SYNTHESIS AND PURIFICATION OF [4'-^{3}H]-MEBENDAZOLE

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SUMMARY

A method for the synthesis and purification of mebendazole specifically labelled with tritium in the 4' position is described.

KEY WORDS Mebendazole, specific tritium labelling.

INTRODUCTION

Mebendazole is a very insoluble compound which is widely used as a broad spectrum intestinal anthelmintic in human and veterinary medicine. Over the past decade considerable interest has developed in its use for the systemic treatment of helminth infections of man[1],[2].

The low solubility of mebendazole in pharmaceutical vehicles, and hence the lack of a suitable intravenous formulation of the drug has hindered the elucidation of the pharmacokinetics and bioavailability of the drug in man, as both an intravenous and oral administration of the drug is necessary to determine such parameters.

A method of obtaining such data is by the administration of a tracer dose of the drug[3]. The label in such molecules should be in a biologically stable position for two reasons. Firstly, pharmacokinetic data for mebendazole and its metabolites can be obtained, and secondly the biological half-life of the label will be that of the drug and its metabolites, which is considerably less than that of H_2O, upon which body burden data for tritium is based.

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RESULTS AND DISCUSSION

Synthesis of methyl-5(6)-(4'-bromobenzoyl)-2-benzimidazole carbamate, (I) This compound was synthesized from 4-bromobenzoic acid and anisole via a six step reaction sequence similar to that described previously[4] for the synthesis of methyl-5(6)-(4'-fluorobenzoyl)-2-benzimidazole carbamate.

Synthesis of methyl-5(6)-[4'-^{3}H]-benzoyl-2-benzimidazole carbamate (II) To 2 mL of a solution of (I) in dioxane (0.45 mg/mL) was added Pd/$CaCO_3$ (10 mg). The resulting suspension was stirred under tritium gas (4 Ci) for 30 minutes at a pressure of 0.06 atmosphere. The catalyst was removed by filtration, and the dioxane removed by evaporation under a gentle stream of nitrogen gas to give a light brown solid. The residue was dissolved in methanol (2 mL) which was again removed by evaporation. This process was repeated five times.

Purification of (II) The product from the reaction above was dissolved in DMSO (500 µL) which was then added to distilled water (30 mL). The resulting solution was passed through a SEP-PAK C18 extraction cartridge (Waters Associates), and subsequently washed with water (40 mL). The radiochemical was eluted by passing 5 mL of methanol through the cartridge. The methanol was evaporated under a gentle stream of nitrogen to give a white solid which was purified by high performance liquid chromatography (HPLC). The solid was dissolved in DMSO (100 µL) and aliquots (25 µL) were injected onto a 150 x 4.6 mm column packed in this laboratory with LiChrosorb RP8. The flow rate was 1 mL/minute which gave a column pressure of 1500 psi. The mobile phase was 55% methanol in 0.05 M ammonium phosphate buffer, pH 6.0. The fractions corresponding to the mebendazole peak were collected, diluted with ten times that volume of distilled water and extracted using the SEP PAK C18 technique

described above. The resulting methanolic solution was evaporated to dryness to give the pure radiochemical as a white solid. (yield 17%, m.p. 1300°C, m/z 295, 297; 218, 220; 105, 107)

Verification of the position of the tritium label and specific activity determination Tritium nuclear magnetic resonance spectroscopy provides a quantitative measurement of the distribution of the isotope at various sites within a labelled molecule[5],[6]. Spectra of [^{3}H-] mebendazole were obtained on a Bruker CXP-300 spectrometer, operating at 320 MHz, with broad band proton decoupling. Tritium chemical shifts were determined by the technique of ghost-referencing from internal non-tritiated TMS, as recommended by Bloxsidge *et al.*[7]. Incorporation of tritium into the 4' position of the mebendazole molecule was confirmed by the detection of a singlet at 7.1 ppm in the tritium spectrum.

The appearance of both $M^{+\bullet}$ + 1 and $M^{+\bullet}$ + 3 for the benzoyl fragment in the mass spectrum of the radiochemical, also indicates the presence of tritium in the benzoyl ring of the molecule (Figure 1).

Specific activity was determined by two methods. Firstly, by calculation of the $M^{+\bullet}$ + 1/$M^{+\bullet}$ + 3 ratio from the mass spectrum of the pure radiochemical. Secondly, by injection of a sample of the radiochemical onto an HPLC column and obtaining from the calibrated integrator/recorder the amount of compound in the sample. The effluent corresponding to the mebendazole peak was collected and the amount of radioactivity present in the effluent was determined by liquid scintillation counting. From the mass and radioactivity data generated, the specific activity of the labelled compound was determined. The calculated specific activity obtained for the labelled material from both methods was 8 Ci/mmol.

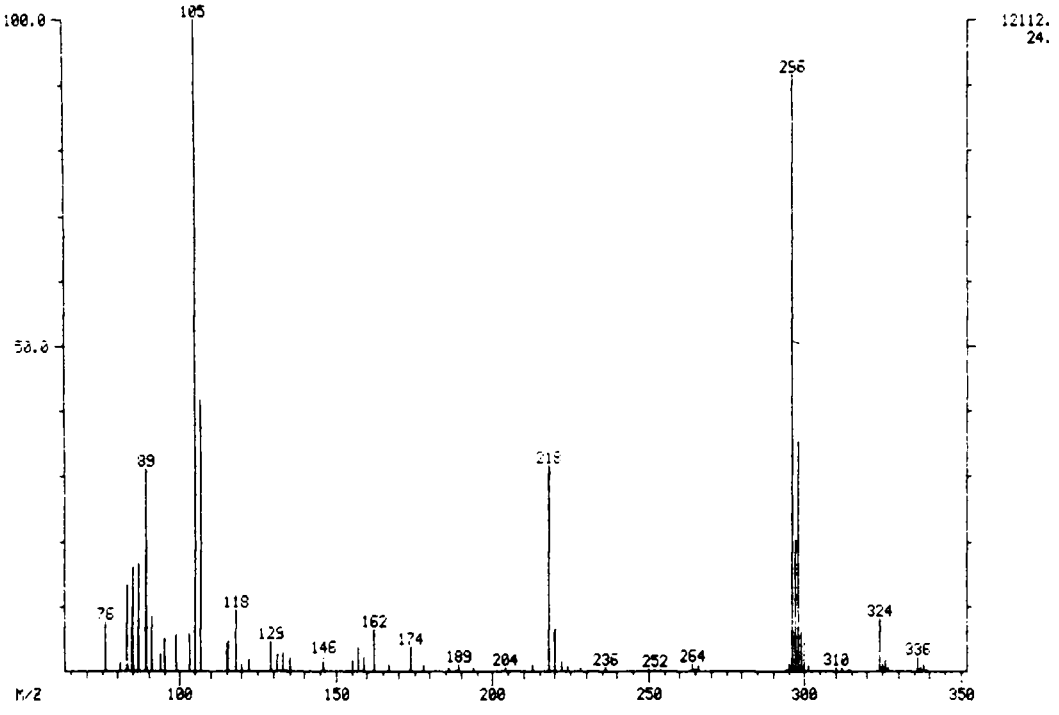

Figure 1. The chemical ionization mass spectrum of tritiated mebendazole

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