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SYNTHESIS OF MITOMYCIN C LABELED WITH MONO-TRITIUM AT THE C6 - METHYL POSITION

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SUMMARY

A first preparation of mitomycin C specifically labeled with mono-tritium at the C6-methyl position is described. The key intermediate in the synthesis, 7,7-ethylenedioxy-6-methylenemitosane (**5**) was made by treating 7,7-ethylenedioxy-6-phenylselenomitosane (**4**) with meta-chloroperbenzoic acid. Subsequent 1,4-addition of [^{3}H]hydride gave 7,7-ethylenedioxymitosane labeled with tritium (**6**). Amination at C7 position and deacetylation of 1a-aziridine nitrogen of **6** gave [C6-CH_3-$^3H_1$] mitomycin C (**2**) with an overall yield of 15%. The specific activity of the final compound was 16.2 mCi/mmol with 13% tritium incorporation.

Key Words : 1,4-Addition of [^{3}H]hydride, [C6-CH_3-$^3H_1$]Mitomycin C

INTRODUCTION

Mitomycin C (**1**) has been extensively used in cancer chemotherapy against a variety of solid tumors, but its use is limited by side effects, such as severe bone marrow suppression or gastrointestinal damage (*1*). Consequently, a number of derivatives targetting less toxicity or more effective activity have been synthesized in our laboratory (*2*) or by other groups (*3*). For evaluation of these new mitomycin derivatives, drug metabolism and pharmacokinetic studies including a comparison with those of **1** seemed to be very important. In such studies, isotopically labeled drugs such as tritium or carbon-14 have been used in a general way. In the case of **1**, only two methods for labeling, i.e, Wilzbach method (*4*) and biosynthetic method (*5*), were reported to date, but both methods were not practical for selective and effective labeling. Thus the

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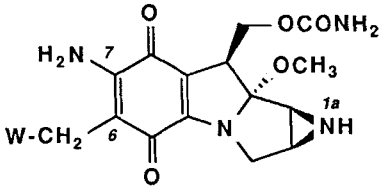
pharmacokinetics of **1** has not been clear, and a practical method for labeling at a metabolically stable position such as C6-methyl group has long been desired. Herein, we describe a first synthesis of mitomycin C specifically labeled with tritium at the C6-methyl position utilizing the methodology developed by us (*6*).



1, Mitomycin C (W=H)
2, [C6-CH_3-$^3H_1$]Mitomycin C (W=^{3}H)

RESULTS AND DISCUSSION

C6-Methyl group, a metabolically stable position of mitomycins, could be specifically labeled with tritium in three steps from readily available **4** (*6*). Selenoxide elimination of **4** with meta-chloroperbenzoic acid, and subsequent 1,4-addition of [^{3}H]hydride with [^{3}H]sodium borohydride were carried out in one-pot reaction to afford the labeled **6**. Amination of C7 position and deacetylation of the 1a-aziridine nitrogen of **6** was accomplished by a treatment with ammonia in methanol. The crystalline [C6-CH_3-$^3H_1$]mitomycin C (**2**) was obtained in 15% chemical yield based on **4**. Reverse phase HPLC analysis of this material showed a radiochemical purity of 98.8% and a specific activity of 16.2 mCi/mmol with 13% tritium incorporation. However all experimental conditions were not optimized, lower incorporation of tritium seemed to result from some proton sources in this reaction mixture or an isotope effect.

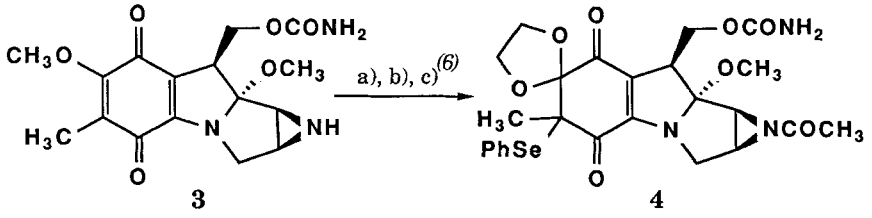
MATERIALS

[^{3}H]Sodium borohydride (TRA. 45) was purchased from Amersham Corporation. All chemicals and solvents used were purchased commercially and used without any further purification. Radioactivity was measured on a Packard Tri-carb 4530 Liquid Scintillation Counter. Thin layer chromatography (TLC) was done with E. Merck silica gel (Art 5719). All compounds had identical Rf to that of authenic unlabeled standards.

EXPERIMENTAL

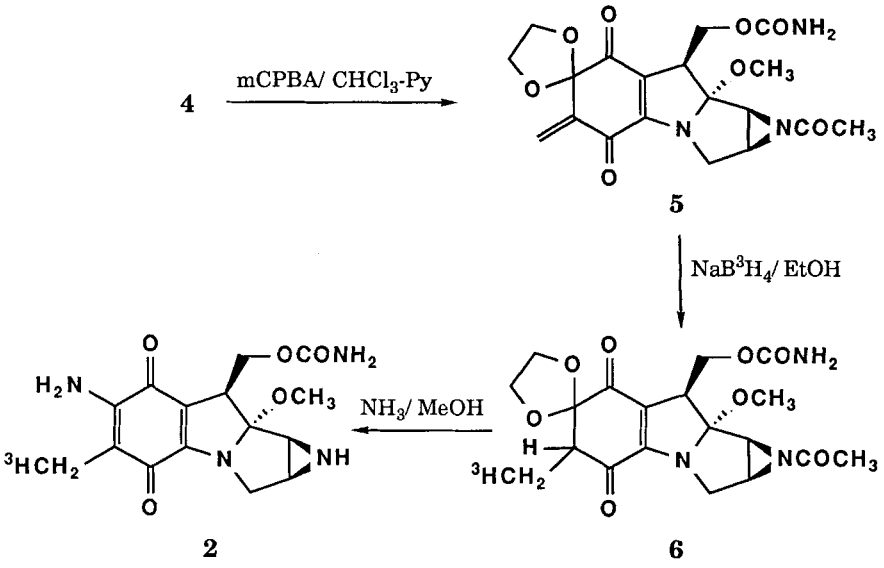
The starting material, phenylselenomitosane (**4**) was obtained according to the method developed by us (*6*) in 68% yield from mitomycin A (**3**). A diastereomeric mixture of **4** (45 mg, 0.078 mmol) was dissolved in a mixture of chloroform (2.0 ml) and pyridine (0.5 ml). To

Preparation of [C6-CH_3-$^3H_1$]Mitomycin C (2) from Mitomycin A (3)



3 4

a) Ac_2O/Py, b) KOH (catalytic)-Ethylene glycol/ THF, c) PhSeBr-Et_3N/ THF



the resulting solution was added meta-chloroperbenzoic acid (30 mg, 70% purity) and the mixture was stirred at 20°C for 10 min. [^{3}H]Sodium borohydride (1.9 mg, 25 mCi) at 500 mCi/mmol was dissolved in ethanol (0.8 ml) and added to the reaction solution. The solution was stirred at 20°C for 15 min and diluted with chloroform (2.0 ml) and 1/15 M phosphate buffer (2.0 ml, pH 4.0). The chloroform layer was washed with aqueous sodium bicarbonate (1.5 ml), followed by drying with anhydrous sodium sulfate. After removal of the solvent by evaporation, the residue was purified by silica gel chromatography using 3% methanol in chloroform to afford **6**. The production of **6** was confirmed by the observed Rf 0.52, by TLC using a solvent system of 10% methanol in chloroform, identical with that of the authentic standard. The compound **6** was dissolved in methanol (2.0 ml) and 6.8M-ammonia in methanol solution (0.5 ml). The solution was stirred at 25°C for 2 hr and then the solvent was removed *in vacuo*. The crude product was purified by silica gel chromatography using 10% methanol in chloroform. Recrystallization from chloroform/n-hexane afforded [C6-CH_3-$^3H_1$]mitomycin C (**2**) (4 mg, radiochemical purity of 98.8% and specific

activity of 16.2 mCi/mmol) with an overall yield of 15%. TLC : Eluent - chloroform (90%) : methanol (10%), Visualization - purple with Rf 0.27. HPLC was carried out on a Shimadzu Liquid Chromatograph with the following parameters : Eluent - methanol (35%) : water (65%), Flow rate - 0.8 ml/min, Detector - ultraviolet of 360 nm, Temperature - 25°C, Column - YMC A-312 (ODS), 5μ, 150 x 6 mm i.d., Retention time - 5.14 min.

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REFERENCES

1. (a) Carter S. K. and Crooke S. T. - Mitomycin C : Current status and New Developments, Academic Press, New York, 1978, (b) Remers W. A. - The Chemistry of Antitumor Antibiotics, Wiley, New York, 1979.
2. Kono M., Saitoh Y., Kasai M., Sato A., Shirahata K., Morimoto M. and Ashizawa T. - Chem. Pharm. Bull. 37: 1128 (1989)
3. Sami S. M., Iyenger B. S., Tarnow S. E., Remers W. A., Bradner W. T. and Schuring J. E. - J. Med. Chem. 27: 701 (1984) and references cited therein.
4. (a) Weissbach A. and Lisio A. - Biochemistry 4: 196 (1965), (b) Ørstavik J. - Acta Path. Microbiol. Scand. Section B 82: 270 (1974)
5. Hornemann U. and Cloyd J. C. - J. Chem. Soc., Chem. Commun. 301 (1971)
6. Kanda Y. and Kasai M. - J. Org. Chem. 1990 (in press)