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The Synthesis of [^{14}C]Pantoprazole - SK&F 96022Z

An H^+/K^+ ATPase Inhibitor

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Summary

Pantoprazole 1, SK&F 96022Z, an H^+/K^+ ATPase inhibitor has been prepared, radiolabelled with carbon-14, in two positions, for drug metabolism studies. [*Benzimidazole*-2-^{14}C]Pantoprazole in 4 steps from potassium ethyl [^{14}C]xanthate, and [*pyridylmethyl*-^{14}C]pantoprazole from cuprous [^{14}C]cyanide in 8 steps. The overall radiochemical yields of these syntheses were 37.9% and 5.2% respectively.

Keywords: carbon-14 H^+/K^+ ATPase inhibitor, pantoprazole, proton pump inhibitor, carbon-14 pyridine, lithiation.

Introduction

The proton pump inhibitors, e.g. omeprazole (ref. 1) are an increasingly important class of pharmaceutical agent for the treatment of gastric acid related gastrointestinal disorders. Pantoprazole, SK&F 96022Z is an irreversible H^+/K^+ ATPase inhibitor under joint development by SmithKline Beecham and Byk Gulden Pharmaceuticals for the treatment of such disorders.

In order fully to investigate the metabolic fate of pantoprazole (sodium 5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridyl)methylsulphinyl]-1H-benzimidazole) 1, it was necessary to radiolabel 1 with carbon-14 in each of the two distinct halves of the molecule. As the introduction of carbon-14 into position-2 of benzimidazole thiols, *via* reaction of an appropriately substituted phenylene diamine with potassium ethyl [^{14}C]xanthate is a robust and well documented process (refs. 2,3), and since it was envisaged that the methylene carbon at position-2 of the pyridine ring would be amenable to isotopic substitution, those positions were chosen for labelling.

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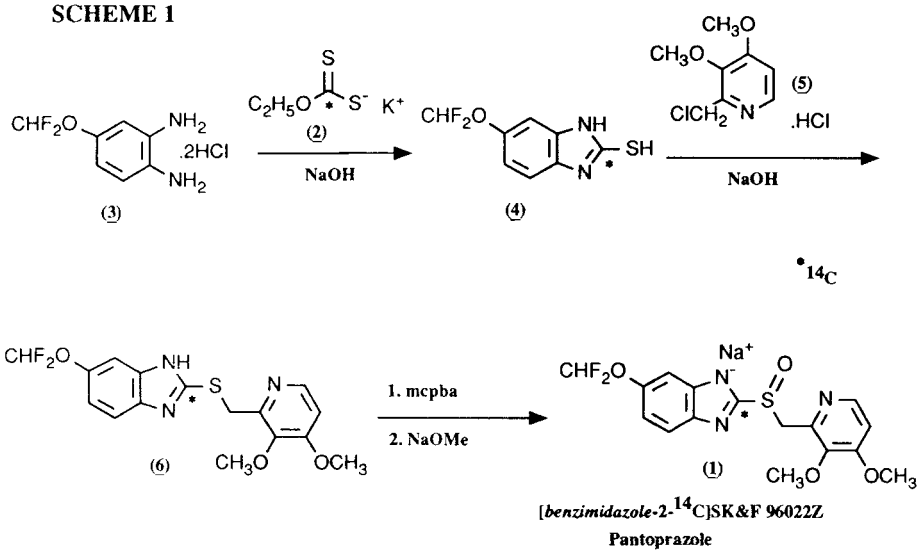
Discussion

The synthesis of [*benzimidazole*-2-^{14}C]pantoprazole 1 was achieved by minor modifications of the methods previously described for the synthesis of [^{14}C]omeprazole (ref. 3), and is shown in Scheme 1. The final compound was purified by semi-preparative h.p.l.c. prior to formation of the sodium salt. This synthesis gave [*benzimidazole*-2-^{14}C]pantoprazole, 108.6mg at 2179Bq/mg (58.9μCi/mg), with a radiochemical purity of >98.2% as assessed by h.p.l.c and t.l.c., in 37.9% overall radiochemical yield from potassium ethyl[^{14}C]xanthate 2.

SCHEME 1

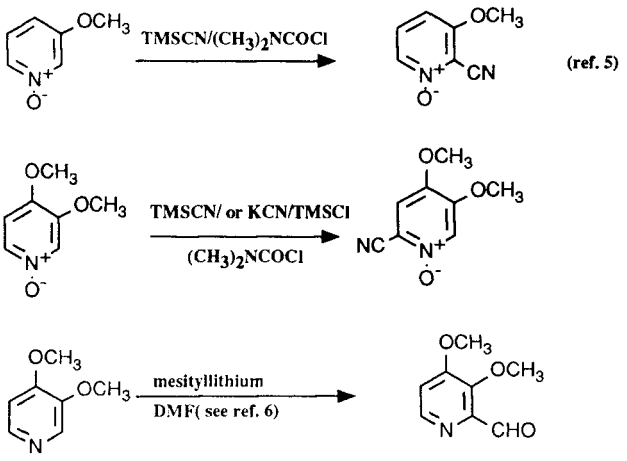


[*benzimidazole*-2-^{14}C]SK&F 96022Z
Pantoprazole

The preparation of [*pyridylmethyl*-^{14}C]pantoprazole necessitated the synthesis of 2-chloromethyl-3,4-dimethoxypyridine 5 labelled with carbon-14 in the chloromethyl group. The initial strategy was to introduce regioselectively into position-2 of 3,4-dimethoxypyridine (refs. 4,5) a radiolabelled one carbon unit suitably functionalised to allow ready conversion to the required chloromethyl group. Clearly it was nesessary to be able to differentiate the 2 and 6 positions of this molecule in order to achieve the desired regio control.

Fife (ref. 6) has reported that the reaction of 3-methoxypyridine-N-oxide with trimethylsilyl cyanide and dimethylcarbamoyl chloride proceeds with excellent regioselectivity, and in high yield, furnishing 2-cyano-3-methoxypyridine. It seemed reasonable to expect a similar result with 3,4-dimethoxypyridine-N-oxide (Scheme 2). However, on subjecting 3,4-dimethoxypyridine-N-oxide to the Fife protocol only 6-cyano-3,4-dimethoxypyridine could be isolated. No evidence for the desired 2-cyano product was obtained. The reasons for this unexpected result are unclear, although it may simply be consequence of greater steric demands imposed

SCHEME 2

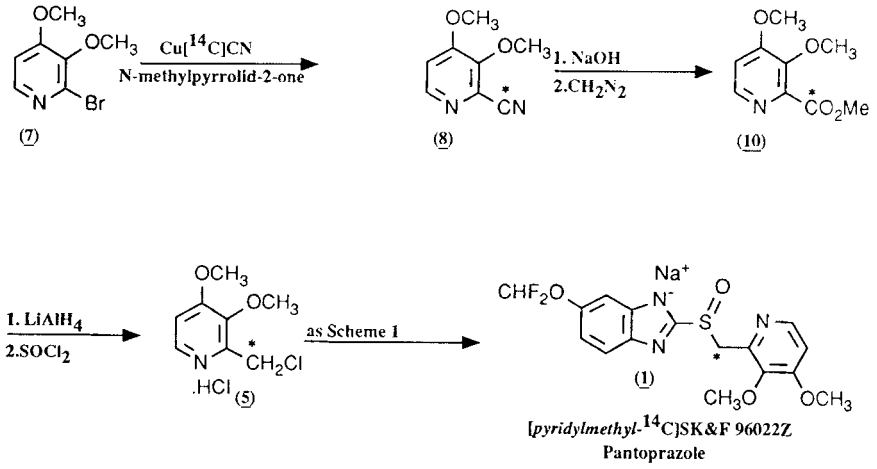


upon the reaction by the disubstituted pyridine. Despite the disappointing outcome in our case, this method should prove generally useful for isotopic labelling of pyridines, particularly as trimethylsilyl cyanide may be generated *in situ* from potassium cyanide, chlorotrimethylsilane and 18-crown-6.

Comins (refs. 7,8) has shown that 3-methoxypyridine can be lithiated, in high yield, by reaction with mesityllithium, and that the resultant 2-lithio species reacts with a variety of electrophiles e.g. DMF, benzaldehyde. 3,4-Dimethoxypyridine lithiates similarly and, on reaction with DMF, gives the corresponding 2-formyl derivative. However, this reaction was found to be rather capricious and the desired 2-formyl compound could not be isolated, reproducibly, in greater than 25% yield (based on DMF) (Scheme 2). In view of the low yield this reaction was considered unsuitable for radiolabelling. It remains to be seen whether an alternative formyl cation equivalent will give better results. No evidence for the formation of 3,4-dimethoxypyridine-2-carboxylic acid could be obtained on reaction of the 2-lithio species with carbon dioxide under a variety of conditions, or 3,4-dimethoxy-2-hydroxymethylpyridine from its reaction with paraformaldehyde.

A third strategy for the introduction of the radiolabel at C-2 proved suceessful and proceeded in good yield. Treatment of 2-bromo-3,4-dimethoxypyridine 7 (ref. 9) with cuprous [^{14}C]cyanide in N-methylpyrrolidin-2-one

Scheme 3



at 175°C gave the required 2[*cyano*-^{14}C]-cyano-3,4-dimethoxypyrididine 8 in 76% yield (Scheme 3). It was noted that the bromopyridine was of low stability under these reaction conditions and, in order to achieve efficient reaction, it was necessary to thoroughly mix all the reagents, before heating, to ensure a homogeneous solution. Base hydrolysis and esterification with diazomethane furnished the required methyl ester 10 in modest yield (14% from the [^{14}C]nitrile). N-Methylation of the pyridine may account in part for this disappointing yield. Reduction of the ester with lithium aluminium hydride in ether at reflux cleanly gave the corresponding alcohol 11 which, in turn, was chlorinated in quantitative yield with thionyl chloride in chloroform at ambient temperature. The resultant 2[*methyl*-^{14}C]-chloromethyl-3,4-dimethoxypyridine hydrochloride 5 was elaborated to [*pyridylmethyl*-^{14}C]pantoprazole as before (Scheme 1).

A number of procedures for the introduction of a carbon-14 labelled one carbon fragment into position-2 of an unsymmetrically substituted pyridine have been investigated. These methods may find applicability in the synthesis of related labelled pyridines and complement our previously described routes (refs. 1,10).

EXPERIMENTAL

Potassium [^{14}C]cyanide and potassium ethyl [^{14}C]xanthate were obtained from ICI TracerCo, Billingham. H.p.l.c. purifications were carried out on Spherisorb 10μ silica (250mm x 22.5mm column) eluted with CH_2Cl_2:(CH_3OH:c NH_4OH 95:5) 96:4 v/v using Gilson 303 pumps and a Holochrome u.v. detector. Radiochemical purities were determined by t.l.c. with a Berthold 2832 linear analyser on Merck 5642 hptlc silica, integration by Berthold Chroma software, in the following systems: i) ethyl acetate/methanol/conc.

ammonium hydroxide (5:1:1 by vol.), ii) dichloromethane/methanol/conc. ammonium hydroxide (90:10:1 by vol.), iii) ethyl acetate/dichloromethane/methanol (2:2:1 by vol.), iv) chloroform/methanol (30:1 v/v), v) ethyl acetate/60-80°petroleum ether (9:1 v/v), vi) chloroform/methanol/conc. ammonium hydroxide (20:10:1 by vol) and vii) ethyl acetate/60-80° petroleum ether/methanol/conc. ammonium hydroxide (20:10:1:1 by vol), and by h.p.l.c. using a Beckman 171 radioisotope detector, integration by Beckman System Gold software, on μ-Bondapak C18 eluted with 20-70% acetonitrile in 0.1M ammonium acetate over 20min at 1ml/min. Quantification of radioactivity was by use of a Beckman LS6800 scintillation counter. All labelled materials were characterised by chromatographic comparison to authentic samples.

5-Difluoromethoxy-2-mercapto-[2-^{14}C]benzimidazole, 4

Potassium ethyl [^{14}C]xanthate 2 (16.875mCi, 624.4MBq, 0.375mmol) was dissolved in absolute ethanol (3ml) and water (3ml). 4-Difluoromethoxy-1,2-phenylenediamine dihydrochloride 3 (ref. 11) (93mg, 0.375mmol) and sodium hydroxide (30mg, 0.75mmol) in ethanol/water (2:1, 1.5ml) were added, and the mixture heated at reflux for 20hour. The cooled solution was diluted with water (3ml), the pH adjusted to 3.5 and the precipitated 5-difluoromethoxy-2-mercapto-[2-^{14}C]benzimidazole 4 filtered off (52mg, 0.256mmol, 11.5mCi, 425.5MBq, 68.1%).

5-Difluoromethoxy-2-[(3,4-dimethoxy-2-pyridylmethyl)thio][2-^{14}C]benzimidazole, 6

5-Difluoromethoxy-2-mercapto-[2-^{14}C]benzimidazole 4 (52mg, 0.256mmol) was dissolved in absolute ethanol (2ml), 2-chloromethyl-3,4-dimethoxypyridine hydrochloride 5 (ref.11) (57mg, 0.256mmol) and sodium hydroxide (0.52mmol, 0.48ml of a 43.4mg/ml solution in water) were added, and the solution stirred at ambient temperature overnight. The solvent was removed *in vacuo*, the residue dissolved in dichloromethane and the inorganics filtered off. The filtrate was evaporated to dryness furnishing 6 (9.84mCi,364.4MBq 85.6%).

5-Difluoromethoxy-2-[(3,4-dimethoxy-2-pyridylmethyl)sulphinyl]-1H-[2-^{14}C]benzimidazole sodium salt, pantoprazole, SK&F 96022Z, (1)

The 5-difluoro-2-[(3,4-dimethoxy-2-pyridylmethyl)thio]-1H-[2-^{14}C]benzimidazole 6 (9.84mCi,364.4MBq) prepared above was dissolved in dichloromethane and cooled to -30°C with stirring. To this was added m-chloroperbenzoic acid (51.8mg, 0.256mmol, 85% pure) portionwise over ~10minutes. Following 4hours reaction a further 10mg of mcpba were added and reaction continued for a 1hour. The reaction was allowed to reach ambient temperature and stirred under an atmosphere of ammonia gas for fifteen minutes. The

precipitate was filtered off and the filtrate evaporated to dryness. The residue was purified by semi-preparative h.p.l.c. (system as described in the introduction) yielding the required 5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridylmethyl)sulphinyl]-1H-[2-^{14}C]benzimidazole, SK&F 96022 (56mg, 7.39mCi, 273.4MBq, 44%). This was diluted with non-radioactive SK&F 96022 and converted to the sodium salt by treatment with one equivalent of sodium methoxide in methanol, the solvent removed and the residue triturated with n-pentane. This preparation furnished [*benzimidazole*-2-^{14}C]pantoprazole (1) (108.6mg, 6.40mCi, 236.8MBq) with a specific activity of 58.9μCi/mg (2179kBq/mg) and a radiochemical purity of ≥98.2% as assessed by t.l.c. and h.p.l.c., in 37.9% overall radiochemical yield from potassium ethyl[^{14}C]xanthate 2.

Copper [^{14}C]cyanide

Copper sulphate (716mg, 2.87mmol) was dissolved in water (2.5ml) containing 0.1% sulphuric acid (0.011ml) and stirred at 60℃. A solution of sodium metabisulphite (201mg, 1.06mmol) in water (0.5ml) was added dropwise, immediately followed by potassium [^{14}C]cyanide (159.9mCi, 5916MBq, 2065MBq/mmol, 2.87mmol) in water (0.6ml). This was stirred at 60℃ for 15min. under nitrogen, then left to stir at room temperature for 1.5hours. The resulting precipitate was filtered, washed with hot water, then absolute ethanol and dried at room temperature under high vacuum to give copper [^{14}C]cyanide (192mg, 117mCi, 4329MBq, 73% radiochemical yield).

2-[*Cyano*-^{14}C]cyano-3,4-dimethoxypyridine, 8

2-Bromo-3,4-dimethoxypyridine 7 (571mg, 2.62mmol, ref. 7), N-methylpyrrolidin-2-one (5ml) and copper [^{14}C]cyanide (192mg, 2.10mmol, 117mCi, 4329MBq), were stirred well at room temperature until thoroughly mixed. The mixture was then stirred at 175℃ under nitrogen for 25minutes., then allowed to cool. A saturated solution of potassium cyanide (15ml) was added and the reaction mixture extracted with ethyl acetate (x3). The combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to give 2-[*cyano*-^{14}C]cyano-3,4-dimethoxypyridine 8 (76.5mCi, 2831MBq, 1.37mmol, RCP 95% in t.l.c. system (iv)).

3,4-Dimethoxy-[*carboxyl*-^{14}C]pyridine-2-carboxylic acid, 9

2-[*Cyano*-^{14}C]cyano-3,4-dimethoxypyridine 8 (76.5mCi, 2831MBq, 1.37mmol) was dissolved in methanol (6ml) and sodium hydroxide solution (0.69ml, 397mg/ml, 6.85mmol) was added. The resulting solution was heated at reflux for 22hours. The reaction mixture was allowed to cool to room temperature

and adjusted to pH3 with concentrated hydrochloric acid. The solvent was evaporated and the residue triturated with absolute ethanol(x3), then dried under high vacuum giving the crude product 9 (RCP 85% in t.l.c. system (vi)).

Methyl 3,4-dimethoxy-[*carboxyl*-^{14}C]pyridine-2-carboxylate, 10

3,4-Dimethoxy-[*carboxyl*-^{14}C]pyridine-2-carboxylic acid 9 (nominally 1.37mmol) was dissolved in methanol (5ml) and a solution of ethereal diazomethane (10mmol in 20ml) was added and the mixture stirred at room temperature for 20hours. Glacial acetic acid was carefully added until effervescence stopped, and the solvent was evaporated. The residue was partitioned between sodium bicarbonate solution and ethyl acetate, and the aqueous layer extracted with ethyl acetate (x3). The combined organics were dried ($MgSO_4$), filtered and evaporated to give the crude product. This material was purified by gravity column chromatography on silica (ethyl acetate/pentane 60:40-90:10 gradient) to give methyl 3,4-dimethoxy-[*carboxyl*-^{14}C]pyridine-2-carboxylate 10 (11.0mCi, 407MBq, RCP 98% in t.l.c. system (v)).

3,4-Dimethoxy-2-[*methyl*-^{14}C]hydroxymethylpyridine, 11

Methyl 3,4-dimethoxy-[*carboxyl*-^{14}C]pyridine-2-carboxylate 10 (11.0 mCi, 407MBq, 0.20mmol) was stirred in dry ether (3ml) with lithium aluminium hydride (13mg, 1.7eq) and stirred at reflux under nitrogen for 5minutes. After cooling to room temperature, saturated aqueous potassium sodium tartrate (4ml) and ether (2ml) were added and the mixture stirred for 5minutes. The layers were separated, the organic phase washed with water and the aqueous thoroughly extracted with ethyl acetate (x4). The combined extracts were dried ($MgSO_4$), filtered and evaporated to give 3,4-dimethoxy-2-[*methyl*-^{14}C]hydroxymethylpyridine 11 (10.0mCi, 370MBq, RCP 87% in t.l.c. system (v)).

2-[*Methyl*-^{14}C]chloromethyl-3,4-Dimethoxypyridine, hydrochloride, 5

3,4-Dimethoxy-2-[*methyl*-^{14}C]hydroxymethylpyridine (10.0mCi, 370MBq, 0.18mmol) was dissolved in chloroform (1ml) containing thionyl chloride (0.4ml) and stirred at room temperature for 30minutes. The solvent was evaporated and the residue thoroughly dried under high vacuum for several hours to give 2-[*methyl*-^{14}C]chloromethyl-3,4-dimethoxy-pyridine, hydrochloride (42.7mg, 10.0mCi, 370MBq, RCP 89% in t.l.c. system (i)).

5-Difluoromethoxy-2-[(3,4-dimethoxy-2-[*methyl*-^{14}C]pyridylmethyl)sulphinyl]-1H-benzimidazole sodium salt, SK&F 96022Z, 1

2-[*Methyl*-^{14}C]chloromethyl-3,4-dimethoxypyridine hydrochloride 5 (42.7mg, nominally 0.18mmol) and 5-difluoromethoxy-2-mercaptobenzimidazole (44.5mg, 0.21mmol) were dissolved in absolute ethanol

(2ml) and sodium hydroxide solution (0.38ml, 1.04M) was added. The resulting solution was stirred for 20hours at room temperature. The reaction mixture was evaporated to dryness and the residue triturated with dichloromethane (10ml),filtered and evaporated. The crude product (nominally 0.18ml, RCP 89% in t.l.c. system (vii)) was redissolved in dichloromethane (1ml), cooled to -30°C, and a solution of m-chloroperbenzoic acid (33mg, 0.19mmol, 80% pure) in dichloromethane (0.5ml) was added portionwise over 20minutes. The reaction was monitored by t.l.c. and a further quantity of m-chloroperbenzoic acid (17mg 0.09mmol) added, over a total of 6hours. The reaction was allowed to reach room temperature and was stirred vigorously under ammonia gas for 5min., filtered through Hyflo, and the filtrate evaporated to dryness. The crude product (66mg, RCP 86% in t.l.c. system (ii)) was purified by normal phase semi-preparative hplc as described above to give [2-*pyridylmethyl*-^{14}C]SK&F 96022 (46mg, 0.12mmol). Non-labelled SK&F 96022 (67.3mg, 0.18mmol) was added to [2-*pyridylmethyl*-^{14}C]SK&F 96022 (46mg) in methanol (2ml) with methanolic sodium methoxide (321µl, 0.92M, 1eq.) and stirred for 10minutes. The solution was evaporated to dryness and triturated with n-pentane to give [2-*pyridylmethyl*-^{14}C]SK&F 96022Z (1) (116mg, 0.30mmol, 6.08mCi, 224.9MBq, 52.4µCi/mg, 1.94MBq/mg, 5.2% overall radiochemical yield, RCP >98.5% in t.l.c. systems (i), (ii), and (iii), and RCP 98.2% by h.p.l.c.

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