LABELLED COMPOUNDS OF INTEREST AS ANTITUMOUR AGENTS. PART II (1). SYNTHESIS OF ^{2}H AND ^{3}H ISOTOPOMERS OF RSU 1069 AND Ro 03-8799 (PIMONIDAZOLE).

PAUL WEBB and MICHAEL D. THREADGILL*

Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxfordshire OX11 ORD, U.K.

SUMMARY

^{2}H- and ^{3}H-Labelled RSU 1069 and Ro 03-8799 (pimonidazole) have been synthesised by reduction of 1-(3-chloro-2-oxopropyl)-2-nitroimidazole with the appropriately labelled sodium borohydride, followed by ring-closure of the chlorohydrins and treatment of the resulting epoxides with aziridine or piperidine. In both cases, the specific activities were 200 mCi $mmol^{-1}$ and the radiochemical yields were 86 %. The parent compounds are radiosensitisers of hypoxic tumour cells.

Key words: Radiosensitiser, Pimonidazole, [^{2}H]-Ro 03-8799, [^{3}H]-Ro 03-8799, [^{2}H]-RSU 1069, [^{3}H]-RSU 1069.

INTRODUCTION

RSU 1069 (1) (2-4) and pimonidazole (Ro 03-8799, 2) (5,6) are members of the second generation of electron-affinic radiosensitisers based on 2-nitroimidazole and have shown some useful additional activity and/or lower toxicity when compared with the archetype misonidazole (3) (Figure 1). The aziridine 1 also exhibits much greater selective toxicity towards hypoxic cells than does misonidazole (7-9), this selectivity arising from the ability of the compound to act as a bifunctional electrophile under hypoxia (10-12). [^{14}C]-RSU 1069, prepared from 2-[^{14}C]-2-nitroimidazole, has been shown to react with DNA in

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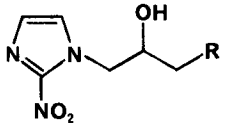
vitro (11). A study (13) of the pharmacokinetics and metabolism of 1, using unlabelled material, has revealed several metabolites of low molecular weight, although this technique does not give information on the nature and location of chemical and metabolic processes involving persistent covalent binding to biological macromolecules. It was therefore of interest to prepare a tritium labelled isotopomer of 1 at moderate specific activity to enable the extension of these studies in vitro and in vivo. The synthetic route employed also facilitated the preparation of a radiolabelled isotopomer of 2 for analogous studies, although electrophilic reaction of this agent with macromolecules would not be expected in the absence of bioreduction.



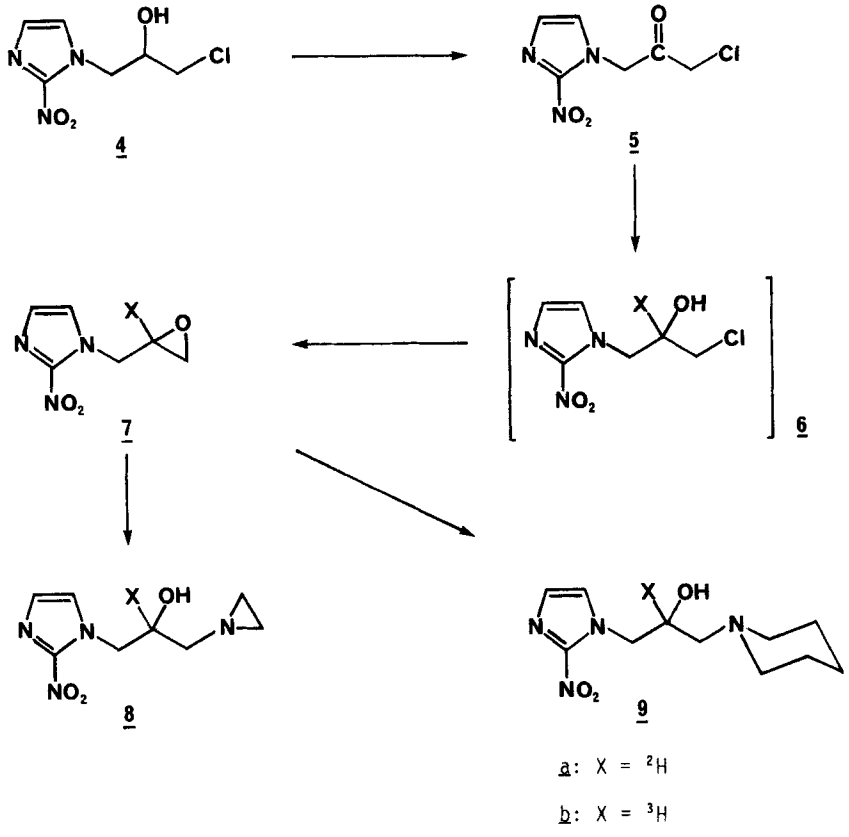
1: R = aziridin-1-yl

2: R = piperidin-1-yl

3: R = OMe

FIGURE 1.

[^{3}H]-Misonidazole has been reported (14,15) to be formed by the reduction of the corresponding ketone (1-(3-methoxy-2-oxopropyl)-2-nitroimidazole). This most direct incorporation of tritium into RSU 1069 by reduction of the corresponding aziridinylmethylketone could not, however, be achieved since attempts to oxidise the secondary alcohol function of 1 resulted in concomitant destruction of the highly labile aziridine moiety. However, oxidation of the chlorohydrin 4 (16) with chromium trioxide in acidic acetone furnished the ketone 5 in modest yield (Scheme 1) in a reaction similar to that reported (16) for the oxidation of 3. Treatment of 5 with 0.29 molar equivalents of sodium boro[^{2}H]hydride gave complete reduction as shown by thin layer chromatography (TLC), indicating that all four hydrogens of the borohydride are available for reaction with this highly electrophilic carbonyl group. The intermediate [^{2}H]-chlorohydrin 6a was not isolated owing to its poor solubility in organic solvents but was cyclised in the presence of aqueous base to give the readily isolable [^{2}H]-epoxide 7a.



SCHEME 1.

In order to investigate whether primary kinetic isotope effects may cause significant preferential incorporation of ^1H rather than ^3H from sodium boro[^3H]hydride, 5 was treated with an excess of a mixture of NaB^1H_4 and NaB^2H_4. Subsequent cyclisation gave a mixture of the [^1H]- and [^2H]-epoxides. From the integral of the multiplet centred at δ 3.44 in the ^1H NMR spectrum (from the 2-H of the side-chain), it was estimated that $k[^1H]/k[^2H] = 1.5$ for the reduction step. Therefore, to avoid poor incorporation due to a significant value of $k[^1H]/k[^3H]$, an excess of 5 was treated with sodium boro[^3H]hydride of high specific activity, followed after a short period by an excess of NaB^1H_4 to complete the reduction. Again, the chlorohydrin 6b was not isolated but converted to the [^3H]-epoxide 7b in near quantitative chemical and radiochemical yield. Thus this 'cold chaser' procedure offers an advantage in incorporation of radiolabel when compared with the lower labelling reported by

Setiabaudi et al (17) for the analogous stoichiometric reduction/cyclisation of phenacyl bromides to phenylepoxides.

The epoxides 7a,b were converted in essentially quantitative chemical and radiochemical yields to the required aziridines 8a,b respectively by brief treatment with aziridine in refluxing ethanol (Scheme 1). The presence of a tertiary amine base was necessary to inhibit the acid-catalysed polymerisation known to occur for the unlabelled analogue. Careful monitoring of the progress of the addition reaction by TLC was also needed for optimum yield. The labelled epoxides reacted smoothly with excess piperidine giving the required isotopomers of pimonidazole (9a,b), again in very high yields.

The use of the above syntheses allowed [^{3}H]-RSU 1069 and [^{3}H]-pimonidazole to be prepared in sufficiently high specific activity for use in experiments designed to examine the selectivity of covalent binding with DNA and other macromolecules of cells *in vitro* in hypoxia and in the presence of oxygen. Similarly, assessment of the extent and selectivity of retention of these radiosensitisers and bioreductively-activated cytotoxins in tumours and in various normal tissues by autoradiography is facilitated. The results of these biological studies will be reported elsewhere.

EXPERIMENTAL

Solutions were dried over anhydrous sodium sulphate and were filtered prior to evaporation of the solvents under reduced pressure. Melting points were uncorrected. NMR spectra were obtained using a Jeol PMX60SI spectrometer with tetramethylsilane as internal standard. IR spectra were recorded on Nujol mulls using a Philips PU9510 instrument. Determination of radioactivity was carried out by liquid scintillation counting using Beckman LS2800 and Beckman LS5000CE instruments. Radiochemical purities were estimated by scintillation counting of appropriate portions of silica scraped from TLC analyses. Sodium boro[^{2}H]hydride was obtained from Aldrich Chemical Co. and sodium boro[^{3}H]hydride was obtained from Amersham International PLC.

1-(3-Chloro-2-oxopropyl)-2-nitroimidazole (5).- 1-(3-Chloro-2-hydroxy-propyl)-2-nitroimidazole (4; 2.06 g, 10 mmol) (prepared from 2-nitroimidazole and 3-chloro-1,2-epoxypropane by the method of Beaman et al (16)) was stirred with chromium trioxide (1.0 g, 10 mmol) and sulphuric acid (2 ml) in acetone (40 ml) for 3 days. Sodium hydrogen carbonate (8 g) was added and the mixture was filtered. The solvent was evaporated from the filtrate and the residue, in ethyl acetate, was washed with water and with saturated aqueous sodium hydrogen carbonate before being dried. The evaporation residue was recrystallised from ethyl acetate to afford 5 (936 mg, 46 %) as white crystals m.p. 93-94°C; v max 1750 cm^{-1}; δ ($CDCl_3$: $(CD_3)_2SO$, 3:1) 4.48 (2 H, s, CH_2Cl), 5.77 (2 H, s, imidazole-CH_2), 7.12 (1 H, brs, imidazole-H), 7.35 (1 H, brs, imidazole-H).

1-(2,3-Epoxy-2-[^{2}H]propyl)-2-nitroimidazole (7a).- The chloromethylketone 5 (203 mg, 1.0 mmol) was stirred with NaB^2H_4 (98 atom %; 12.2 mg, 0.29 mmol) in absolute ethanol (15 ml) for 20 min. Acetone (3 ml) was then added and the mixture was stirred for 1 h before evaporation of the solvents. The residue was stirred vigorously with aqueous sodium hydroxide (10 %, 2.0 ml, 5.0 mmol) for 30 min. The suspension was diluted with water (5 ml) and was extracted with chloroform (3 x 15 ml). The combined extracts were dried and the solvent was evaporated to give 7a (130 mg, 76 %) as an off-white crystalline solid m.p. 53-55°C; δ ($CDCl_3$) 2.55 (1 H, d, J = 4.5 Hz) and 2.90 (1 H, d, J = 4.5 Hz, epoxide-CH_2, 4.25 (1 H, d, J = 14.5 Hz) and 4.95 (1 H, d, J = 14.5 Hz) NCH_2, 7.08 (1 H, s, imidazole-H), 7.20 (1 H, s, imidazole-H).

Experiment to determine k[^{1}H]/k[^{2}H] for the reduction of 5.- A solution of NaB^1H_4 (37.5 mg, 0.987 mmol) and NaB^2H_4 (98 atom %; 410 mg, 0.976 mmol) in ethanol (25 ml) was added to ketone 5 (407 mg, 2.0 mmol) and the resulting solution was stirred for 15 min. Acetone (5 ml) was added and the mixture was stirred for 16 h before evaporation of the solvents. The residue was treated with aqueous sodium hydroxide as above to afford a pale yellow crystalline solid (320 mg, 94 %) which was shown by NMR to comprise a mixture of isotopomers of 1-(2,3-epoxypropyl)-2-nitroimidazole. Integration of the multiplet centred at

δ 3.44 showed that the mixture contained the [^{1}H]-epoxide (61 %) and the [^{2}H]-epoxide (39 %). From these data it was calculated that k[^{1}H]/k[^{2}H] = 1.5 for the reduction step.

1-(2,3-Epoxy-2-[^{3}H]propyl)-2-nitroimidazole (7b).- Sodium borohydride (1 mg, 0.026 mmol) was added to ketone 5 (203 mg, 1.0 mmol) in ethanol (10 ml), followed after 5 min by sodium boro[^{3}H]hydride (1.8 mg; 0.048 mmol, 220 mCi) in ethanol (1 ml) and water (0.5 ml). After a further 20 min, sodium borohydride (40 mg, 1.05 mmol) was added and the mixture was stirred for 30 min. Acetone (3 ml) was then added and the mixture was stirred for 16 h before evaporation of the solvents. The residue was stirred vigorously with 10 % aqueous sodium hydroxide (4.0 ml, 10 mmol) for 35 min. The suspension was diluted with water (10 ml) and was extracted with chloroform (3 x 50 ml). The combined extracts were dried and the solvent was evaporated to give 7b (160 mg, 95 % chemical yield; 189 mCi, 86 % radiochemical yield) which co-chromatographed (TLC, silica, $CHCl_3$/MeOH, 9:1, Rf 0.6) with authentic unlabelled material. The radiochemical purity was > 96 % and the specific activity was 200 mCi mmol^{-1}.

1(3-(Aziridin-1-yl)-2-hydroxy-2-[^{2}H]propyl)-2-nitroimidazole (8a).- The [^{2}H]-epoxide 7a (120 mg, 0.7 mmol) was boiled under reflux in ethanol (2 ml) with aziridine (0.09 ml; 75 mg, 1.7 mmol) and triethylamine (0.03 ml) for 15 min. Evaporation of the solvents and excess reagent gave a solid which was triturated with cold acetone to afford 8a (140 mg, 94 %) as a pale yellow solid m.p. 116-118°C; δ (CD_3OD) 1.4 (2 H, m) and 1.8 (2 H, m) aziridine-H, 2.25 (1 H, d, J = 12 Hz) and 2.45 (1 H, d, J = 12 Hz) aziridine-C$\underline{H}_2$CD(OH)-, 4.30 (1 H, d, J = 14 Hz) and 4.68 (1 H, d, J = 14 Hz) imidazole-C$\underline{H}_2$CD(OH)-, 4.75 (1 H, s, OH), 7.03 (1 H, d, J = 2 Hz, imidazole-H), 7.37 (1 H, d, J = 2 Hz, imidazole-H).

1-(3-(Aziridin-1-yl)-2-hydroxy-2-[^{3}H]propyl)-2-nitroimidazole (8b).- Epoxide 7b (100 mg, 0.59 mmol; 118 mCi) was boiled under reflux in ethanol (5 ml) with aziridine (0.35 ml) and triethylamine (0.1 ml) for 20 min. Evaporation of the

solvents and excess reagent gave 8b (125 mg, quant.; 118 mCi, quant.) which co-chromatographed (TLC, silica, $CHCl_3$/MeOH, 9:1, Rf 0.15) with authentic unlabelled material. The radiochemical purity was > 96 % and the specific activity was 200 mCi $mmol^{-1}$.

1-(2-Hydroxy-3-(piperidin-1-yl)-2-[^{2}H]propyl)-2-nitroimidazole (9a).- The [^{2}H]-epoxide 7a (240 mg, 1.4 mmol) was boiled under reflux in ethanol (20 ml) with piperidine (260 mg, 3.1 mmol) for 15 min. Evaporation of the solvent and excess reagent gave 9a (360 mg, 99 %) as pale yellow crystals m.p. 107-108°C; ν max (Nujol mull) 3320 br, 2160 cm^{-1}; δ ($CDCl_3$) 1.5 (6 H, br, piperidine 3,4,5-CH_2), 2.1-2.8 (6 H, m, $CDCH_2N$ + piperidine 2,6-CH_2, 4.22 (1 H, d, J = 14 Hz) and 4.64 (1 H, d, J = 14 Hz) imidazole-$CH_2CD(OH)$-, 4.28 (1 H, s, OH), 6.98 (1 H, brs, imidazole-H), 7.25 (1 H, brs, imidazole-H).

1-(2-Hydroxy-3-(piperidin-1-yl)-2-[^{3}H]propyl)-2-nitroimidazole (9b).- Epoxide 7b (30 mg, 0.18 mmol; 35 mCi) was boiled under reflux in ethanol (1.5 ml) with piperidine (15 mg, 0.534 mmol) for 5 min. Evaporation of the solvents and excess reagent gave 9b (45 mg, quant.; 35 mCi, quant.) which co-chromatographed (TLC, silica, $CHCl_3$/MeOH, 9:1, Rf 0.2) with authentic unlabelled material. The radiochemical purity was > 96 % and the specific activity was 200 mCi $mmol^{-1}$.

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