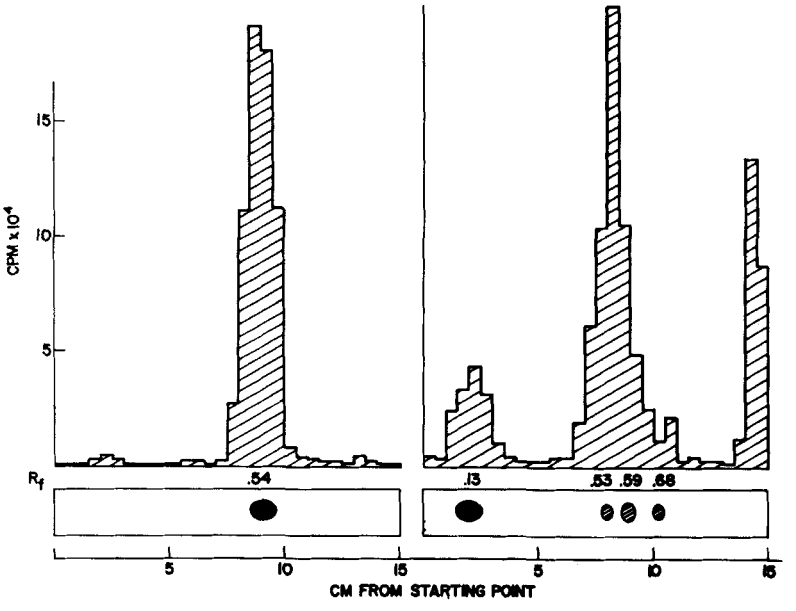

Figure 4 - TLC chromatogram of purified (^{3}H)-DAMP-ES before (left) and after (right) degradation. 10 ul of the solution of purified (^{3}H)-DAMP-ES (about 4 mg/ml) or of the reaction mixture were applied to TLC-silica gel strips. The conditions and procedures were the same as those described in Figure 1.

absorbs UV light and cannot be extracted from aqueous solution with dichloroethane. The three weakly absorbing spots in the middle of the strip represent DAMP (Rf 0.53) and two of its decomposition products of unknown structure, in which the integrity of the molecule was preserved. Similar to DAMP each of these compounds was extractable with dichloroethane from an alkaline but not from an acidic solution. The radioactive material which migrates with the solvent front has the properties of adamantane: It does not absorb UV light, is extractable with dichloroethane from both alkaline and acid solutions, and can be sublimed off from the reaction mixture. The distribution of radioactivity between pyrimidine and adamantane could be estimated to be 3:4.

The radiopurity of different preparations of DAMP, calculated from radioactive TLC profiles varied between 93-99%. The material could be stored in aqueous solution in the refrigerator without signs of decomposition for at least four months.

DISCUSSION

A method for the preparation of (^{3}H)-DAMP has been outlined. The initial approach involving bromination of the 6-methyl group of DAMP, followed by catalytical replacement of bromine with tritium was unsuccessful because of the cleavage of the molecule upon treatment with Br_2-HBr. Among suitable tritium exchange reactions, the catalytic exchange in solution was chosen as the most practical (6). Since most tritium exchange procedures are more or less destructive, and because of the fragility of DAMP (5), conditions had to be carefully chosen to provide the best compromise between high specific radioactivity and minimal degradation of the material. Even so, the product

of this reaction required extensive purification before it was suitable for use in biological or pharmacological experiments.

The data presented here show that it is possible using Sephadex G-25 to obtain (^{3}H)-DAMP in sufficient purity for biological study.

The location of tritium in the molecule was established by splitting the pyrimidine from the adamantane moiety of the molecule. The only nonexchangeable hydrogens in DAMP molecule which can retain tritium are in the 6-methyl group of the pyrimidine and in the adamantane moiety. The data showed that both are radioactive in a ratio of 3:4.

Because of the poor solubility of DAMP in aqueous solvent especially at neutral pH, it was advantageous to prepare the ethanesulfonate salt of DAMP which is soluble in water, and thus more suitable for biological and pharmacological experiments.

This preparation of (^{3}H)-DAMP-ES was used successfully in a study of pharmacokinetics of this drug in dogs.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the capable technical assistance of Mrs. J. Drobniak and Mr. M. Marcus.

This work was supported in part by United States Public Health Service Grant CA-21071.

REFERENCES

1. Jonak, J.P., Zakrzewski, S.F., Mead, L.H. and Hakala, M.T., J. Med. Chem., 13, 1170(1972).
2. Zakrzewski, S.F., Dave, C., and Rosen, F., J. Natl. Cancer Inst., 60, 1029(1978).

3. Ho, Y.K., Hakala, M.T. and Zakrzewski, S.F., Cancer Res. 32, 1023(1972).

4. Zakrzewski, S.F., Dave, C., Mead, L.H. and Delluomo, D.S., J. Pharmac. Exptl. Therapeutics, 205, 19(1978).

5. Zakrzewski, S.F. and Delluomo, D.S., Proc. Am. Assoc. Cancer Res., 17, 73(1976).

6. Evans, E.A., p.271, in Tritium and Its Compounds, 2nd edition Butterworths, London 1974.