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SYNTHESIS OF MUTAGENIC 2,4-DINITRO-[7-^{14}C]BENZALDEHYDE

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SUMMARY

2,4-Dinitro-[7-^{14}C]benzaldehyde (4), a mutagenic substance which may have an unique metabolic activating pathways, was synthesized as a tracer for the detection of adducts of 4 with macromolecules. The preparation was performed in four steps from [7-^{14}C]toluene to give an overall radiochemical yield of 20% after purifying by TLC. The radiochemical purity was over 98%.

Key Words: 2,4-dinitro-[7-^{14}C]benzaldehyde, mutagen

INTRODUCTION

2,4-Dinitrobenzaldehyde (4), which is a biliary metabolite of 2,4-dinitrotoluene (1)(1) and is formed by incubating 2,4-dinitrobenzyl alcohol (3) with rat liver microsomal preparations (2), has been shown to be mutagenic without metabolic reaction system in the Ames assay using Salmonella typhimurium strains TA 98 and TA 100 (3). In addition, 4 is found to elicit marked cell transformation in the cultivation with C3H/10T1/2 cell line not containing nitroreductase activity (4).

These results led to the assumption that the biological activities of 4 mentioned above might be more dependent on the presence of CHO group in the molecule than on the bacterial and cellular reductions of the nitro groups. This postulate was reinforced by the finding that the mutagenic activity of 4 in TA 98/1,8-DNP$_6$, an O-acetylase-deficient mutant of TA 98, was lower than that in TA 98 (5), suggesting that a hydrate of 4 is responsible for the mutagenicity.

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Since it is probable that the reduction of the nitro group is an essential step in the activation of organic nitro compounds to ultimate mutagens (6,7,8), the biological activities of 4 seen in the bacterial and cellular systems are thought to be unique. Thus, the preparation of ^{14}C-labeled 4 was attempted as a tracer for the detection of adducts of 4 with bacterial and cellular macromolecules including nucleic acids. The preparation of ^{14}C-labeled 4 was performed using [7-^{14}C]toluene as a starting material.

DISCUSSION

[7-^{14}C]Toluene was nitrated with nitric acid and trifluoromethanesulfonic acid (9) to give isomers of 1 in 66% yield. The precise proportion of the isomers was not determined in this step. Major isomers obtained by the nitration of toluene are reported to be 2,4-dinitrotoluene (82.84%), and its 2,6-isomer (15.70%) under the same condition as in this study (9). Oxidation of methyl group of 1 in acetic acid with chromium(VI) oxide and sulfuric acid provided a crude product of 2,4-dinitro-[7-^{14}C]benzoic acid (2) in the calculated yield of 41% from toluene. Carboxylic acid 2 accounted for 97.3% of isomers of dinitrobenzoic acid, since methyl group of 2,6-isomer of 1 was hard to be oxidized. The reduction of carboxylic acid 2 to alcohol 3 was accomplished using borane-THF (10). Following oxidation of alcohol 3 with activated manganese(IV) oxide (11) gave a crude product of 4. Purification of this product by preparative TLC gave a final product of 4 with a radiochemical purity of over 98% .

Unlabeled aldehyde 4 is commercially prepared from 2,4-dinitrotoluene and *p*-nitroso-N,N-dimethylaniline with a yield of 24-32% (12). The procedures applied for the preparation of 4 in this study were carried out under mild conditions with convenient reagents, which appears to be suitable for the small-scale preparation.

The preparation of mutagenic 4 was accomplished with a specific activity of 131 MBq/mmol and an overall yield of 20% using a four step synthetic route from [7-^{14}C]toluene. Hepatic macromolecular covalent binding studies are currently in progress using this labeled compound.

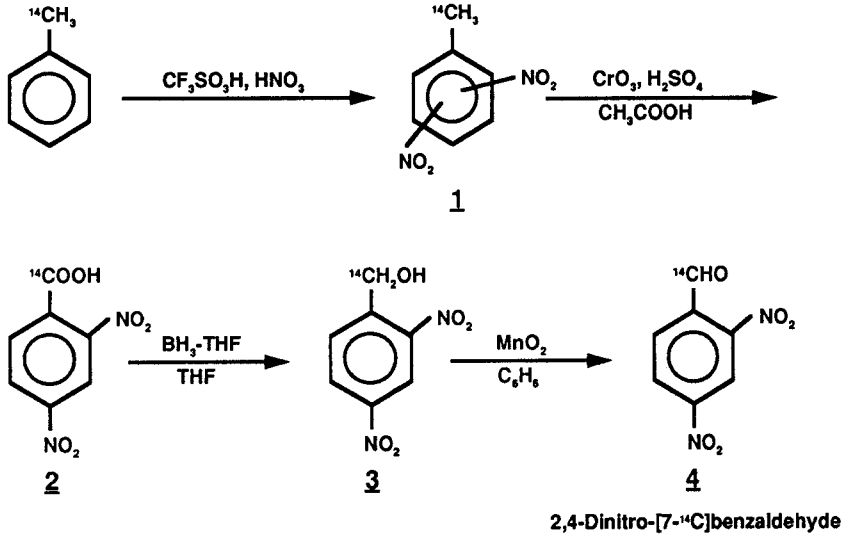
EXPERIMENTAL

[7-^{14}C]Toluene was obtained from Amersham. All other chemicals were purchased commercially and used with no further purification.

Radioactivity determinations were carried out with a Beckman LS3801 liquid scintillation counter using ACS II scintillation cocktail. HPLC analysis was performed using a Hitachi Liquid Chromatograph 655 with a Toso TSKgel ODS-80T$_M$ column (4.6 mm ID x 15 cm). HPLC radioactivity measurements were achieved with Aloka Radioanalyzer RLC 551. Analytical TLC was performed using silica gel 60 F-254 (Merck glass plates, 0.25 mm thickness, 5 cm x 20 cm) followed by radioactivity measurements with Ambis Radioanalytic Imaging System. Preparative TLC was carried out using silica gel 60 F-254 (Merck glass plates, 0.5 mm thickness, 20 cm x 20 cm).

Labeled intermediates and the final product were identified by co-elution of the labeled substance with authentic unlabeled compounds via HPLC or TLC.

SCHEME



Dinitro-[7-^{14}C]toluene (1)

A total of 1.9 mg (0.021 mmol, 1.78 GBq/mmol) of [7-^{14}C]toluene supplied under vacuum in break seal ampoules was collected at the bottom by cooling in a liquefied nitrogen bath. The ampoules were opened by breaking glass seals with a glass stick, and a total of 24 mg (0.26 mmol) of toluene was added. Ampoules were closed by ground glass caps and brought to room temperature. To this was added 400 mg (2.7 mmol) of trifluoromethanesulfonic acid and 63 mg (1.0 mmol) of fuming nitric acid.

The resulting mixture was left at room temperature for 90 min. To the reaction mixture, 20 ml of ether was added followed by 2 ml of 2 N sodium hydroxide. The ethereal layer was separated and the aqueous layer was extracted further with ether. The combined ethereal extract was dried over anhydrous magnesium sulfate and filtered. Removal of solvent gave 33.7 mg (24.5 MBq, 66%) of a crude mixture of isomers of 1. This material was used directly in the next step without purification.

2,4-Dinitro-[7-^{14}C]benzoic acid (2)

A crude mixture of isomers of 1 (33.7 mg, 0.185 mmol, 24.5 MBq) was dissolved in 0.4 ml of glacial acetic acid, and 129 mg (1.3 mmol) of sulfuric acid and 70 mg (0.70 mmol) of chromium(VI) oxide were added. The reaction mixture was stirred at room temperature for 24 hr. The resulting solution was extracted with ether, dried over anhydrous magnesium sulfate, filtered and evaporated to give a crude mixture of isomers of 2. HPLC analysis indicated that this product contained 24.6 mg (15.3 MBq) of 2, and the relative proportion of 2, its 2,6-isomer and other isomers was 97.3%, 1.0% and 1.7%, respectively. The calculated yield of 2 from toluene was 41%. This material was used in the next step after drying.

2,4-Dinitro-[7-^{14}C]benzyl alcohol (3)

The crude product containing 24.6 mg (0.116 mmol, 15.3 MBq) of 2 was dried under vacuum and dissolved in 0.025 ml of THF followed by adding 0.6 ml (0.6 mmol) of 1.0 M solution of borane-THF complex in THF slowly with stirring and the resulting solution was stirred at room temperature for 16 hr. Excess hydride was carefully destroyed by dropping water slowly and the aqueous layer was saturated with potassium carbonate. The THF layer was separated and the aqueous layer was extracted with ether. The combined organic extracts was dried over anhydrous magnesium sulfate. Removal of solvents gave a crude product of 3, and this material was used in the next step without further purification.

2,4-Dinitro-[7-^{14}C]benzaldehyde (4)

The crude product of 3 was dissolved in 1.7 ml of benzene followed by the addition of 174 mg (2.0 mmol) of activated manganese(IV) oxide. The reaction mixture was stirred at room temperature for 24 hr. Separation of manganese oxide from the

suspension by centrifugation and subsequent decantation gave a benzene solution containing 4. Solvent evaporation gave a crude product of 4. The purification of 4 from other isomers and unreacted intermediates was carried out on a preparative TLC in chloroform to give 11.3 mg (0.058 mmol, 7.6 MBq) of final product 4 in 20% overall yield from toluene. A radiochemical analysis by TLC indicated a radiochemical purity of over 98%.

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