Note

Synthesis of 3'-Hydroxymethyl-4-(dimethylamino)azobenzene[carbinol- ^{14}C] †*

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1. Introduction

3'-Methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) and 3'-methyl-4-methylaminoazobenzene (3'-Me-MAB) are much more potent hepatocarcinogens than DAB and MAB.¹⁾ However, the effect of a ring methyl group on the carcinogenic action of the aminoazo dyes is not clear. Recent our studies have demonstrated that the metabolites oxidized at the 3'-methyl group of 3'-Me-DAB or 3'-Me-MAB, such as 3'-CH₂OH-, 3'-CHO-, and 3'-COOH-aminoazo dyes, were newly detected from the rat bile; the metabolism of these aminoazo dyes in the rat involves oxidation of the ring methyl group²⁾⁻⁴⁾. In these metabolites, 3'-CH₂OH-DAB was found to be a potent hepatocarcinogen to the rats, while 3'-CHO-DAB and 3'-COOH-DAB were not⁵⁾. In order to examine whether a two-step enzymic activation suggested by Kadlubar et al.⁶⁾ is involved in the metabolic change of 3'-hydroxymethyl group of 3'-CH₂OH-DAB, further studies on the metabolism of 3'-CH₂OH-DAB at the 3'-hydroxymethyl group in animals were required. Therefore, it became desirable for the detailed metabolic studies to prepare 3'-CH₂OH-DAB labeled with carbon-14 at a specific position (carbinol- ^{14}C). Benzyl alcohol[7- ^{14}C] and benzoic acid[carboxyl- ^{14}C] are the commercially available starting

materials for the purpose. In this paper, the synthesis of 3'-CH₂OH-DAB[carbinol- ^{14}C] (I) starting from benzoic acid[carboxyl- ^{14}C] (II) was investigated.

2. Results

The synthetic scheme of I from II was shown in Fig. 1. 3-Nitrobenzoic acid[carboxyl- ^{14}C] (III) was obtained by nitration of II with nitric acid in a 51% yield. III was effectively converted to 3-aminobenzoic acid[carboxyl- ^{14}C] (IV, 99%) by catalytic hydrogenation using 5% palladium on charcoal as a catalyst. Reduction of a mixture of IV and unlabeled 3-aminobenzoic acid added as a carrier with lithium aluminum hydride gave 3-aminobenzyl alcohol[carbinol- ^{14}C] (V) in a good yield (94%). Purification of each product in these steps was omitted. Direct reduction of III to V by several ways was failed.

The preparation of 3'-CH₂OH-DAB[carbinol- ^{14}C] (I) was carried out by adding N,N-dimethylaniline to a solution of diazotized V. This coupling of the diazonium salt gave I in 34% yield after intensive purification by thin-layer chromatography on silica gel plate and by recrystallization from aqueous ethanol. Consequently, the overall yield for this four-step synthesis of I from II amounted to 16.3%. The product similarly synthesized from unlabeled benzoic acid as shown in Fig. 1 was identified by melting point (122°), mass spectrometry (M^+ , 255 m/e), and infrared spectroscopy; the spectrum is consisted with that of an authentic sample. Radiochemical purity was shown to be 97% by radioscannography of thin-layer chromatogram.

† 3'-ハイドロキシメチル-4-(ジメチルアミノ)アゾベンゼン[カルビノール- ^{14}C]の合成。森幸雄，丹羽俊朗，豊吉一美：岐阜薬科大学，502 岐阜市三田洞東5-6-1

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Fig. 1 Synthesis of 3'-hydroxymethyl-4-(dimethylamino)azobenzene [carbinol-¹⁴C].

The specific activity of the product was 0.364 mCi/mmol and the radiochemical yield was 15.5 % based on benzoic acid[carboxyl-¹⁴C]. Accordingly, it was considered that the use of I should permit detailed studies on the *in vitro* or *in vivo* metabolism of 3'-CH₂OH-DAB in animals.

3. Experimental

3.1 3-Nitrobenzoic acid[carboxyl-¹⁴C] (III)

A mixture of benzoic acid[carboxyl-¹⁴C] (II, 3.26 mg, 1.5mCi, 57mCi/mmol, The Radiochemical Centre) and benzoic acid (350.0mg) was slowly added to 2 ml of nitric acid (d. 1.52). The reaction temperature was kept below 20° with an ice bath. After the addition was completed, the reaction mixture was stirred for 1 hr at a room temperature. The mixture was then poured into ice water and the precipitated III was filtrated. The yield of a crude III was 213 mg (51%).

3.2 3-Aminobenzoic acid[carboxyl-¹⁴C] (IV)

III (213mg) in ethanol (20 ml) was hydrogenated at ordinary pressure and temperature over 5% Pd-C (44 mg) in a Wilsbach apparatus for 1 hr. Hydrogenation of the mixture was continued for further 2 hr when the uptake of hydrogen ceased, and the catalyst filtered off. The filtrate was evaporated under reduced pressure to give a crude IV. The yield was 173 mg (99%).

3.3 3-Aminobenzyl alcohol[carbinol-¹⁴C] (V)

Into a flask fitted with a reflux condenser were placed lithium aluminum hydride (242 mg, Metallgesellschaft AG) and anhydrous tetrahydrofuran (30 ml). To the mixture was added dropwise with stirring a solution of a mixture of IV (173 mg) and 3-aminobenzoic acid (100mg) in anhydrous tetrahydrofuran (20 ml). After the complete addition, the mixture was gently refluxed for 22 hr. After cooling, the mixture was extracted with ethyl acetate, and the extract washed with water. Drying over sodium sulfate, the extract was evaporated under reduced pressure to give a crude V. The yield was 231 mg (94%).

3.4 3'-Hydroxymethyl-4-(dimethylamino)azobenzene [carbinol-¹⁴C] (I)

To 231 mg of V dissolved in 4.6 ml of 4N hydrochloric acid and cooled to 0-5° with an ice bath was slowly added 0.92 ml of a 2M solution of sodium nitrite (129 mg) while maintaining the temperature between 0° and 5°. After the addition was completed, stirring was continued for 15 min. N,N-Dimethylaniline (227 mg) was added to the diazonium salt solution in one portion. The solution was then neutralized by addition of sodium acetate (7.3 g) in portions, and the precipitated aminoazo dye was extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate, and evaporated under reduced pressure to give a residue. The residue was subjected to thin-layer chromatography on silica gel (Wakogel B-5F) plates (20×20 cm) with benzene-acetone (9:1 v/v) 2 times. Elution of the main fraction with methanol, evaporation of the eluate under a gentle stream of nitrogen, and recrystallization from aqueous ethanol gave I (0.232 mCi, 163 mg, 34%); radiochemical purity 97% on radioscanography of thin-layer chromatogram (silica gel, benzene-acetone=9:1 and chloroform-methanol=5:1, R_f values 0.54 and 0.85 respectively; being identical with an unlabeled authentic sample).

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