The Synthesis of [^{3}H] Renzapride (BRL 24924)

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SUMMARY

Renzapride, a $5HT_3$ receptor antagonist and gastric motility stimulant has been reported[1].

In order to undertake receptor binding studies, high specific activity tritiated renzapride (BRL 24924) was synthesised.

This was achieved by catalytic tritio dehalogenolysis of a corresponding halogenated analogue to produce [^{3}H] renzapride with a specific activity of 16.3Ci.mmol^{-1}.

INTRODUCTION

Renzapride (1), is a $5HT_3$ antagonist and gastric motility agent and its synthesis and pharmacological activity have been reported[1]. The basis of its conception was to mimic higher energy conformations of quinolizidine and indolizidine.

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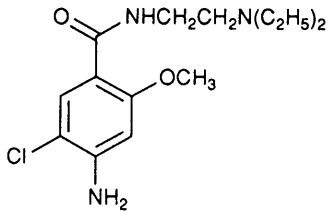
We considered two alternatives in preparing [^{3}H] renzapride; label the bicyclic ring system or more attractively, label the aromatic ring. The latter could be more easily prepared from a more accessible precursor.

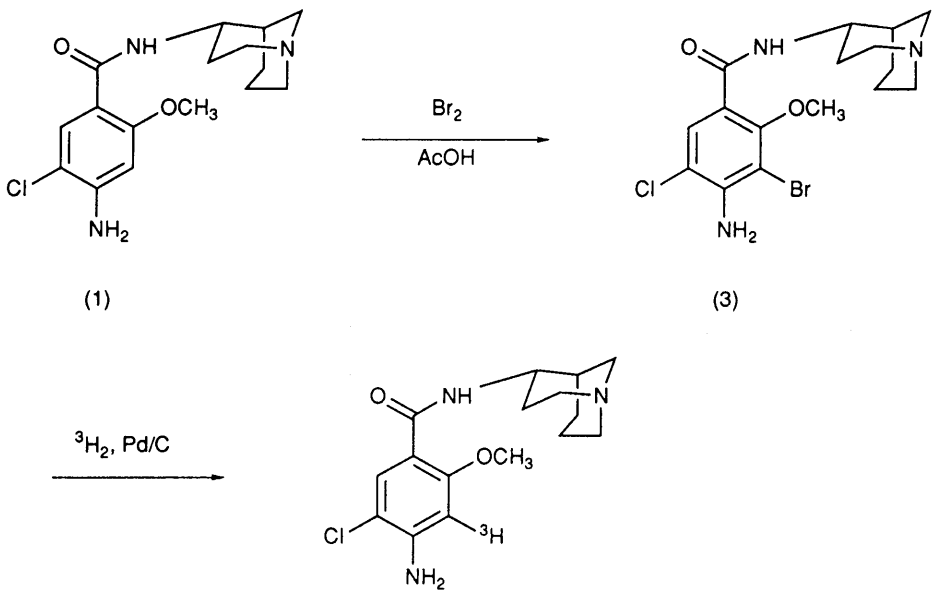
The synthesis of analogous tritium labelled benzamide drugs, in particular metaclopramide (2), has been reported[2].



(2)

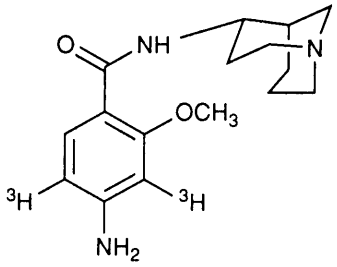
This was achieved by catalytic tritiodehalogenation of a corresponding brominated analogue to produce [^{3}H] metaclopramide (2). The authors had assumed that the position of the tritium label was regiospecific, however, this was not substantiated experimentally.

We rationalised that the expected tritiodebromination of (3) (Scheme 1) would give the desired product (1).



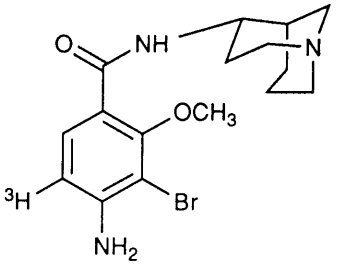
Scheme 1

However, if further dehalogenation to produce compound (4) were to occur its



(4)

formation could have been *via* the dechlorination of [^{3}H] renzapride (Scheme 1) or *via* the intermediate (5).



(5)

The presence of (4) and (5) in the reaction mixture was therefore a possibility. Preliminary unlabelled studies of reaction mixtures by mass spectroscopy had not indicated the presence of compound (5). Interestingly, the mass spectrum of compound (3) did show a molecular fragment of mass 323 (renzapride) presumably due to the lability of bromine. We subsequently reasoned that the presence of (5) in the mixture could therefore not be confidently quantified by mass spectroscopy.

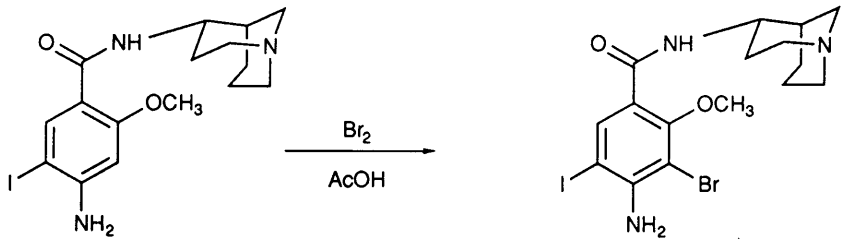
Accordingly, we devised a synthesis as shown in Scheme 1, to prepare [^{3}H] renzapride (1) and in addition prepare the potential dechlorinated impurities (4) and (5) for analytical purposes to establish the purity of the final product (1).

DISCUSSION

The synthesis of the bromo analogue (3) was performed using bromine in acetic acid in 87% yield (Scheme 1).

Preliminary studies using deuterium gas and palladium catalyst indicated the method cited[2] for metaclopramide did not convert the substrate (3) to [^{2}H] renzapride. Those authors[2] had reported that the addition of pyridine as a catalyst poison inhibited the dehalogenation of the aromatic chlorine atom. However, we found it was necessary to use a reaction solvent mixture of dioxan/DMF/triethylamine to prepare [^{2}H] renzapride and subsequently the tritiated analogue.

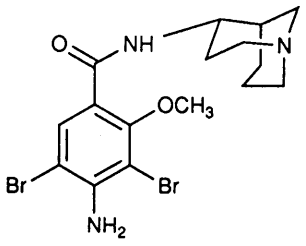
The synthesis of the potential impurity (5) was achieved as shown in Scheme 2, starting from the 5-iodo compound (6) which had been prepared using standard methodology.



(6) (7)

Scheme 2

Hplc monitoring of the reaction mixture indicated the formation of two compounds. Mass spectroscopic analysis of the mixture indicated that compounds of molecular weights 447 and 494 were present. The molecular weight of 494 could be assigned to (7). The structure of the other component (M.wt. 447) was found to be the dibromo compound (8).



(8)

However, this mixture was difficult to separate and the subsequent conversion of this mixture (7) and (8) to the bromo analogue (5) was performed smoothly by catalytic dehalogenation in aqueous acid.

The non-radiolabelled potential impurity (4) was prepared by catalytic dechlorination of renzapride (1).

Using the conditions described previously, the synthesis of [^{3}H] renzapride (1) was performed by a contractor[3]. (See Experimental)

The crude [^{3}H] renzapride returned by the contractor was assayed by hplc. The sample, prior to injection, was "spiked" with non-radiolabelled compounds (1), (4) and (5). The radioactivity corresponding to the eluant of the "spiked" components was determined, the result of which is in the following table:-

Component	% of component in mixture
(5)	0.5 - 1
(4)	21
(1)	63
Components of unknown identity	15

The crude [^{3}H] renzapride (1) (100mCi) was purified by hplc (see Experimental) yielding (52.3mCi) of [^{3}H] renzapride (1) and stored as an aqueous/ethanol solution at -20°C. The radiochemical purity of the product was 97.8% as determined by hplc and 97.0% by tlc (mean of three systems).

EXPERIMENTAL

General

Thin layer chromatography (tlc) was performed on Merck silica gel 60 F254 plates (part no. 5735).

Three solvent systems were used:-
Chloroform:acetone:diethylamine (2:2:1 v/v)
Ethyl acetate:methanol:ammonia (0.88) (7:3:1 v/v)
Chloroform:methanol:acetic acid (10:1:1 v/v)

Analytical scale high performance liquid chromatography (hplc) was performed on a Waters μBONDAPAK C_{18} column (3.9mm id x 300mm) eluted with 0.1M pH4.5 sodium dihydrogen phosphate buffer, acetonitrile (85:15 v/v) at a flow rate of 2.0ml. min^{-1} with UV detection at 230nm.

The purification of the crude [^{3}H] renzapride as supplied[3] was performed by hplc using the system previously described with the exception of UV detection being monitored at 280nm.

Liquid scintillation counting was performed using a Packard Tri-Carb 2660 liquid scintillation counter and Packard ES 299 scintillant. Autoradiography was performed on ^{3}H Hyperfilm (Amersham International plc) using Pentelex developer and Perfix fixative.

Mass spectra were recorded on a Jeol JMS-DX303 mass spectrometer.

The specific activity of [^{3}H] renzapride (1) (16.3mCi.mmol) was determined using a Jeol DX-303 Mass Spectrometer (Fast Atom Bombardment) and also by liquid scintillation counting of solutions of known mass concentration as determined by hplc peak areas referenced against a standard.

Proton nuclear magnetic resonance spectra (NMR) were run in deuteromethanol using a Jeol JNM-GX 270 FT NMR spectrometer with tetramethylsilane as internal standard. For clarity, only the assignments corresponding to the aromatic protons are quoted for the ^{1}H-NMR spectra of non-radiolabelled compounds. Proton coupled ^{3}H NMR (500MHz) spectroscopy was performed by the late Dr. A. Derome, University of Oxford. 1,4-Dioxan was predried by passage through neutral alumina. Dimethylformamide was redistilled from phosphorus pentoxide. Triethylamine was dried over 3Å molecular sieves. All other solvents/reagents were used without prior treatment.

exo-4-Amino-5-iodo-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (6), was supplied by Dr. F. King, Medicinal Chemistry Dept., SmithKline Beecham Pharmaceuticals, Harlow, Essex. Non-radiolabelled *exo*-4-Amino-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (4), was supplied by Dr. D. Rowles, Synthetic Chemistry Dept., SmithKline Beecham Pharmaceuticals, Harlow, Essex. Our thanks are extended to them both.

Synthesis

[^{3}H] Renzapride {(*exo*-3-[^{3}H]-4-amino-5-chloro-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (1)}.

exo-4-Amino-3-bromo-5-chloro-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (3) (10mg, 0.025mmol) was dissolved in 1,4-dioxan (5ml), dimethylformamide (0.6ml) and triethylamine (0.025ml). 10% Palladium on charcoal catalyst (10mg) in 1,4-dioxan (1ml) was added and the mixture stirred under an atmosphere of tritium gas (25Ci, 0.43mmol) for 48 hours. The catalyst was removed by filtration and labile tritium removed by additions of methanol (3 x 10ml) and subsequent removal by evaporation *in-vacuo*.

The crude [^{3}H] renzapride (1) was then purified by hplc using the conditions described previously.

^{3}H NMR 6.76 ppm (s).
MS *m/z* 325.

exo-4-Amino-3-bromo-5-chloro-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (3).

exo-4-Amino-5-chloro-2-methoxy-*N*-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (1) (610mg, 1.89mmol) was dissolved in glacial acetic acid (25ml). Bromine (303mg, 1.89mmol) in acetic acid (6ml) was added dropwise and the mixture stirred at room temperature for 3.5 hours. The reaction mixture was diluted with chloroform (100ml) and washed with sodium metabisulphite solution (10% w/v 55ml). The mixture was then basified with sodium hydroxide solution (20% w/v) (pH 10) The chloroform extract was removed and the aqueous further extracted with chloroform (40ml). The combined chloroform extracts were washed with water (30ml) and saturated brine (30ml) and dried over anhydrous sodium sulphate. Filtration and removal of the solvent by evaporation *in vacuo* yielded a solid 627mg.

Purification by column chromatography (alumina; eluant:- ethyl acetate: chloroform 1:1 v/v grading to chloroform) afforded (3) as a white solid 623mg (1.54mmol 82%).

^{1}H NMR 7.68 ppm (s)
MS *m/z* 401/ 403

exo-4-Amino-3-bromo-5-iodo-2-methoxy-_N_-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (7).

exo-4-Amino-5-iodo-2-methoxy-_N_-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (6) (100mg, 0.24mmol) was dissolved in glacial acetic acid (10ml). Bromine (502mg, 3.14 mmol) in acetic acid (10ml) was prepared. An aliquot of this solution (760µl, 0.24mmol) was added to the reaction mixture and stirred at room temperature for 1.5 hours. (It was necessary to add a further aliquot (100µl) of the bromine/acetic acid solution and stirred for an additional 1.5 hours. The reaction mixture was diluted with chloroform (40ml) and washed with sodium metabisulphite solution (10% w/v 10ml) and water (10ml). The pH was adjusted to 10 with sodium hydroxide solution (20% w/v). The chloroform extract was removed and the aqueous extracted with chloroform (40ml). The chloroform extracts were combined and washed with saturated brine (10ml) and dried over sodium sulphate. Filtration and removal of the solvent by evaporation _in vacuo_ yielded an oil (109mg). Purification of the crude product by column chromatography (alumina; eluant:- chloroform) yielded a solid (55mg) consisting of (7) and _exo_ -4-amino-3,5-dibromo-2-methoxy-_N_- (1-azabicyclo [3.3.1] nonan-3-yl) benzamide (8) as determined by hplc/mass spectroscopy, using the conditions described previously.

exo-4-Amino-3-bromo-2-methoxy-_N_-(1-azabicyclo [3.3.1] nonan-3-yl) benzamide (5)

The mixture of (7) + (8) (45mg) was dissolved in water (25ml) containing concentrated hydrochloric acid (250µl). Palladium on charcoal catalyst (50mg) was added and the mixture stirred under a hydrogen atmosphere for 1.75 hours. The catalyst was removed by filtration and the pH of the filtrate adjusted to 10 with sodium hydroxide solution. Chloroform (50ml) was added and the product extracted. The aqueous was further extracted with chloroform (50ml). The combined chloroform extracts were washed with brine (20ml) and dried over anhydrous sodium sulphate. Filtration and removal of the solvent by evaporation _in vacuo_ yielded an oil which on trituration with ethyl acetate yielded (5) as a white solid (30mg 0.08mmol).

^{1}H NMR 6.56 ppm (d, 1H, J = 8Hz); 7.47 (d, 1H, J = 8Hz)
MS _m/z_ 367/ 369

REFERENCES

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2. I. Pri-Bar and O. Buchman, *J. Label. Compounds and Radiopharm*, **XX**, (10), 1153, (1983).

3. The tritium labelling was performed by Amersham International plc.