The synthesis of 5'-[$^{14}C_1$] and 3a, 4-[$^{13}C_2$] labelled Panadiplon (U-78875; 3-(5'-cyclopropyl-1,2,4-oxadiazol-3-yl)-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one).

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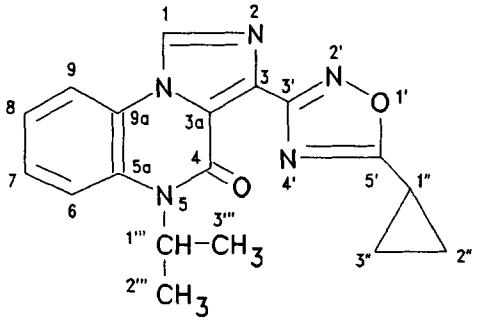
SUMMARY

5'-[$^{14}C_1$]Panadiplon was prepared in 3 steps starting from [$^{14}C_1$]cyclopropane carboxylic acid and 3-(5'-cyano-1,2,4-oxadiazol-3-yl)-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one. 3a, 4-[$^{13}C_2$]Panadiplon was prepared in two steps from $^{13}C_2$-oxalic acid and N-1-(1-methylethyl)-o-phenylenediamine. The position of labelling was confirmed by the appearance of two coupled resonances (J_{C-C} = 80.59 Hz) at 121.95 and 154.39 ppm in the assigned ^{13}C-NMR spectrum.

Keywords
3-(5'-cyclopropyl-1,2,4-oxadiazol-3-yl)-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one; panadiplon; carbon-13 labelling; carbon-14 labelling.

INTRODUCTION

Panadiplon[1] (1) is a non-benzodiazepine anxiolytic agent for treating "General Anxiety Disorder" and "Panic Disorder" in man. Recent clinical studies have revealed unexpected hepatotoxicity in man, possibly due to metabolic degradation of the drug to cyclopropane carboxylic acid, which is a known inhibitor of β-oxidation[2]. To test this hypothesis the drug



(1)

was required specifically labelled in the 5' position, which would subsequently release [$^{14}C_1$]cyclopropane carboxylic acid and enable tracer studies to be performed. In addition, a

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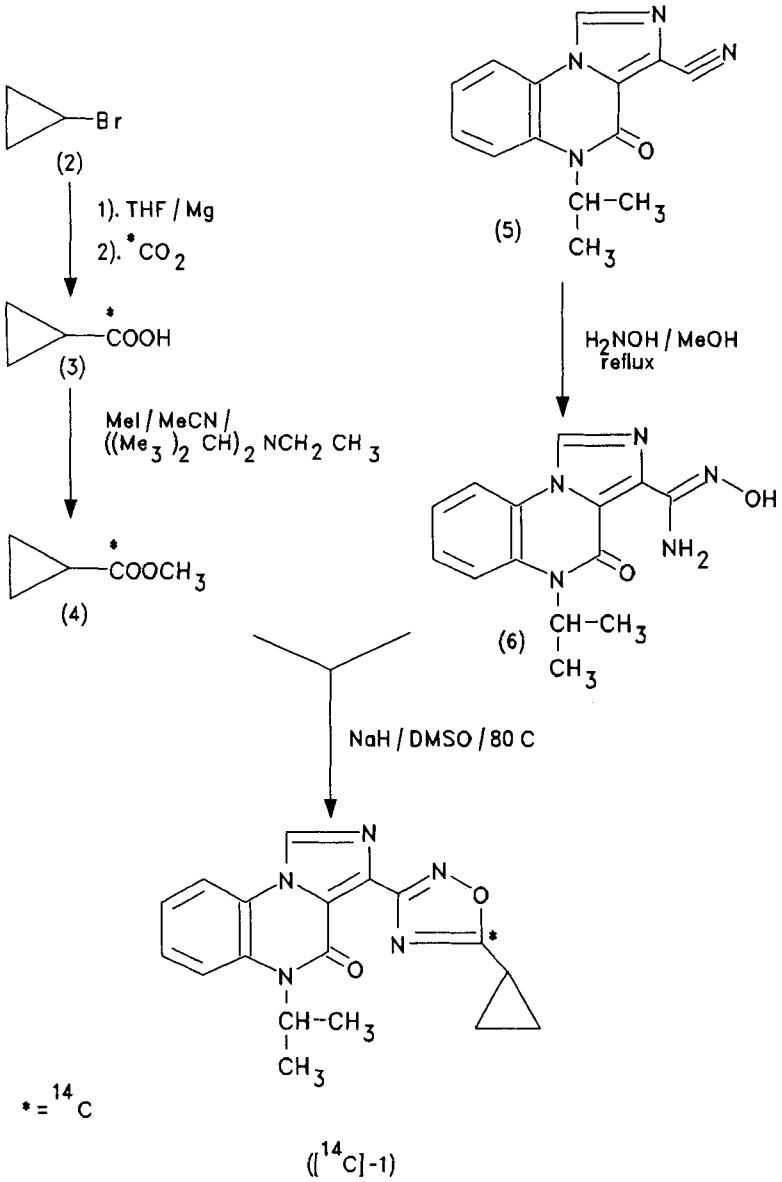
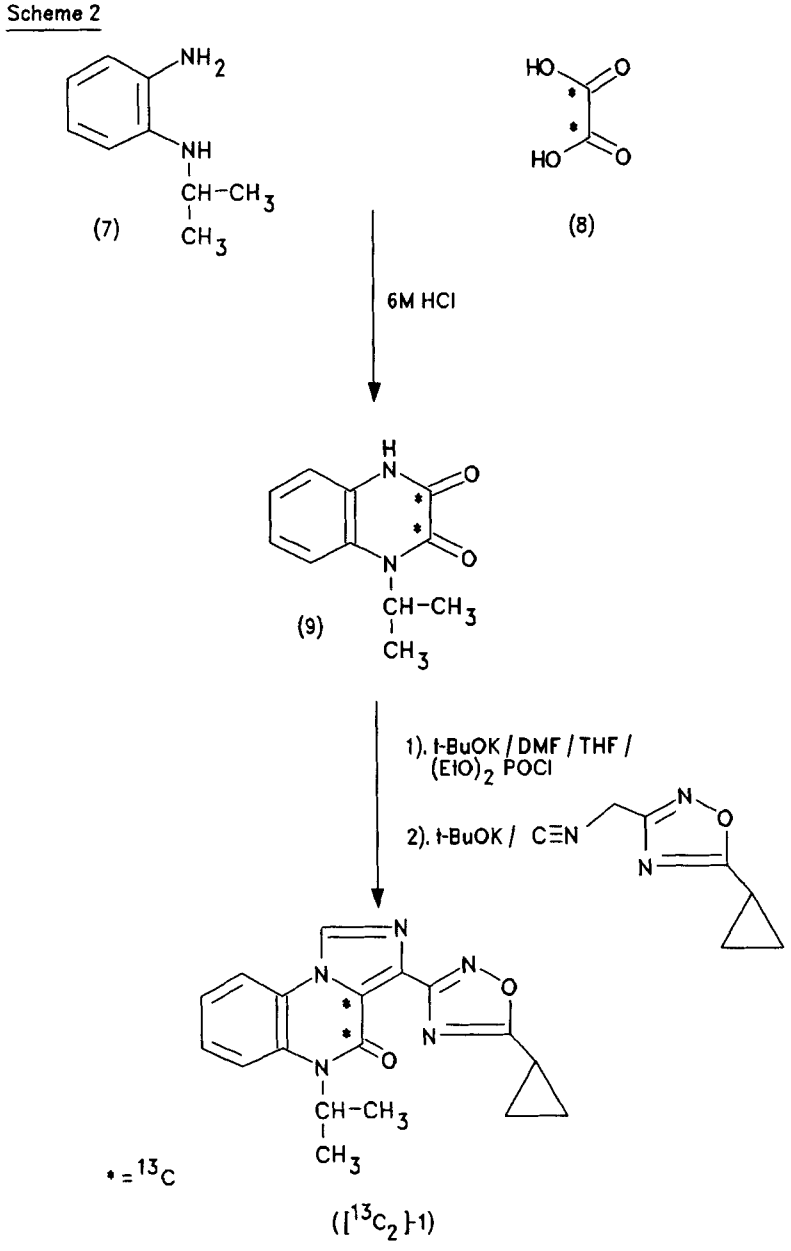
Scheme 1.



([^{14}C]-1)

more generally labelled heavy isotope version of the drug ([$^{13}C_2$]-1) was required to pursue identification of metabolites associated with other parts of the molecule. This compound, administered as a 1:1 mix with unlabelled drug, would assist mass spectral identification of resultant metabolites by giving a "tell tale" doublet signal two mass units apart for drug related entities. This paper therefore describes the synthesis of 5'-[$^{14}C_1$]-1 and 3a,4-[$^{13}C_2$]-1.

Scheme 2



RESULTS AND DISCUSSION

The synthesis of 5'-[$^{14}C_1$]Panadiplon ([$^{14}C_1$]-1) is described in scheme 1. [$^{14}C_1$] Cyclopropane carboxylic acid methyl ester[3] (4) was prepared commercially (Cambridge Research Biochemicals, Cleveland, UK) from bromocyclopropane (2). Treatment with magnesium in THF followed by quenching with [$^{14}C_1$]carbon dioxide gave the free acid (3) which was

esterified with methyl iodide and base to give the ester (4). 3-Cyano-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one (5) was treated with hydroxylamine in methanol to give amidoxime (6). Treatment of this with [$^{14}C_1$]cyclopropane carboxylic acid methyl ester (4; 10 mCi; 13.4 mCi/mmole) gave 5'-[$^{14}C_1$]Panadiplon ([$^{14}C_1$]-1; 1.13 mCi; 13.4 mCi/mmole). The compound had identical retention characteristics to that of a reference sample and was 99.6% and 98.6% pure using two different thin layer chromatographic methods and 99.8% pure by HPLC analysis.

The synthesis of 3a,4-[$^{13}C_2$]Panadiplon ([$^{13}C_2$]-1) is depicted in scheme 2. N-1-(1-Methylethyl)-o-phenylenediamine (7) was treated with [$^{13}C_2$]oxalic acid (8) in 6M hydrochloric acid to give 1,4-[$^{13}C_2$]-1,4-dihydro-1-(1-methylethyl)-2,3-quinoxalinedione (9) in good yield (95%). Treatment of this with diethylchlorophosphate and potassium-t-butoxide followed by addition of 5'-cyclopropyl-3-isocyanomethyl-1,2,4-oxadiazole gave 3a,4-[$^{13}C_2$]Panadiplon ([$^{13}C_2$]-1) in 38% yield. The spectroscopic and microanalytical results of the product were in accordance with those of ^{12}C-material. The parent compound had been assigned previously[4] using 1-D hydrogen, 1-D ^{13}C, 2-D single bond heteronuclear [HETCOR] and 2-D multiple bond heteronuclear [COLOC] experiments. A comparison of the ^{13}C-NMR spectrum for labelled and unlabelled material (see table 1) showed the expected enrichment of carbon atoms C-3a and C-4, the resonances appeared as coupled doublets (J_{C-C} = 80.59 Hz) at 121.95 and 154.39 ppm respectively.

METHODS & MATERIALS

Melting points were obtained on a Koeffler hot stage microscope (Reichert, Wien, Austria).

Anhydrous [$^{13}C_2$]oxalic acid was obtained from MSD Isotopes (Cambrian Gases, K&K Greeff Ltd, Croydon, UK). [$^{14}C_1$]Cyclopropane carboxylic acid methyl ester was obtained from Cambridge Research Biochemicals (Cleveland, U.K.). Panadiplon and the intermediates N-1-(1-methylethyl)-o-phenylenediamine and 5-cyclopropyl-3-isocyanomethyl-1,2,4-oxadiazole were obtained from Chemical Research Preparations (The Upjohn Company, Kalamazoo, MI, USA). 3-Cyano-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one was obtained from Ferrosan (Malmo, Sweden).

Carbon Number	Carbon Shift (ppm)
1	132.93
3	131.45
*3a	121.95
*4	154.39
5a	130.72
6	117.64
7	128.32
8	123.84
9	117.41
9a	122.24
3'	164.92
5'	181.86
1"	7.85
2"	10.08
3"	10.08
1"'	47.89
2"'	19.75
3"'	19.75

• = ^{13}C-labelled (coupling 80.59 Hz).

Table 1. Carbon numbers and chemical shift values. Referenced to TMS = 0.

All organic solvents were of analytical grade or better and were obtained from Fisons Scientific Apparatus Ltd (Loughbrough, Leicestershire, UK).

Normal phase chromatography was performed according to the method of Still, Khan and Mitra[5] commonly known as 'flash chromatography'. Silica gel (230-400 mesh ASTM, E.Merck, Darmstadt, Germany; obtained through Aldrich Chemical Company Ltd, Gillingham, Dorset, UK) was employed as the stationary phase.

Characterisation and purity check of radiolabelled materials was performed on glass tlc plates (Kieselgel 60 F_{254}; 20 x 20 cm plates; 0.25 mm coating). Radioactive areas were located using a RITA-90 linear analyzer (Raytest, Sheffield, UK).

Scintillation counting was performed using Optiphase HiSafe II scintillant (3 ml; Pharmacia Ltd, Milton Keynes, Bucks, U.K.) and an LKB 1218 Rackbeta scintillation counter (Pharmacia Ltd). The external standard ratio method was used to correct for quenching.

Proton and carbon NMR spectra were obtained on a Bruker 500 MHz spectrometer (Bruker Ltd, Coventry, Warwickshire, U.K.). Spectra were run at a probe temperature of 50°C in d_7-dimethylformamide and were referenced to the shifts of deuterated dimethylformamide at 8.01, 2.91 and 2.74 for hydrogen and 162.7, 35.2 and 30.1 for carbon-13 (vs 0 for TMS). Comparisons were made with spectra recently assigned[4] using 1-D hydrogen, 1-D ^{13}C, 2-D single bond heteronuclear [HETCOR] and 2-D multiple bond heteronuclear [COLOC] experiments.

Mass spectra were obtained on a Finnigan Mat 4610B quadrupole mass spectrometer (Finnigan MAT Ltd, Hemel Hempstead, Hertforshire, UK).

Infra red spectra were run on a 157G grating infra-red spectrometer (Perkin Elmer, Beaconsfield, Bucks, UK). The samples were prepared in chloroform and air dried.

Microanalyses were performed by Butterworth Laboratories Ltd (Teddington, Middlesex, UK). The water contents were determined on a Mitsubishi moisturemeter (CA-05; Anachem, Luton, Bedfordshire, U.K.).

[$^{14}C_1$]Cyclopropane carboxylic acid methyl ester (4).

The compound had identical retention characteristics to that of a genuine sample and was 97% pure by gas chromatography on two separate columns: 1). 20% SE30 on Chromosorb W.AW 80-100# 2m x 3 mm ID 110 °C and 15 psig Argon. 2). 20% Carbowax 20M on Chromsorb W.AW 80-100# 2m x 3 mm ID 130 °C and 15 psig Argon. The product had an identical E.I mass spectral fragmentation pattern to that of a genuine sample of cyclopropane carboxylic acid acid methyl ester. MS m/z 100 (M^+; 8%), 99 (50%), 72 (10%, 69 (95%), 59 (40%), 41 (100%) mass units.

5'-[$^{14}C_1$]Panadiplon ([$^{14}C_1$]-1).

Sodium (0.09 g; 3.86 x 10^{-3} moles) was dissolved in anhydrous methanol (1.0 ml) under a

nitrogen atmosphere at room temperature and a solution of hydroxylamine hydrochloride (0.243 g; 3.51 x 10^{-3} moles) in anhydrous methanol (2.2 ml) added, this gave a white precipitate of sodium chloride. The resultant hydroxylamine solution was filtered and added to a suspension of 3-cyano-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one (0.886 g; 3.51 x 10^{-3} moles) in methanol (8.9 ml) and the mixture refluxed for 8 hours. The mixture was allowed to cool and the precipitate filtered, washed with chilled methanol and dried at 50 °C under high vacuum for one hour to give the 3-amidoxime-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one (0.675 g) which was used without further purification. A sample of amidoxime (0.379 g; 1.33 x 10^{-3} moles) was suspended in dry dimethylsulphoxide (4.5 ml) and heated to 80°C under a nitrogen atmosphere. Sodium hydride (0.042 g; 80% dispersion in oil; 1.4 x 10^{-3} moles) was added over 5 minutes to give an orange-red solution, which was stirred for a further 30 minutes. A solution of [^{14}C]cyclopropane carboxylic acid methyl ester (0.133 g; 10mCi; 15.1 mCi/mmole; 1.33 x 10^{-3} moles) in anhydrous dimethylsulphoxide (0.57 ml) was slowly added over 3 minutes. After an hour the mixture was cooled to room temperature and left for a further 16 hours. Acetic acid (1.8 ml; 3.14 x 10^{-2} moles) was added and the solvent removed *in vacuo* to give a yellow solid. Flash chromatography (20 x 150 mm column; solvent: diethyl ether/methanol, (75:5, v/v; 10 ml fractions) gave product in fractions 15-23. The fractions were pooled and solvent removed *in vacuo* to give 3-(5'-[$^{14}C_1$] 5'-cyclopropyl-1,2,4-oxadiazole-3-yl-5-(1-methylethyl)-imidazo-[1,5a]-quinoxalin-4(5H)-one (5'-[$^{14}C_1$]Panadiplon; 1.13 mCi; 13.4 mCi/mmole by mass spectral analysis) as a white solid, which was immediately dissolved in methanol (5 ml) for storage. The compound had identical retention characteristics to that of a reference sample and was 99.6% and 98.6% pure by thin layer chromatography (solvent: diethylether/methanol, 9:1, v/v and butanol/acetic acid/water, 15:3:6, v/v respectively) and 99.8% pure by HPLC analysis (Zorbax phenyl column, solvent: 0.2% trifluoroacetic acid in water/tetrahydrofuran, 3:1, v/v; flow rate 1 ml/min; uv detection at 315 nm).

1,4-[$^{13}C_2$]-1,4-Dihydro-1-(1-methylethyl)-2,3-quinoxalinedione (9).

To a solution of N-1-(1-methylethyl)-o-phenylenediamine (1.8g; 1.2 x 10^{-2} moles) in 6M hydrochloric acid (25 ml) was added [$^{13}C_2$]oxalic acid (1g, 1.1 x 10^{-2} moles). The reaction mixture was heated to 100°C and the temperature maintained for one hour. The mixture was cooled to 0°C and the resulting solid collected by filtration and washed thoroughly with water

until the pH of the filtrate was 6. The material was dried at 50°C under high vacuum to give crude product (2.11g; 94% yield). A sample was recrystallised from methanol to give pure 1,4-[$^{13}C_2$]-1,4-dihydro-1-(1-methylethyl)-2,3-quinoxalinedione. Mp 229-231°C (lit.,[1] 227-228°C for ^{12}C). (Found C, 63.90; H, 5.71; N 13.45. $^{13}C_2$ $C_9H_{12}N_2O_2$. 0.2 H_2O requires C, 63.91; H, 5.95; N, 13.35.); υ_{max} 1645 (s), 1630 (s), 1500 (w), 1450 (s), 1220 (m), 740 (s), 700 (s) cm^{-1}; δ (d_7-dimethylformamide) 1.6 (6H, d, 6.9 Hz, $(\underline{CH_3})_2$-CH), 5.1 (1H, m, -$\underline{CH}$-$(CH_3)_2$), 7.18 (2H, m, Ar-$\underline{H}$), 7.34 (1H, m, Ar-$\underline{H}$), 7.59 (1H, m, Ar-$\underline{H}$) ppm; M.S m/z 206 (M^+; 63%), 164 (87%), 135 (100%) and 106 (30%).

3a,4-[$^{13}C_2$]Panadiplon ([$^{13}C_2$]-1).

1,4-[$^{13}C_2$]-1,4-dihydro-1-(1-methylethyl)-2,3-quinoxalinedione (0.954g; 4.63 x 10^{-3} moles) was suspended in dry dimethylformamide (0.64 ml) and dry tetrahydrofuran (0.6 ml). To this was added a solution of potassium-t-butoxide (5.1 ml; 1M; 5.1 x 10^{-3} moles) in tetrahydrofuran at room temperature and under a nitrogen atmosphere. The orange suspension was then cooled to -10°C and diethylchlorophosphate (0.88 ml; 1.05g; 6.1 x 10^{-3} moles) added. The reaction was stirred at -5°C for a further hour. 5-Cyclopropyl-3-isocyanomethyl-1,2,4-oxadiazole (0.91g; 6.1 x 10^{-3} moles) was dissolved in dry tetrahydrofuran (2.55 ml) and added to the reaction mixture. The reaction was then cooled to -25°C and a solution of potassium-t-butoxide (5.5 ml; 1M; 5.1 x 10^{-3} moles) in THF added slowly. The temperature was maintained at -20°C for 30 minutes and then allowed to warm to room temperature. The crude mixture was added to a solution of sodium hydroxide (0.374g; 9.35 x 10^{-3} moles) in water (19 ml) and the THF removed *in vacuo*. The mixture was extracted with chloroform (3 x 100 ml) and the pooled organic phase washed with a saturated solution of sodium chloride (100 ml), dried over sodium sulphate, filtered and the solvent removed *in vacuo* to give a dark oil. The oil was subjected to normal phase column chromatography (50 x 100 mm column; 50 ml fractions; solvent: dichloromethane/methanol, 98.5 : 1.5, v/v for first 11 fractions and 98 : 2, v/v for the remaining fractions) to give a crude oil (1.19g) in fractions 9-16. This was dissolved in methanol (25 ml) and stirred at room temperature for 30 mins with activated charcoal (0.061g). Filtration through Celite followed by removal of solvent gave semi-pure material which was recrystallised from isopropanol/toluene to give 3a,4-[$^{13}C_2$]Panadiplon ([$^{13}C_2$]-1) 0.596g; 38%). Mp 172-174°C. Found: C, 63.81; H, 4.84; N 20.56. $^{13}C_2$ $C_{16}H_{17}N_5O_2$. 0.25 H_2O requires C, 63.82; H, 5.16; N, 20.48. υ_{max} 1620 (s), 1570 (s), 1510 (m), 1450 (m), 1330 (m), 1300 (m), 740

(s) cm^{-1}; δ (d_7-dimethylformamide) 1.2, 1.3 (4H, mm, 2"-CH_2-, 3"-CH_2-), 1.6 (6H, d, 6.96 Hz, 2"'-CH_3, 3"'-CH_3), 2.4 (1H, m, 1"-CH-), 5.25 (1H, m, 1"'-CH-), 7.35 (1H, m, 8-CH), 7.5 (1H, m, 7-CH), 7.8 (1H, dd, 1.1 Hz, 8.6 Hz, 6-CH), 8.38 (1H, dd, 1.5 Hz, 8.1 Hz), 9.19 (1H, s, 1-CH) ppm; M.S. m/z 337 (M^+; 100%), 295 (75%), 254 (40%), 227 (75%).

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