LABELLED COMPOUNDS OF INTEREST AS ANTITUMOUR AGENTS. PART III (1). SYNTHESIS OF ^{2}H AND ^{3}H ISOTOPOMERS OF ETANIDAZOLE (SR 2508).

PAUL WEBB and MICHAEL D. THREADGILL*

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

SUMMARY

Chemically and radiochemically efficient syntheses of N-(2-hydroxy-2-[^{2}H]ethyl)-2-(2-nitroimidazol-1-yl)acetamide and N-(2-hydroxy-2-[^{3}H]ethyl)-2-(2-nitroimidazol-1-yl)acetamide, isotopomers of the hypoxic cell radiosensitiser etanidazole (SR 2508), have been achieved by reduction of the corresponding aldehyde with isotopically labelled sodium borohydride. The primary kinetic deuterium isotope effect for the process is 1.5.

Key words: [^{2}H]-Etanidazole, [^{3}H]-Etanidazole, Etanidazole, SR 2508.

INTRODUCTION

Etanidazole (SR 2508; 1; Figure 1) (2,3) is a member of the second generation of electron-affinic compounds based on 2-nitroimidazole, designed for the sensitisation of hypoxic tumour cells to radiation. Particularly, it elicits less neurotoxicity than the archetype misonidazole (2). It is also significantly more polar than the latter. It was therefore of interest to assess the selectivity of uptake and covalent retention of 1 in hypoxic and oxic regions of tumours and in normal tissues, including brain. Radiolabelled material was required for this purpose .

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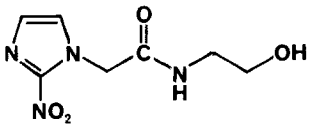

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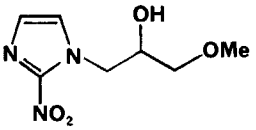

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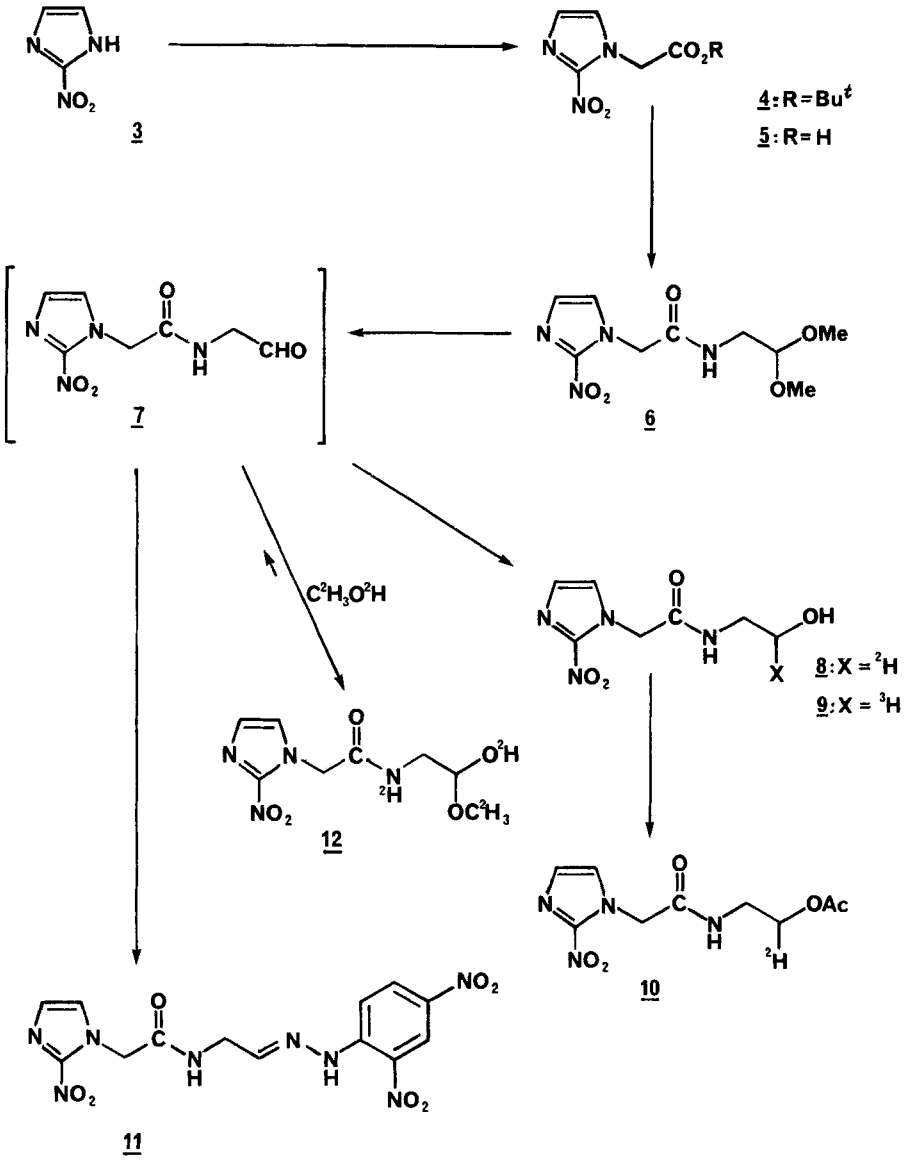
FIGURE 1.

We have reported (1) the efficient and rapid incorporation of ^{2}H and ^{3}H by the reduction of a doubly activated ketone by the appropriate isotopically labelled sodium borohydride. It was therefore considered highly likely that the analogous reduction of the activated aldehyde 7 would be successful in providing the required isotopically labelled materials.

Synthesis of 7 was achieved in four steps as shown in Scheme 1. 2-Nitroimidazole (3) was alkylated with t-butyl bromoacetate under basic conditions in refluxing dimethylformamide and the imidazoleacetic acid 5 was formed by treatment of the intermediate t-butyl ester 4 with anhydrous trifluoroacetic acid. This route was found to avoid many of the previously reported problems (4) of isolation and purification associated with the great aqueous solubility and acid strength of 5. The acid chloride of 5 proved to be difficult to form and to be unstable, so a carbodiimide method was used to couple this acid with 2,2-dimethoxyethylamine, albeit in modest yield. Hydrolysis of the acetal function of 6 was achieved using an ion-exchange resin as catalyst. The resultant aldehyde 7 was found to be highly electrophilic, being extensively hydrated in water and forming fully the corresponding hemiacetal 12 when an attempt was made to record the ^{1}H NMR spectrum in solution in [$^{2}H_{4}$]methanol. The dinitrophenylhydrazone 11 was prepared for characterisation.

For the purpose of the reductions with sodium boro[^{2}H]hydride, 7 was not isolated but was used as the neutral aqueous solution given by removal of the catalytic resin by filtration; the [^{2}H]-primary alcohol 8 was obtained in good yield. To determine the kinetic isotope effect for this reduction step, the aqueous solution of 7 was reduced with an excess of an equimolar mixture of



SCHEME 1.

NaB^1H_4 and NaB^2H_4. Analysis of the resulting mixture of isotopomers by NMR was facilitated by acetylation, thus removing the overlapping of the $NC^1\underline{H}_2C^1H^2HOH$ and $NC^1H_2C^1\underline{H}^2HOH$ resonances in the spectrum of 8. Comparison of the integrals of signals at δ 3.45 ($CONC^1H_2$) and δ 4.10 ($C^1\underline{H}^2HOAc$ + $C^1\underline{H}_2OAc$) enabled the estimation $k[^1H]/k[^2H] = 1.5$ for the reduction step. To avoid possible poor incorporation of ^3H from sodium boro[^3H]hydride through unfavourable competition

with ^{1}H, the 'cold chaser' technique (1) was used and the radiochemical yield of 9 was excellent. Interestingly, Coleman et al (5) report that the corresponding unlabelled acetate ester is a good prodrug for 1.

The development of this facile and high-yielding preparation of radiolabelled etanidazole permits studies of selectivity in uptake and retention of the drug and its metabolites in normal tissues and in tumours. The results of these experiments will be published elsewhere.

EXPERIMENTAL

DMF refers to dimethylformamide. Tetrahydrofuran (THF) was distilled from calcium hydride before use. Solutions were dried with anhydrous sodium sulphate and were filtered prior to evaporation of the solvents under reduced pressure. Melting points are uncorrected. NMR spectra were obtained using a Jeol PMX60SI spectrometer with tetramethylsilane as internal standard. IR spectra were recorded using a Philips PU9510 instrument. Determinations of the radioactivity were carried out by the liquid scintillation method using Beckman LS2800 and Beckman LS5000CE instruments. The radiochemical purity of 9 was checked by scraping appropriate portions of an analytical thin layer chromatography plate (silica gel; $CHCl_3$: MeOH, 4:1) and assaying radioactivity by liquid scintillation counting. Sodium boro[^{2}H]hydride was obtained from Aldrich Chemical Co. and sodium boro[^{3}H]hydride was obtained from Amersham International PLC.

t-Butyl 2-(2-nitroimidazol-1-yl)acetate (4).- 2-Nitroimidazole (3; 4.57 g, 40.4 mmol) was stirred at reflux in DMF (40 ml) with potassium t-butoxide (4.85 g, 43.3 mmol) for 5 min before being cooled to 120°C. t-Butyl bromoacetate (7.90 g, 40.5 mmol) was added and the whole was stirred at reflux for a further 5 min. The cooled mixture, in ethanol (100 ml) was filtered and the solvents were evaporated at 1 torr. The residue, in dichloromethane, was washed with water (4 x) and saturated aqueous sodium chloride before being dried. The

solvent was evaporated to give 4 (6.38 g, 70 %) as a pale yellow oil; δ ($CDCl_3$) 1.45 (9 H, s, $C(CH_3)_3$), 5.02 (2 H, s, CH_2), 7.1 (2 H, br, 2 x imidazole-H).

2-(2-Nitroimidazol-1-yl)acetic acid (5).- The t-butyl ester 4 (6.29 g, 27.7 mmol) was stirred with trifluoroacetic acid (40 ml) for 16 h before evaporation of the reagent. The residual solid was washed with a mixture of diethyl ether and hexane (1:1) to afford 5 (4.74 g, quant.) as a pale yellow solid m.p. 145-146°C (lit. (4) m.p. 159-160°C (expl.)); δ ($CDCl_3$) 5.20 (2 H, s, CH_2), 7.52 (1 H, d, J = 1.5 Hz, imidazole-H), 7.58 (1 H, d, J = 1.5 Hz, imidazole-H).

N-(2,2-Dimethoxyethyl)-2-(2-nitroimidazol-1-yl)acetamide (6).- The carboxylic acid 5 (510 mg, 3 mmol) was stirred with N,N´-dicyclohexylcarbodiimide (630 mg, 3 mmol) and 2,2-dimethoxyethylamine (360 mg, 3 mmol) in THF (13 ml) for 3 days before the mixture was filtered and the solvent was evaporated. Centrifugally-accelerated preparative layer chromatography (silica gel, ethyl acetate:hexane, 1:1, followed by ethyl acetate) gave a crude solid which was recrystallised from ethyl acetate:hexane to afford 6 as an off-white solid m.p. 116-117°C; δ ($CDCl_3$:$(CD_3)_2CO$, 1:2) 3.30 (6 H, s, 2 x OCH_3), 3.35 (2 H, t, J = 5 Hz, NCH_2CH), 4.35 (1 H, t, J = 5 Hz, NCH_2CH), 5.15 (2 H, s, imidazole-CH_2), 7.07 (1 H, d, J = 1.5 Hz, imidazole-H), 7.17 (1 H, d, J = 1.5 Hz, imidazole-H), 7.55 (1 H, br, NH).

N-(2-Hydroxy-2-[^{2}H]ethyl)-2-(2-nitroimidazol-1-yl)acetamide (8) and N-(2-Acetoxy-2-[^{2}H]ethyl)-2-(2-nitroimidazol-1-yl)acetamide (10).- The acetal 6 (258 mg, 1 mmol) was boiled under reflux with Dowex 50X4 (H^+ form; 300 mg) in THF (5 ml) and water (3 ml) for 1 h before the suspension was filtered to give a solution of the aldehyde 7. Sodium boro[^{2}H]hydride (NaB^2H_4; 42 mg, 1 mmol), in water (2 ml) was added to the cooled filtrate and the mixture was stirred for 5 min before addition of acetone (3 ml). After a further 16 h, the solvents were evaporated and the residue, in water (5 ml), was applied to a short column of Amberlite IRA-400 (^-OH form; 1.0 g) and was eluted with a water (10 ml). This

eluate was then passed through a short column of Dowex 50X4 (H^+ form; 1.0 g) and the water was evaporated from the final eluate to give 8 (160 mg, 74 %) as an off-white solid m.p. 133-136°C; ν max (Nujol mull) 3400, 3300, 2120 , 1665 cm^{-1}; δ ($CDCl_3$ + $(CD_3)_2SO$; 1:1) 3.2-3.6 (3 H, m, NCH_2CHD), 5.15 (3 H, brs, imidazole-CH_2 + OH), 7.10 (1 H, brs, imidazole-H), 7.45 (1 H, br imidazole-H) and 8.20 (1 H, brt, J = 6 Hz, NH). A small sample was treated with acetic anhydride and pyridine to afford 10 as a pale yellow solid 2.02 (3 H, s, $COCH_3$), 3.45 (2 H, t, J = 7 Hz (becomes d, J = 7 Hz on decoupling at δ 8.32), $CONCH_2$), 4.10 (1 H, brt, J = 7 Hz, CHD), 5.15 (2 H, s, imidazole-CH_2), 7.13 (1 H, brs, imidazole-H), 7.43 (1 H, brs, imidazole-H) and 8.30 (1 H, brt, J = 6 Hz, NH).

N-(2-Hydroxy-2-[^{3}H]ethyl)-2-(2-nitroimidazol-1-yl)acetamide (9).- Acetal 6 (136 mg, 0.51 mmol) was hydrolysed with Dowex 50X4 in aqueous THF and the resin was removed by filtration as described above. Sodium borohydride (1 mg, 0.026 mmol) was added, followed after 5 min by an aqueous solution of sodium boro[^{3}H]hydride (0.0005 mmol, 2.5 mCi; 0.5 ml). After a further 5 min, sodium borohydride (19 mg, 0.5 mmol) was added and the mixture was stirred for 15 min before addition of acetone (2 ml). After a further 16 h, the solvents were evaporated and the residue, in water (5 ml), was applied to a short column of Amberlite IRA-400 ($^-$OH form; 0.7 g) and was eluted with a water (7 ml). This eluate was then passed through a short column of Dowex 50X4 (H^+ form; 1.0 g) and the water was evaporated from the final eluate to give 9 (95 mg, 2.0 mCi, chemical yield 87 %, radiochemical yield 80 %) with TLC properties identical to those of an authentic sample of 1 (silica gel, $CHCl_3$:MeOH, 4:1, Rf 0.2). The radiochemical purity was > 97 % and the specific activity was 4.5 mCi $mmol^{-1}$.

N-(2-Oxoethyl)-2-(2-nitroimidazol-1-yl)acetamide 2,4-Dinitrophenylhydrazone (11).- A solution of the aldehyde 7 (0.7 mmol) was prepared by hydrolysis of 6 with Dowex 50X4 in aqueous THF as described above. Treatment with 2,4-dinitrophenylhydrazine (150 mg, 0.75 mmol) in ethanol for 5 min afforded, after recrystallisation from water, 10 as bright yellow-orange crystals m.p. 239-242°C; δ ($(CD_3)_2SO$) 4.05 (2 H, dd, J = 5 Hz, J = 3.5 Hz (becomes d, J = 5 Hz on

decoupling at δ 8.00; becomes d, J = 3.5 Hz on decoupling at δ 8.75), $NC\underline{H}_2CHN$), 5.25 (2 H, s, imidazole-CH_2), 5.7 (1 H, br, ArNH), 7.25 (1 H, d, J = 1 Hz, imidazole-H), 7.70 (1 H, d, J = 1 Hz, imidazole-H), 7.95 (1 H, d, J = 9 Hz, Ar 6-H), 8.00 (1 H, m (becomes s on decoupling at δ 4.05), $NCH_2C\underline{H}N$), 8.40 (1 H, dd, J = 9 Hz, J = 2 Hz, Ar 5-H), 8.75 (1 H, t, J = 5 Hz (becomes s on decoupling at δ 4.05), $CON\underline{H}CH_2$), 8.85 (1 H, d, J = 2 Hz, Ar 3-H).

Experiment to determine $k[^1H]/k[^2H]$ for the reduction of 7.- Acetal 6 was hydrolysed with Dowex 50X4 in aqueous THF, reduced and treated with acetic anhydride in pyridine as above, except that the reductant was an excess of an equimolar mixture of NaB^1H_4 and NaB^2H_4 (98 atom %). The residue was shown by NMR to comprise a mixture of isotopomers of N-(2-acetoxyethyl)-2-(2-nitroimidazol-1-yl)acetamide. Integration of the triplet centred at δ 4.10 showed that the mixture contained 65 % of the ^1H-compound and 35 % of the ^2H-compound. From these data it was calculated that $k[^1H]/k[^2H]$ = 1.5 for the reduction step.

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