NOTES

SYNTHESIS OF ^{14}C-LABELLED SHOWDOMYCIN, 3-(β-D-RIBOFURANOSYL)-3-PYRROLINE-2,5-DIONE-5-^{14}C

The C-glycosyl nucleoside antibiotic, showdomycin, was first isolated from the culture filtrate of Streptomyces showdoensis by Nishimura et al.[1,2] of our laboratory. On the basis of spectroscopic studies, chemical transformations, and X-ray crystallographic examination, showdomycin was confirmed to be 2-(β-D-ribofuranosyl)maleimide[3] (I). The antibiotic inhibited growth of several Gram-positive and Gram-negative bacteria[2] and was particularly active against Streptococcus haemolyticus. As the compound showed significant antibacterial and antitumor[2,4,5] activities, it was the subject of numerous biochemical studies.[6] Hatanaka reported that showdomycin showed excellent radiopotentiating activity[7] against Ehrlich ascites tumor in mouse, and further stated at the 1972 Japan Conference on Cancer that its administration gave good results in four cases of inoperable glioblastoma.[8]

The ^{14}C-labelled form of showdomycin (I) was needed in order to conduct metabolic and distributive studies in animals. Showdomycin was synthesized by Trummlitz and Moffatt[9] and Kalvoda et al.,[10] and ^{14}C-labelled showdomycin by Pichat[11] from potassium cyanide-^{14}C by the Moffatt procedure. The labelled compound was 3-(β-D-ribofuranosyl)-3-pyrroline-2,5-dione-2-^{14}C (II).

For a facile synthetic route to ^{14}C-labelled showdomycin, we used chloroacetic acid-1-^{14}C as starting material to obtain a ^{14}C-labelled triphenylphosphonium chloride (VI), from which ^{14}C-labelled showdomycin, 3-(β-D-ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (III) was smoothly obtained according to the Moffatt procedure. The labelled compound was synthesized as shown in the synthetic scheme.

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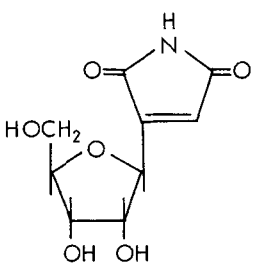
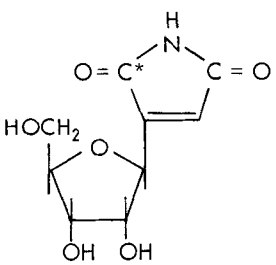
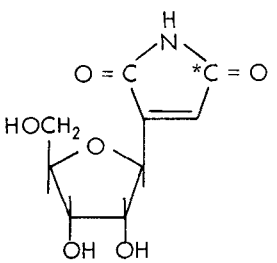
Methyl chloroacetate-1-^{14}C prepared from chloroacetic acid-1-^{14}C, 5 mCi was treated with ammonium hydroxide to give chloroacetamide-1-^{14}C. The acid amide was converted into (carbamoyl-^{14}C) methylenetriphenylphosphonium chloride (VI) by treatment with triphenyl phosphin in 80% yield. Methyl 3,6-anhydro-4,5,7-tri-O-benzyl-D-glycero-D-allo-heptonate[9] (IV) was oxidized to give the keto ester (V), which was condensed with the Wittig reagent obtained from VI to give a maleimide (VII) in an overall yield of 59% from the hydroxy ester (IV). Removal of the benzyl ether groups from the maleimide (VII) was performed by treatment with boron trichloride in methylene dichloride at -75°, giving ^{14}C-labelled showdomycin, 3-(β-D-ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (III), in 22.8% radiochemical yield. The overall radiochemical yield was 9.8% based on chloroacetic acid-1-^{14}C. Cycloshowdomycin acetonide-^{14}C[3] (VIII) was obtained as a byproduct.



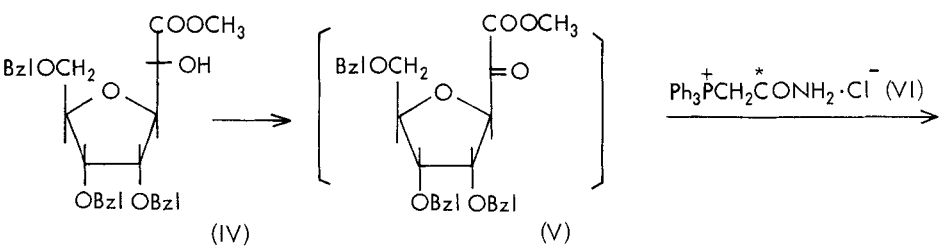
(I)



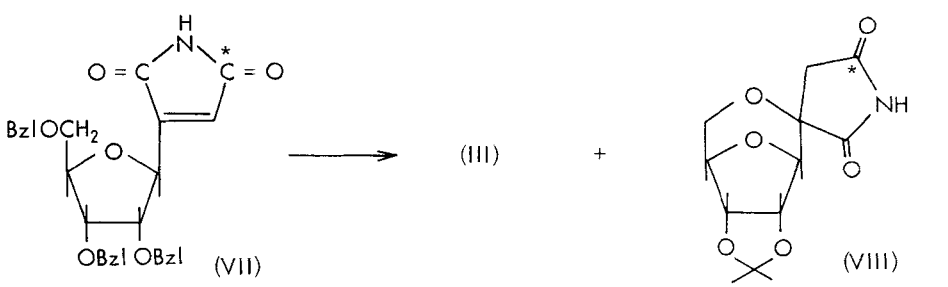
(II)



(III)



(IV) (V)



(VII) (III) + (VIII)

EXPERIMENTAL

Chloroacetamide-1-^{14}C

Chloroacetic acid-1-^{14}C, 5 mCi (specific activity: 44 mCi/m mol) was dissolved in ether (2 ml) and diluted with a carrier, cold chloroacetic acid (195 mg, 2.066 m mol) in ether (2 ml). An excess of diazomethane-ether solution was added to the acid solution with stirring in an ice bath and left for 10 min. Concentrated ammonium hydroxide (4 ml) was added to the reaction solution with stirring and the mixture was stirred for 1 hr. in an ice bath. The mixture was evaporated and extracted with methylene dichloride. The extract was dried (Na_2SO_4) and evaporated to leave a residue (185 mg, 4.55 mCi), chloroacetamide-1-^{14}C, in 91% radiochemical yield, which was used for the next reaction without purification.

(Carbamoyl-^{14}C) methylenetriphenylphosphonium chloride (VI)

Chloroacetamide-1-^{14}C (4.55 mCi, 185 mg, 1.98 m mol) and triphenylphosphin (786 mg, 3 m mol) were dissolved in benzene (5 ml) and heated under reflux for 56 hr. After cooling, crystals were separated from the solution and collected. They were washed with cold benzene and dried to give (carbamoyl-^{14}C) methylenetriphenylphosphonium chloride (VI) (564 mg, 3.64 mCi) in 80% radiochemical yield.

3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (VII)

Dichloroacetic acid (120 mg) was added to a solution of methyl 3,6-anhydro-4,5,7-tri-O-benzyl-D-glycero-D-allo-heptonate[9] (IV, 940 mg, 1.9 m mol) and dicyclohexylcarbodiimide (980 mg) in a mixture of anhydrous dimethyl sulfoxide (10 ml) and benzene (10 ml) with stirring in an ice bath. The mixture was stirred for 30 min. at room temperature and cooled to 0°, and then a solution of oxalic acid (360 mg) in water (2 ml) was added with stirring. The mixture was kept at room temperature for 30 min., diluted with ethyl acetate (40 ml), and filtered. The filtrate was washed with water (50 ml x 5), dried (Molecular Sieve 4A), and evaporated to leave a syrup. The residue was dissolved in chloroform and filtered. The filtrate was dried and evaporated, leaving the crude keto ester (V) as a syrup.

A mixture of the syrup and (carbamoyl-^{14}C) methylenetriphenylphosphonium chloride (VI) (3.64 mCi, 564 mg, 1.59 mmol) in anhydrous tetrahydrofuran (23 ml) was stirred at room temperature to give a suspension. Sodium hydroxide solution [270 µl of a solution of NaOH (473 mg) in water (1 ml)] was added to the suspension with stirring in an ice bath and stirred for 1 hr. at the same temperature. Dowex 50 (H^+) (4 g) was added to the mixture and filtered. The resin was washed with tetrahydrofuran. The filtrate and the washings were combined and evaporated to leave a residue. The residue was dissolved in methylene dichloride (30 ml), washed with water (30 ml x 4), dried (Na_2SO_4), and evaporated, leaving a residue. The residue was dissolved in 100 ml of hexane - ether (2 : 1) and chromatographed on 15 g of silicic acid (Mallinckrodt, 100 mesh) to give the crude maleimide (VII). The product was crystallized from ether-hexane to give crystals (450 mg). The mother liquor was evaporated and a residue was separated to obtain VII (135 mg) by preparative t.l.c. on silica gel (Merck, KGF plate). Combined crystals were recrystallized from ether-hexane to give 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (VII), m.p. 64-65° (469 mg, 2.15 mCi), in 59% radiochemical yield based on VI.

3-(β-D-Ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (III) (showdomycin-^{14}C)

A solution of boron trichloride (8.8 g) in methylene dichloride (30 ml) at -75° was added dropwise to a solution of VII (2.15 mCi, 469 mg, 0.94 mmol) in methylene dichloride (17 ml) with stirring at -75° under nitrogen atmosphere and stirred for 1 hr. at the same temperature. A mixture of methanol (50 ml) and methylene dichloride (50 ml) at -75° was added dropwise to the reaction mixture with stirring at below -60° over a period of 1 hr. and stirred for 1 hr. at -75°. The temperature was allowed to slowly rise to -20°, and the mixture was stirred for 1 hr. at -20° and stored overnight at -25°. The mixture was evaporated in vacuo at below 10° and the residue was coevaporated with methanol (5 ml) two times. Because the final residue (400 mg) showed two radioactive spots, R_F 0.33 and 0.68 on t.l.c. (silica gel: Merck KGF plate; solvent system: acetone-ethyl acetate = 1 : 1), it was separated by preparative t.l.c. The R_F 0.33 fraction was extracted with acetone from silica gel and the solvent was evaporated to leave a residue (138 mg), which was crystallized from

acetone-benzene to give 3-(β-D-ribofuranosyl)-3-pyrroline-2,5-dione-5-^{14}C (III) as colorless needles, m.p. 154-155° (48.1 mg, 490 μCi, 2.34 mCi/m mol), in 22.8% radiochemical yield. The overall radiochemical yield was 9.8% based on chloroacetic acid-1-^{14}C. This compound was confirmed to be pure by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel (Merck KGF plate), solvent: acetone-ethyl acetate = 1 : 1].

The R_F 0.68 fraction was extracted with acetone from silica gel and evaporated to leave a residue (155 mg), which was crystallized from ether-acetone to give colorless needles (85 mg), m.p. 218-220° . This compound was confirmed to be ^{14}C-labelled cycloshowdomycin acetonide (VIII) upon mixed m.p. determination with an authentic cold sample[3] and by comparison of spectroscopic data and t.l.c.

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H. Minato, Y. Katsuyama, T. Nagasaki, J. Irisawa, and K. Igarashi

Shionogi Research Laboratory, Shionogi & Co., Ltd.,

Fukushima-ku, Osaka, 553 Japan

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