Synthesis of ^{14}C-Labelled 1-β-D-Arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU)

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SUMMARY

^{14}C-Labelled 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU) was synthesized in 26.6% radiochemical yield from 1-β-D-arabinofuranosyl-5-formyluracil and ^{14}C-labelled malonic acid for the purpose of the metabolic studies in animals.

Key Words: antiviral agent, 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil, malonic acid, synthesis, carbon-14

1-β-D-Arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU) is a novel potent antiviral agent against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV).[1-4] The clinical studies on the efficacy of BV-araU treatment are now being studied. ^{125}I-Labelled iodovinyl-deoxyuridine, a structurally related compound of BV-araU, as effectively used to study its metabolic fate in VZV-infected cells.[5] Then, we have synthesized ^{14}C-labelled BV-araU (3), whose labelled position is C-2 in 2-bromovinyl side chain at C-5 position of uracil base. The labelled ^{14}C-BV-araU is going to be employed for the biochemical and pharmacological studies on BV-araU. The synthetic pathway of ^{14}C-labelled BV-araU is shown in scheme 1.

^{14}C-Labelled carboxyvinyl derivative (2) was obtained by the reaction of 1-β-D-arabinofuranosyl-5-formyluracil[6](1) with [2-^{14}C]-malonic acid in the presence of piperidine. After thorough removal

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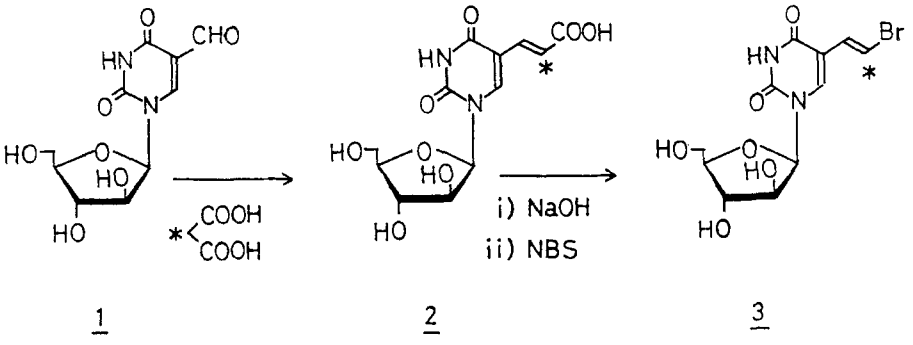


Scheme 1

of the solvent by co-evaporation with aqueous ethanol, the sodium salt of compound (2) was treated with N-bromosuccinimide (NBS) in aqueous N,N-dimethylformamide (DMF) to give crude compound (3). After usual work-up, 3 was isolated as crystals. The yield based on ^{14}C-labelled malonic acid was 26.6%. The radiochemical purity of 3 was confirmed to be greater than 98% by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses. In a preliminary study most of ^{14}C-BV-araU administered orally into rats was found in blood and recovered from the urine as unchanged form.

EXPERIMENTAL

[2-^{14}C]Malonic acid was diluted to 137 mCi/2.58 mmol with unlabelled malonic acid. TLC was performed on pre-coated silica gel 60 F254 plates (Merck). Solvent systems were as follows: A, chloroform-methanol-acetic acid (9:3:1 v/v/v); B, 2-propanol-water-acetic acid (7:2:1 v/v/v); and C, 2-propanol-water-ammonium hydroxide (7:2:1 v/v/v). The ^{1}H-NMR spectra were recorded on a JEOL FX-270FT spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet) and m (multiplet). HPLC was performed on an Inertsil ODS-5μ (Gasukuro Kogyo Co., Ltd.) reversed

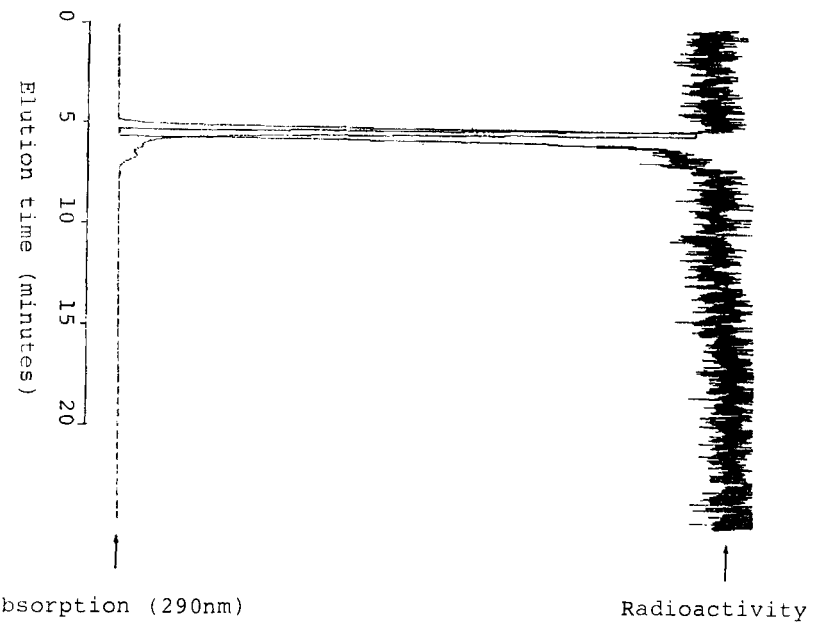
phase column. The solvent was a mixture of water, acetonitrile and 1 M triethylammonium acetate buffer (pH 7.0) (75:20:5 v/v/v). The flow rate was 2 ml/min. An ultraviolet detector at a wavelength of 290 nm and a radiodetector were used in the analyses. The radioactivity of the labelled compound as measured by a Liquid Scintillation Counter using an AQUASOL-2 (Du Pont/NEN Research Products) as a scintillator.

1-β-D-Arabinofuranosyl-*E*-5-(2-carboxy-[2-^{14}C]vinyl)uracil (2) was obtained as follows. The aldehyde (1) (743 mg, 2.73 mmol) was dissolved in anhydrous pyridine (5 ml) and mixed with [2-^{14}C]-malonic acid (2.58 mmol, 137 mCi) and freshly prepared piperidine (0.05 ml). The mixture was stirred at 100 °C for 20 min, then cooled in an ice bath. This mixture was directly subjected to the next step.

1-β-D-Arabinofuranosyl-*E*-5-(2-bromo-[2-^{14}C]vinyl)uracil (3)

One normal aqueous sodium hydroxide (2.7 ml) was added to the solution of the crude 2, and the mixture was evaporated to dryness under reduced pressure. The residual syrup was co-evaporated with 50% (v/v) aqueous ethanol (7.5 ml). This procedure was repeated four times. The resulting syrup was dissolved in 80% (v/v) aqueous DMF (12 ml) and mixed with NBS (513 mg, 2.9 mmol). The mixture was stirred at room temperature for 10 min, and then diluted with distilled water (50 ml). The pH of the mixture was adjusted to 2.0 with 6 N hydrochloric acid. The solution was applied to a column of Diaion Sepabeads SP-206 (Mitsubishi Chemical Co., Ltd., 2.0 cm x 9.5 cm), which was washed with distilled water (50 ml). The product was eluted stepwise with 20% (v/v) and 40% (v/v) aqueous ethanol. Appropriate fractions were combined and evaporated to dryness. Then 405 mg of unlabelled BV-araU was added to the residue. Final product (3) (682 mg, 36.5 mCi) was obtained from 50% (v/v) aqueous methanol as white crystals. The radiochemical yield was 26.6%.

Fig. 1 HPLC analysis of the final product (3)



^{1}H-NMR (DMSO-d_6) δ : 3.62 - 3.67 (2H, m, H-5'), 3.73 - 3.76 (1H, m, H-4'), 3.89 - 3.92 (1H, m, H-3'), 4.01 - 4.06 (1H, m, H-2'), 5.99 (1H, d, J=4.7 Hz, H-1'), 6.88 (1H, d, Ja,b=13.7 Hz, -CHa=CHbBr), 7.24 (1H, d, Ha), 7.89 (1H, s, H-N^3).

IR (KBr) : 3250, 2830, 1690, 1470, 1290, 1090, 1030, 950 cm^{-1}. TLC of the radioactive compound showed only one spot (Rfs : 0.36, 0.75 and 0.61 in the systems A, B and C, respectively). The Rfs were identical to those of authentic BV-araU. The radiochemical purity was more than 98 and 99.9% by TLC and HPLC (Fig. 1) analyses, respectively.

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