Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 9

SYNTHESIS OF ^{14}C- AND ^{35}S-LABELLED 2-MERCAPTOBENZIMIDAZOLES

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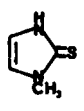
SUMMARY

^{14}C- and ^{35}S-labelled 2-mercaptobenzimidazole and 1-methyl-2-mercaptobenzimidazole were synthesized from ^{14}C-carbon disulfide, ^{35}S-thiourea, ^{14}C-methyl iodide and ^{35}S-thiourea, respectively, for use in studies on the mechanism of action of antithyroid drugs. The products were purified by chromatography on silica and isolated with radiochemical purities of greater than 98%, yields of 45-77% and specific activities of 2.1-5.3 mCi/mmole.

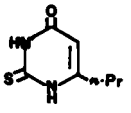
INTRODUCTION

Derivatives of 2-mercaptobenzimidazole (1) are model compounds for the therapeutic antithyroid drugs e.g. 1-methyl-2-mercapto-imidazole (MMI) and 6-propyl-2-thiouracil (PTU) (1) (see Scheme 1).

SCHEME 1.



MMI



PTU

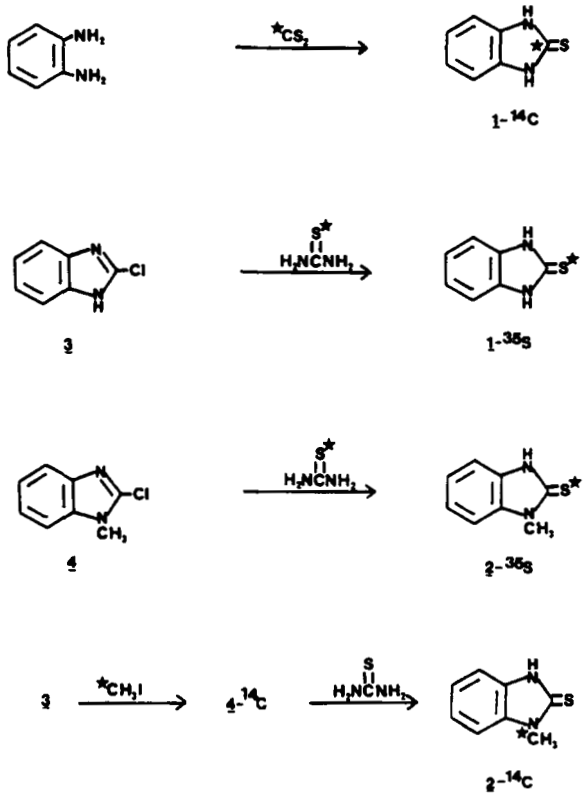
Previous research from this laboratory identified these compounds as suicide inactivators of lactoperoxidase (LPX) (2,3). LPX is readily available and a good model for the thyroid peroxidase (TPX) by many structural and catalytic criteria, including inhibition by thiocarbamides (4). The binding of ^{14}C-MMI and ^{35}S-PTU to TPX was previously measured but the conclusions of this study were limited since no single inhibitor containing ^{14}C- and ^{35}S-isotopes was available (5). In the present study, the syntheses of two thiocarbamide inhibitors (1 and 2), each

0362–4803/88/090985–06$05.00

Received July 24, 1987

singly-labelled with ^{14}C and ^{35}S, were performed in order to determine the mechanism of LPX suicide inactivation.

SCHEME 2.



RESULTS AND DISCUSSION

The radiosynthetic routes to 1 and 2 are shown in Scheme 2. These pathways represent practical methods for the production of 2-mercaptobenzimidazoles which are labelled with ^{14}C and ^{35}S in yields, purity and specific activities which are sufficient for applications in studies on the mechanism of action of antithyroid drugs.

EXPERIMENTAL

Radiochemical precursors

^{14}C-carbon disulfide (21.17 mCi/mmole) was supplied by

Pathfinder Labs, now Sigma Chemical Co. (St. Louis, MO); ^{14}C-methyl iodide (56 mCi/mmole) and ^{35}S-thiourea (30-45 mCi/mmole) were supplied by Amersham Co. (Arlington Hts., IL).

Chemical precursors

1,2-Diaminobenzene, obtained from Eastman Kodak Corp. (Rochester, NY), was purified by recrystallization from aqueous sodium dithionite (6). 2-Chlorobenzimidazole (3) was synthesized by the method of Harrison et al. (7). 2-Chloro-1-methylbenzimidazole (4) was synthesized by the method of Kikugawa (8). The reaction methods were validated with regard to yield and separation method using unlabelled precursors prior to each radiosynthesis.

Chromatographic methods

Radiosyntheses were monitored by thin layer chromatography on silica (Merck F254, 0.25 mm) using 5% methanol/chloroform (solvent A) or 50% benzene/ethyl acetate (solvent B). Following radiosynthesis, the products were purified by flash chromatography on silica (Davisil 30-40 micron particle size, Alltech Associates, Deerfield, IL). The primary concern in the chromatographic separations was the purity of the product and therefore the yields reported are not maximized. Chemical purity of products and starting materials was determined by tlc or HPLC. Radiochemical purity of all products was determined by tlc in one dimension using both solvents A and B followed by scraping silica zones from the plates and liquid scintillation counting (Beckman DPM-100 liquid scintillation system). Radiochemical purities were at least 98% for all compounds synthesized. The concentration of 1 and 2 in acetone solution was determined by reversed phase HPLC on Novapak C-18 (5 micron, Waters Associates, Milford, MA) using UV absorbance at 305 nm for detection, 20% acetonitrile/water for 1 and 25% acetonitrile for 2 at a flow rate of 1.5 ml/min.

Synthesis of 1-^{14}C

The reaction was performed by the method of Van Allen and Deacon (9). Unlabelled carbon disulfide (0.5 mmole) was added to a cooled (-77°C) break-seal tube which contained ^{14}C-carbon disulfide (1 mCi, 0.05 mmole). A solution of 1,2-diaminobenzene (0.56 mmole) in 1 ml of ethanol containing potassium hydroxide (0.05 mmole) was added and the tube resealed. After heating for 4 hr at 100°, the tube was cooled and 2 ml water and 0.1 ml acetic acid were added. The resulting suspension was extracted with two 5 ml portions of ethyl acetate and the combined extracts dried with anhydrous sodium sulfate. The product was purified by flash chromatography using solvent B. The yield of purified 1-^{14}C was 65% with a specific activity of 2.1 mCi/mmole.

Synthesis of 1-^{35}S

The reaction was performed by the method of Harrison and Ralph (10). An ethanolic solution of ^{35}S-thiourea (0.5 mCi 0.01 mmole) was added to 3 (0.2 mmole) and unlabelled thiourea (0.2mmole) in a total volume of 5 ml. The solution was refluxed for 2.5 hr and tlc analysis showed a single product band and no remaining 3. The solvent was removed in vacuo and 5 ml of a 2% sodium carbonate solution added. After stirring at room temperature for 1 hr, the reaction mixture was cautiously acidified with 4 M hydrochloric acid. The resulting suspension was extracted with two 5 ml portions of ethyl acetate and dried. Following flash chromatography using solvent A, the yield of purified 1-^{35}S was 62% with a specific activity of 2.7 mCi/mmole.

Synthesis of 2-^{14}C

One ml of an acetone solution containing ^{14}C-methyl iodide (0.5 mCi, 0.02 mmole) and unlabelled methyl iodide (0.2 mmole) was added to a solution of 3 (0.22 mmole) in 3 ml dry acetone containing powdered potassium hydroxide (1.8 mmole). The reaction was stirred vigorously for 10 min then 5 ml dichloromethane was

added. The suspension was washed with 5 ml water followed by 5 ml of a saturated salt solution. The organic phase was dried and the solvent removed in vacuo to yield a colorless oil. Unlabelled thiourea (0.26 mmole) dissolved in 5 ml ethanol was added and the mixture refluxed until tlc analysis showed the disappearance of starting material (16 hr). The product 2-^{14}C, worked-up as described for I-^{35}S, was isolated with a yield of 45% with a specific activity of 3.0 mCi/mmole.

Synthesis of 2-^{35}S

The synthesis was performed as described for 1-^{35}S using ^{35}S-thiourea (1 mCi, 0.03 mmole) and IV. The yield of purified 2-^{35}S was 77% and the specific activity was 5.3 mCi/mmole.

ACKNOWLEDGEMENTS

This work was supported by a Research Starter Grant from the Pharmaceutical Manufacturers Association Foundation and is contributed as Journal Series No. 3198 from the Hawaii Institute of Tropical Agriculture and Human Resources.

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