NOTE

THE SYNTHESIS OF TRITIUM-LABELLED MISONIDAZOLE

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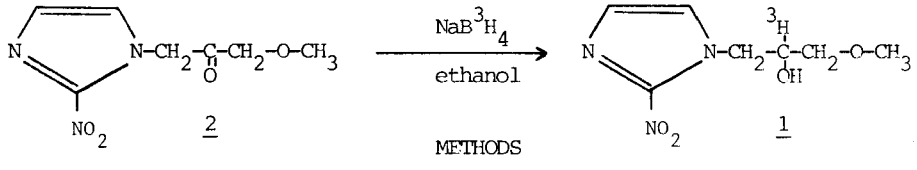
SUMMARY

The synthesis of tritium-labelled misonidazole via the NaB^3H_4 reduction of 1-(3-methoxy-2-oxopropyl)-2-nitro-1H-imidazole is described. Purification of labelled misonidazole was accomplished by recrystallization. The purity of the labelled compound was verified by HPLC.

Key Words: Misonidazole, Radiosensitizer, NaB^3H_4, 1-(3-methoxy-2-oxopropyl)-2-nitro-1H-imadazole.

INTRODUCTION

Misonidazole, 1-(2-hydroxy-3-methoxypropyl)-2-nitro-1H-imidazole (1), is a 2-nitroimidazole which is undergoing clinical trials to evaluate its effectiveness as a hypoxic cell radiosensitizer[1,2]. Misonidazole also causes peripheral neuropathy[3], it is preferentially toxic to hypoxic cells[4], and it is mutagenic[5]. The biochemical mechanism of the toxicity of misonidazole to hypoxic cells has not been conclusively determined, but reduced metabolites have been implicated in the mutagenicity and toxicity of the compound. To facilitate metabolic studies, we have devised a method to synthesize ^{3}H-1 with high specific activity via the reduction of 1-(3-methoxy-2-oxopropyl)-2-nitro-1H-imidazole (2) with NaB^3H_4, as shown below.



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METHODS

Ultraviolet spectra were obtained from a Perkin-Elmer Coleman Model 124 spectrometer. Radioactive disintegrations were measured on a Beckman Model LS

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100 liquid scintillation counter. High performance liquid chromatography was done on a Varian Model (5000) chromatograph equipped with a Varian Micropak MCH-10 column. The UV absorbance of the column effluent was monitored at 330 nm with a Varian Vari-Chrom detector Model VUV-10. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. NMR spectra were determined on a Perkin-Elmer Hatachi Model R24B using TMS as the internal standard. IR spectra were obtained on a Beckman Model Accu-Lab 6.

Misonidazole was a gift from Dr. W. E. Scott, Hoffman-LaRoche, Inc., (Nutley, NJ). Sodium boro[^{3}H]hydride was purchased from Amersham (Chicago, IL).

1-(3-methoxy-2-oxopropyl)-2-nitro-1H-imidazole (2) was prepared by the oxidation of 1 with Jones reagent as described by Beaman, et al.[6] MP 67°-68° [lit. 65°-67°] ^{1}H NMR ($CDCl_3$) δ7.21 (s,1), δ7.05 (s,1), δ5.43 (s,2), δ4.19 (s,2), δ3.51 (s,3). IR (Nujol) 1725 cm^{-1}.

Synthesis of 2-^{3}H-1(2-hydroxy-3-methoxypropyl)-2-nitro-1H-imidazole (1)

A vial of NaB^3H_4 (100 mCi, 14 Ci/mmol) was opened and 150 µl of absolute ethanol containing 8 mg (0.4 mmol) 2 was added to the contents of the vial. The top portion of the vial was washed with 100 µl of ethanol which was added to the reaction mixture. The combination of the NaB^3H_4 and 2 produced an immediate violet color which did not dissipate. After two and one-half hours at room temperature, the reaction mixture was filtered through glass wool into a test tube which contained 250 mg (1.24 mmol) 1. The glass wool was washed with 700 µl of ethanol and the resulting mixture heated in a water bath to produce a yellow solution. This solution was cooled in an ice bath to insure complete crystallization. The crystals were removed and labelled lot one. The supernatant from lot one was similarly treated to yield a second crop of crystals (lot two). The crystals from both lots were dried *in vacuo* and then dissolved in 7 ml absolute ethanol.

The concentration of 1 in each lot of material was determined spectrophotometrically at 330 nm. The amount of 1 in lots one and two were 0.166 mmol and 0.189 mmol respectively.

Lot one contained 81.8 mCi of activity and lot two contained 11.1 mCi of activity. The specific activities of the two lots were 69.9 mCi/mmol and 8.41 mCi/mmol, respectively.

The radiochemical purity of each lot was determined by HPLC. A 1 µl aliquot was dissolved in 25 ml of non-radioactive 1 x 10^{-4}M 1, and then chromatographed at a 1 ml/min flow rate using a methanol:H_2O gradient which was initially 0% methanol and changed linearly to 20% methanol at twenty minutes, and then to 100% methanol at forty minutes. One-half ml fractions were collected and the radioactivity determined. The tritium labelled misonidazole eluted from the column in fractions 53 through 57. Minor radiochemical impurities eluted in fractions 33, 46 and 47. The only peak observed by monitoring the absorbance of the effluent at 330 nm was that of 1 which had a retention time identical to the major radiochemically labelled peak. The radiochemical purity was 99.48% for lot one and 97.83% for lot two.

The stability of radiolabeled 1 at various temperatures and pH's was evaluated to insure the appropriateness of the label for drug metabolism studies. A sample of lot number one was diluted with 0.1mM 1 and added to the appropriate buffer (.01M, phosphate) and following an incubation period, the materials were chromatographed using the conditions reported for the determination of radiochemical purity. The results of these experiments are listed in Table 1.

TABLE 1. The effect of temperature and pH on the radiochemical purity of 2-[^{3}H]-misonidazole.

pH	Time	Temperature	% Radiochemical Purity[a]
2.3	4 h	37°C	99.3
2.3	10 min	100°C	98.45
7.4	4 h	37°C	99.34
7.4	10 min	100°C	97.8
9.0	4 h	37°C	99.17

[a]The initial radiochemical purity was 99.48 %.

DISCUSSION

The NaB^3H_4 reduction of 2 provides excellent yields of high specific activity tritium labelled 1. The lack of sterospecificity of the borohydride reduction procedure produces tritium labelled 1 which is racemic as is the 1 utilized as a radiosensitizer. The radiochemical purity of lot one was greater than 99%, the specific activity of the product was 69.9 mCi/mmol. Total incorporation of the ^3H from NaB^3H_4 was greater than 90%.

The labelled misonidazole is appropriate for metabolic studies, as ^3H-exchange does not occur under physiological conditions or at acidic or basic pHs. Furthermore, our initial investigations using perfused rat livers have demonstrated that ^3H-exchange from misonidazole metabolites also does not occur (B. R. Smith and J. L. Born, unpublished results). The compound is less stable at elevated temperatures, however, and boiling samples in in vitro studies to stop enzymatic reactions is not recommended.

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