SYNTHESIS OF ^{14}C-LABELLED ISOPHOSPHAMIDE

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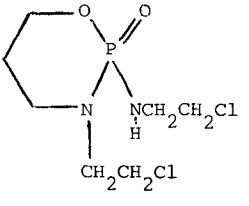
SUMMARY

Isophosphamide labelled with ^{14}C in the chloroethyl group attached to the exocyclic nitrogen has been synthesised by treatment of N-3-hydroxypropyl-aziridine with phosphorus oxychloride and reaction of the resulting 2-chloro-3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide with [1-^{14}C]-2-chloroethylamine.

Key Words: Carbon-14, Isophosphamide, Synthesis, Cancer Chemotherapy.

INTRODUCTION

Isophosphamide [2-(2-chloroethylamino)-3-(2-chloroethyl)-tetrahydro-2H,1,3,2-oxazaphosphorine-2-oxide, 1], an isomer of the established antitumour agent cyclophosphamide 2, is currently undergoing clinical investigation. The superiority of isophosphamide over conventional chemotherapy in the treatment of testicular tumours, particularly teratomas, and also hypernephromas has been reported (1). We now describe a synthesis of specifically ^{14}C-labelled isophosphamide 9 required for metabolic studies. Although the use of a side-chain ^{14}C-labelled isophosphamide has been reported (2) the precise location of the label was not given and a synthesis does not appear to have been published.



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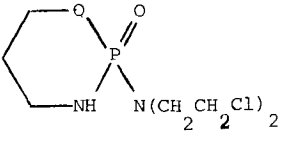

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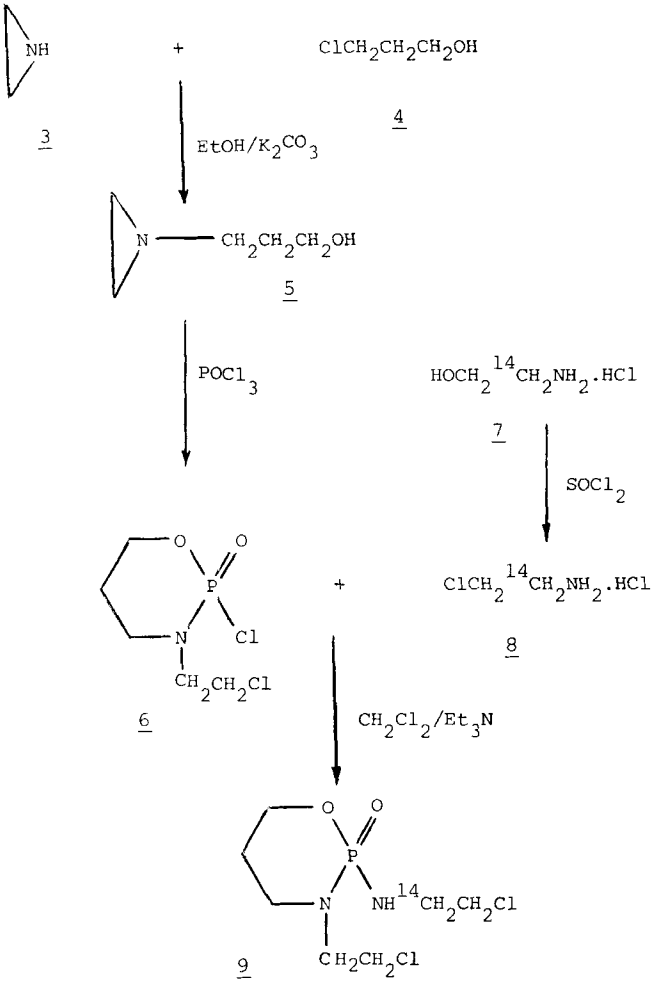

DISCUSSION

Reaction of aziridine 3 with 3-chloropropanol 4 gave N-3-hydroxypropylaziridine 5 (3). A 10:1 ratio of 3 and 4 was used to obtain complete alkylation without further reaction (cf. ref. 4) of 5 with 4. Reaction of 5 with phosphorus oxychloride gave 2-chloro-3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide 6. The aziridino group in 5 is cleaved in situ by the hydrochloric acid generated resulting in the formation of the chloroethyl group attached to the ring nitrogen of 6 (5). In the conventional synthesis of 6, N-(2-chloroethyl)-N-(3-hydroxypropyl)amine was used instead of 5, resulting in a longer overall synthetic route to isophosphamide (6,7).

Treatment of [1-^{14}C]-2-hydroxyethylamine hydrochloride 7 with a 12.6 molar excess of thionyl chloride gave [1-^{14}C]-2-chloroethylamine hydrochloride 8 which was co-crystallised with unlabelled material. Condensation of 6 and 8 (molar ratio 0.8:1) was effected in boiling dichloromethane in the presence of triethylamine to give ^{14}C-isophosphamide 9 with an activity (3.03 μCi/mg) suitable for metabolism studies.

EXPERIMENTAL

Melting points were determined with a Köfler hot-stage apparatus and were corrected. Kieselgel GF_{254} (Merck 7730) was used for thin-layer chromatography (t.l.c.) and Kieselgel 60 (Merck 7734) for column chromatography. N.m.r. spectra (60 MHz) were obtained for solutions in $CDCl_3$ (internal Me_4Si) with a Perkin-Elmer R-12B spectrometer and mass spectra with an AEI MS-12 spectrometer using a direct insertion method with an ionizing voltage of 70 eV, an ion-source temperature of 100°, and a trap current of 100 μamp. Radioactivity determinations were carried out with a Packard 3320 liquid scintillation counter and radiochromatograms were examined with a Berthold LB 2723 Radiochromatogram Scanner. [1-^{14}C]-2-Hydroxyethylamine hydrochloride (2.2 mg, 1 mCi) was obtained from the Radiochemical Centre, Amersham, England (CFA.329).

N-3-Hydroxypropylaziridine 5

To a stirred mixture of 3-chloropropanol (15 g, 0.16 mol), anhydrous K_2CO_3 (26.6 g, 0.19 mol), and ethanol (457 ml), aziridine (68.9 g, 1.6 mol) was added. The mixture was then boiled under reflux for 43 h, filtered, and concentrated. A solution of the residual oil in ether (450 ml) was filtered through Celite, concentrated, and the residue was distilled to give 5 (12.55 g, 78%), b.p. 78-80°/8 mm. N.m.r. data: δ 3.80 (t, 3H, CH_2OH; 2H in $CDCl_3/D_2O$), 2.40 (t, 2H, N-CH_2-), 1.80 (m, 4H, aziridine ring), 1.20 (m, 2H, -C-CH_2-C-). Mass spectrum: m/z 101 (5.2%, M^+), 42 (100%, $H_2C{=}\overset{+}{N}{=}CH_2$).

2-Chloro-3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide 6

Compound 6 (m.p. 34-35°C) was a gift from Asta-Werke AG, Bielefeld,

W.Germany. It was also prepared as follows.

A solution of 5 (6 g, 59.4 mmol) and triethylamine (6 g, 59.4 mmol) in dioxane (36 ml) was added during 2 h to a solution of phosphorus oxychloride (9.1 g, 59.4 mmol) in dioxane (93 ml) at 5-20°C. The mixture was then stirred at room temperature for 18 h, filtered, and concentrated under reduced pressure. The residue was extracted with ether (4 x 30 ml), the combined extracts were diluted with ether (500 ml), filtered through silica, and concentrated under reduced pressure. The residue was crystallised from ether-cyclohexane (10:6) to give 6 (6.4 g, 49.2%), m.p. 34-35°C [previously reported (7) as an oil]. N.m.r. data δ 4-5 (m, 2H, H-6,6'), 2.5-4.0 (m, 6H, $-CH_2CH_2Cl$ and H-4,4'), 1.4-2.4 (m, 2H, H-5,5'). Mass spectrum: m/z 217 (4.7%, M^+), 182 (4.5%, $[M-Cl]^+$), 168 (100%, $[M-CH_2Cl]^+$).

Anal. Calc. for $C_5H_{10}Cl_2NO_2P$ C, 27.54; H, 4.62; Cl, 32.52; N, 6.43; P, 14.21. Found: C, 27.78; H, 4.78; Cl, 31.96; N, 6.21; P, 14.64.

2-(2-Chloro-1-^{14}C-ethylamino)-3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide 9

(a) Conversion of [1-^{14}C]-2-hydroxyethylamine hydrochloride 7 into [1-^{14}C]-2-chloroethylamine hydrochloride 8.

A solution of 7 (2.2 mg, 1 mCi) in methanol was concentrated to dryness under reduced pressure in a 1 ml reacti-vial (Pierce Chemical Co., Rockford, Ill., U.S.A.). Thionyl chloride (20 µl, 12.6 molar excess) was then injected through a septum cap at room temperature, and the mixture was kept at 80°C for 35 min. T.l.c. (butanol-acetic acid-water, 5:2:3), then showed the reaction to be complete. The thionyl chloride was removed under reduced pressure at room temperature. A solution of the crystalline residue in methanol (2 ml), had 91.7% of the initial radioactivity. T.l.c. showed the product to be radiochromatographically pure.

(b) Reaction of 8 with 2-chloro-3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide 6.

The product 8 from (a) was diluted with unlabelled 2-chloroethylamine

hydrochloride (128.6 mg), dissolved in acetone (1.5 ml) and ethanol (0.15 ml) and cocrystallised by the addition of light petroleum (b.p. 40-60°). The mother liquor was removed with a Pasteur pipette and the crystalline product (128.4 mg, 97.7%) was dried in a desiccator. The mother liquor contained 4.34% of the original radioactivity (39.82 μCi).

To a suspension of 8 (128.4 mg, 1107 μmol) in methylene chloride (10 ml), 6 (193.1 mg, 886 μmol) and triethylamine (310 μl, 2215 μmol) were added at 0°. This mixture was heated under reflux at 45°C for 4.5 h (conditions under which unlabelled materials were completely converted into isophosphamide) then applied to a column (13 x 1.5 cm) of Kieselgel 60 and eluted with methylene chloride-methanol (9:1). The elution was monitored by t.l.c. using chloroform-methanol (9:1) and the fractions containing ^{14}C-isophosphamide were subjected to re-chromatography to remove a trace of triethylamine hydrochloride. A solution of the product in ether (10 ml) was applied to a column (13 x 1.5 cm) of Kieselgel 60 and eluted with ether-ethanol (9:1). The fractions containing ^{14}C-isophosphamide were combined, concentrated and the residue was crystallised from ether (5-7 ml) at -40°C. T.l.c. (chloroform-methanol, 9:1) showed the product 9 (174.5 mg, 75.5%), which had a specific activity of 3.03 μCi/mg (0.79 mCi/mmol), to be radiochromatographically pure. The theoretical specific activity was 3.39 μCi/mg.

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