NOTE

SYNTHESIS OF [^{3}H] PORFIROMYCIN

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SUMMARY

The synthesis of the title compound (1) is described. The reaction of a specific amount of [^{3}H] methyl iodide under controlled conditions with naturally occurring mitomycin C produced [^{3}H] porfiromycin with an overall yield of 61% and a radiochemical purity of 98%.

Key Words: [^{3}H] porfiromycin, gram-positive bacteria, gram-negative bacteria.

INTRODUCTION

Mitomycins possess strong activities against gram-positive and gram-negative bacteria, as well as against several kinds of tumors. These structures[1,2] are unique not only in natural products chemistry, but also among antitumor substances. Many derivatives of natural mitomycins were synthesized in a search for less toxic and more effective substances. In this note, we describe a synthesis for metabolism and pharmacokinetic studies of [^{3}H] porfiromycin utilizing the chemistry existing in the literature,[3] but modified to optimize conditions for the incorporation of radiolabel.

MATERIALS

[^{3}H] Methyl iodide was purchased from Amersham Corporation. All chemicals used in the synthesis were purchased commercially and used without any further purification. All other solvents were either distilled or analytical reagent

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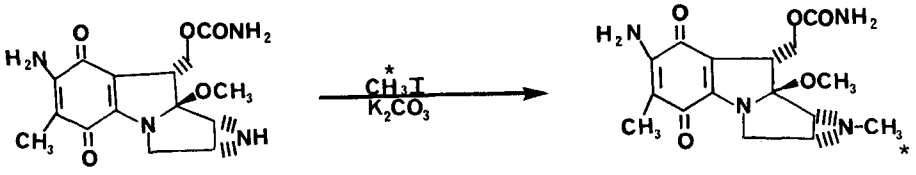
quality. Thin layer chromatography plates used were Analtech silica gel GF, scored 10 x 20 cm, 250 microns and high pressure liquid chromatography was carried out on Waters Associates instrumentation. Radioactivity was measured by a Beckman LS9000 liquid scintillation counter. Nuclear magnetic resonance was measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and Mettler Microanalytical M5AS balance.

SYNTHETIC PATHWAY



*Position of radiolabel

1

EXPERIMENTAL

[^{3}H]Porfiromycin (1)

Mitomycin C (5.41 g, 16 mmoles) was dissolved in dry acetone (325 ml). To this was added potassium carbonate (27.03g) followed by [^{3}H]methyl iodide (1.5 Ci) at 16 Ci/mmol in 2 ml of toluene and then cold methyl iodide (2 ml). This mixture was refluxed gently for 20 hrs. The reaction was filtered hot to remove potassium carbonate and potassium iodide. The filtrate was concentrated under reduced pressure to a purple solid which was purified by column chromatography on silica gel (Wöelm 63-200 mesh) in a mixture of ethyl acetate (70); acetone (30).

[^{3}H]porfiromycin (3.433 g) yield = 61%, radiochemical purity = 98% and specific activity = 103.3 μCi/mg was obtained.

Thin Layer Chromatography: Eluent-ethyl acetate (70); acetone (30), Analtech silica gel plates, Visualization- compound purple in color, with Rf = 0.45.

High Pressure Liquid Chromatography was carried out on Waters Associates instrumentation with the following parameters: Eluent - methanol (30%), water (70%). Flow Rate - 2 ml/min. Detector - Ultraviolet of 254 nm. Temperature - 22.5°C. Column - International Business Machines C-18. Retention Time - 14.02 min.

RESULTS AND DISCUSSION

The reaction of methyl iodide with mitomycin C goes smoothly and in good yields when a twenty fold excess of methyl iodide was used.[3] Because [^{3}H]methyl iodide at high specific activity is expensive, new methodology had to be worked out to provide maximum yields using minimum amounts. With this in mind, several experiments were carried out varying the amounts of methyl iodide and the reaction times. It was found that one equivalent of methyl iodide, refluxing for 4 hrs gave a 10% yield of product. Using the same amount of methyl iodide but increasing the reaction time to refluxing for 16 hrs yielded 30% of product. When two equivalents of methyl iodide was used, and refluxing continued for 16 hrs, a 60% yield was produced. This was the equalization point which was the minimum amount of methyl iodide that would provide a maximum amount of [^{3}H]porfiromycin. All experimental conditions were optimized using non-radiolabeled materials.

REFERENCES

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