SYNTHESIS OF ^{14}C-LABELLED 4-HYDROPEROXYISOPHOSPHAMIDE, AN EFFECTIVE ANTITUMOR AGENT

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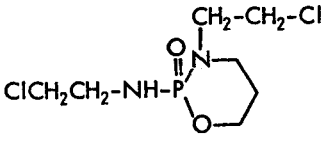
SUMMARY

4-Hydroperoxyisophosphamide (II), an effective antitumor agent has been labelled with carbon-14. The carbon-14 label was incorporated into the C-6 position of the molecule to give (VII). The overall radiochemical yield was 4.4% based on barium carbonate-^{14}C. The labelled compound obtained was used for absorption and metabolism studies.

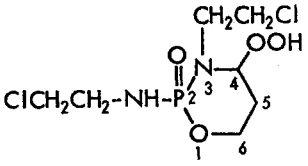
Isophosphamide (I) is an experimentally effective antitumor agent and is thought to exert cytotoxicity after in vivo metabolic transformation. Some recent studies have suggested that the activation of isophosphamide is caused by enzymatic C-4 hydroxylation in animal liver. Takamizawa[1,2] in our laboratory has synthesized 4-hydroperoxyisophosphamide (II) as the suggested active species of isophosphamide, and reported that it exhibited marked antileukemic activities in both in vivo and in vitro experiments and that there were significant differences not only in in vivo activity but also in in vivo metabolic behavior between isophosphamide and its active form (II).

For studies of the incorporation of this compound (II) into Yoshida sarcoma ascites cells and L 1210 leukemia cells and of the organ distribution in mice, radioactive forms

of the drug were required.

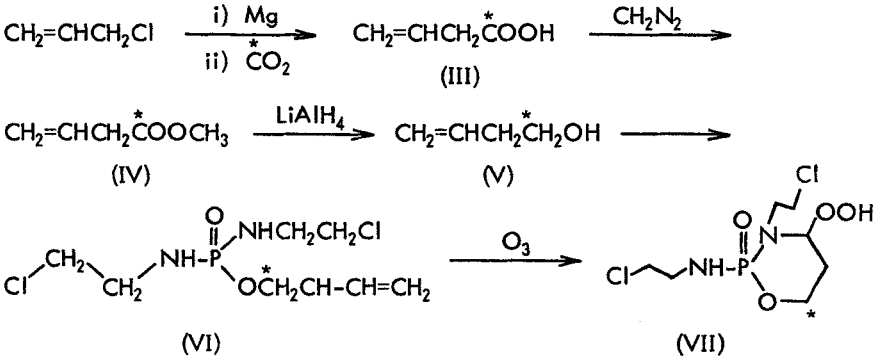


(I)



(II)

Although the compound (II) may be able to be labelled with tritium or phosphorus-32, the carbon-14 label appeared to be the most desirable. We therefore tried labelling the carbon at the 4-, 5-, or 6-position of the molecule with carbon-14.



(III) (IV) (V) (VI) (VII)

As 4-hydroperoxyisophosphamide[2] (II) was synthesized by ozonolysis of O-3-butenyl N,N'-bis(2-chloroethyl)phosphorodiamidate (VI) derived from 3-buten-1-ol and 2-chloroethylamine, 3-buten-1-ol-1-^{14}C (V), -2-^{14}C, or -3-^{14}C was required.

^{14}C-carboxylation of allylchloride or bromide is undesirable due to low yields reported in literature.[3] However, as the reaction of allylmagnesium chloride with ^{14}C-carbon dioxide afforded butenoic acid-^{14}C (III) in ca. 53% yield, 4-hydroperoxyisophosphamide-^{14}C (VII) was synthesized by the route indicated in the above scheme. The overall radiochemical yield was 4.4% based on barium carbonate-^{14}C.

The labelled compound obtained was used for absorption and metabolism studies.[4]

EXPERIMENTAL

Methyl butenoate-^{14}C (IV)

Grignard reagent was prepared from 1.83 g (24 mM) of allylchloride and 583 mg (24.3 mM) of magnesium in 35 ml of anhydrous ether in a vacuum manifold Grignard apparatus. Carbon dioxide-^{14}C derived from 197 mg (50 mCi, 1.0 mM) of barium carbonate-^{14}C and 2.36 g (12 mM) of a carrier, cold barium carbonate and 60% perchloric acid solution (10 ml) were induced into the Grignard reagent at -15~-20° with stirring. Unreacted carbon dioxide was collected by cooling with liquid nitrogen and again induced into the reaction mixture. To acidify this mixture, 6N H_2SO_4 was added dropwise in an ice bath. This mixture then was extracted with ether (20 ml x 3). A carrier, cold butenoic acid (III, 20 mg), was added to the aqueous layer and the mixture was extracted with ether (15 ml x 2). The combined ether extract was extracted with $NaHCO_3$ solution with cooling. The aqueous layer was neutralized with 6N H_2SO_4, salted out with NaCl and extracted with ether (20 ml x 3). The ether extract was dried over Na_2SO_4, and the solvent was slowly evaporated at 50~55° (bath temperature) leaving 10 ml of butenoic acid-^{14}C solution, which had 26.4 mCi of total activity (52.8% radiochemical yield). The solution of butenoic acid-^{14}C (III) in ether was esterified to give methyl butenoate-^{14}C (IV) with ca. 19 ml of a solution of diazomethane in ether (1 mM/1.5 ml). The residual ether solution was used for lithium aluminium hydride reduction without isolation of methyl butenoate-^{14}C (IV).

3-Buten-1-ol-1-^{14}C (V)

Lithium alminium hydride (400 mg) was added to a solution of methyl butenoate-^{14}C (IV, 26.4 mCi) in 25 ml of ether during 10 min. with stirring in an ice bath, and stirred for 50 min. at room temperature. To this mixture, 6N H_2SO_4 was added with stirring in an ice bath, and the mixture was salted out with NaCl and extracted with ether. The ether extract was dried over Na_2SO_4, and the solvent was slowly evaporated at 50° (bath temperature) leaving 1 ml of 3-buten-1-ol-1-^{14}C solution, which had 19.8 mCi of total activity (75.0% radiochemical yield) and was used for the next

reaction without isolation of 3-buten-1-ol-1-^{14}C (V).

O-3-Butenyl N,N'-bis(2-chloroethyl)phosphorodiamidate-^{14}C (VI)

The above-mentioned 3-buten-1-ol-1-^{14}C solution [19.8 mCi, 9.5 mM (calculated value based on radioactivity)] was diluted with 0.5 ml of ether, and added dropwise to a solution of phosphorous oxychloride (1.6 g, 10.5 mM) in dichloromethane (3 ml) with stirring at -10~0°. The mixture was stirred for 4 hr. at the same temperature. The solvent and excess phosphorus oxychloride was evaporated in vacuo at below 30° leaving an oily residue. The residue was dissolved in 4 ml of dichloromethane, and added dropwise to a solution of chloroethylamine in dichloromethane, which had been derived from addition of triethylamine (6.07 g, 60 mM) into a suspension of chloroethylamine hydrochloride (2.7 g, 23.2 mM) in dichloromethane (30 ml) with stirring at -7~-5° followed by further stirring for 15 min. at -2~0° and for 10 min. at -5~5°, and being left overnight at 0°. The reaction mixture was filtered off, and the filtrate was washed with water, dried (Na_2SO_4), and evaporated in vacuo leaving a viscous oil (VI, 1.7 g).

4-Hydroperoxyisophosphamide-6-^{14}C (VII)

The viscous oil (VI, 1.7 g) was dissolved in 25 ml of aqueous acetone (acetone-water = 2 : 1) and ozonized with 3 equivalent moles of ozone stream at 0° for 1 hr. Thirty percent hydrogen peroxide (5 ml) was added to the reaction mixture, which was then left standing for 2 days at 0~2°. Acetone in the reaction mixture was evaporated in vacuo, and the residue was salted out with NaCl, extracted with chloroform (15 ml x 3), dried (Na_2SO_4), and evaporated, leaving a viscous oil. The residue was crystallized from ether to give colorless prisms (270 mg). The mother liquid was evaporated, and the residue was dissolved in aqueous acetone and again oxidized with 30% hydrogen peroxide to give 100 mg of colorless prisms. The combined crystals (370 mg, m.p. 99.5~100° (dec.), total activity: 3.58 mCi) were recrystallized from methanol-ether (1 : 8) to give 4-hydroperoxyisophosphamide-6-^{14}C (VII), m.p. 113~114° (dec.) (223 mg, 2.16 mCi, specific activity: 9.7 μCi/mg) in 4.4% overall

radiochemical yield based on barium carbonate-^{14}C. This compound was confirmed to be pure by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel KGF plate, solvent system = chloroform-methanol (8 : 2)] .

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