SYNTHESIS OF [^{14}C]WIN 54954

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Summary

A synthetic procedure for producing 5-[5-[2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)-(U-^{14}C)-phenoxy]pentyl]-3-methylisoxazole, [^{14}C]WIN 54954 is described. The synthesis is achieved in seven steps with an overall radiochemical yield of 13.7%, using [U-^{14}C]phenol as a source of radiolabel.

Key Words: Anti-viral, Carbon-14, Synthesis, WIN 54954.

Introduction

5-[5-[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl) phenoxy]pentyl]-3-methlisoxazole, WIN 54954 (1), is an antiviral agent with broad spectrum antirhinovirus activity both *in vitro* and *in vivo*, plus activity against several enteroviruses.[1] Mode of action studies on compounds from this series indicate that such compounds inhibit virus (rhinovirus type-2 and poliovirus type-2) replication by preventing viral uncoating.[2] As part of a development programme, a quantity of ^{14}C-labelled material was required for metabolism and pharmacokinetic studies.

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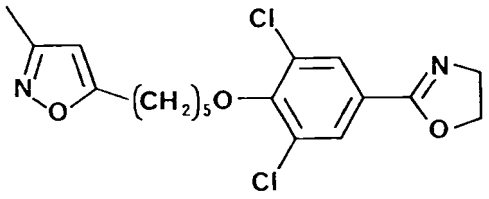
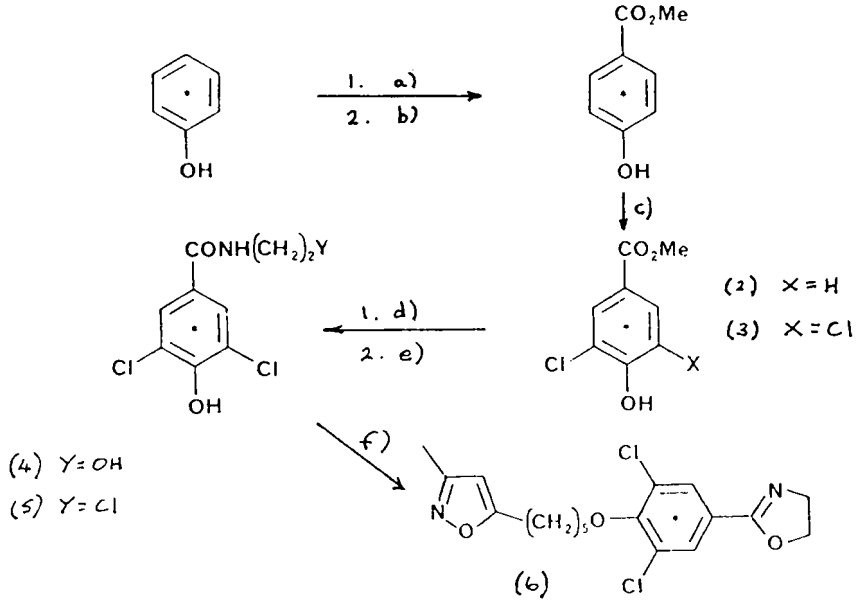


(1)

Results and Discussion

[^{14}C]WIN 54954 was synthesised as outlined in Scheme 1.

Scheme 1



Reagents a) NaOH, Cu, CCl_4, β-cyclodextrin, Δ

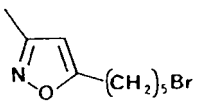
b) $SOCl_2$, MeOH

c) SO_2Cl_2

d) $NH_2(CH_2)_2OH$, Δ

e) $SOCl_2$

f) K_2CO_3, DMF,



Conversion of phenol to 4-hydroxybenzoic acid was accomplished via the method of Komiyama and Hirai[3] and the product methylated to afford 4-hydroxybenzoic acid methyl ester in 74% overall yield. Treatment of this ester with sulfuryl chloride in dichloromethane gave, via the monochloride (2), the desired dichloride (3) in 73% yield. The compound (3) was added to ethanolamine and heated under reflux to afford the hydroxyethyl amide (4), identical with that described previously in the unlabelled synthesis [1], in 86% yield. Treatment of (4) with an excess of thionyl chloride gave, after work-up, the chloroethyl amide (5) in virtually quantitative yield. This chloroethyl amide (5) was treated with potassium carbonate in dimethylformamide, followed by addition of 5-(5-bromopentyl)-3-methyl-isoxazole[4], to afford crude product, which after chromatography gave [^{14}C]WIN 54954 (6) in 29.6% yield.

Using this methodology, [^{14}C]WIN 54954 (specific activity 26.0 mCi/mmol) of radiochemical purity >98% was prepared from [U-^{14}C]phenol (specific activity 60 mCi/mmol), supplied by ICI Tracerco, Billingham, Cleveland, U.K., in a radiochemical yield of 13.7%.

Intermediates in the synthesis were characterised in trial experiments with unlabelled material. Reactions in the radiolabelled synthesis were monitored by t.l.c. using unlabelled reference compounds.

Experimental

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. N.m.r. spectra were recorded on a Bruker AC 80 and are reported relative to internal tetramethylsilane. I.r. spectra (KBr dispersion) were recorded with a Pye-Unicam PV 9516 spectrophotometer.

4-Hydroxy-[U-^{14}C]benzoic acid methyl ester

[U-^{14}C]Phenol (54mCi, 84mg, 0.9mmol) in 20% aqueous sodium hydroxide (5ml) was diluted with phenol (111.3mg, 1.18mmol) in further 20% aqueous sodium

hydroxide (1ml). To this solution was added β-cyclodextrin hydrate (255mg, 0.225mmol), copper powder (40mg, 0.63mmol) and carbon tetrachloride (1ml). This mixture was heated under reflux (oil bath temperature 100°C) in a nitrogen atmosphere for three hours. After this time, TLC (Et_2O:AcOH, 99:1) revealed complete conversion to 4-hydroxy-[U-^{14}C]benzoic acid. The reaction mixture was cooled in an ice-bath and distilled water (10ml) and concentrated hydrochloric acid (10ml) added with vigorous stirring. The solution was extracted with ether, dried over anhydrous magnesium sulphate and the solvent removed by distillation at atmospheric pressure. The residue was dissolved in methanol (10ml) and cooled in an ice-bath. To the vigorously stirred methanolic solution, thionyl chloride (500μl, 6.85mmol) was added dropwise via a syringe. The reaction mixture was allowed to stand at room temperature for two days after which excess thionyl chloride and methanol were removed by distillation at atmospheric pressure. The residue was partitioned between chloroform and distilled water. The aqueous phase was further extracted with chloroform and the combined organic phases dried over anhydrous magnesium sulphate. The solvent was removed by distillation at atmospheric pressure to yield a residue which was chromatographed on silica gel in increasing proportions of diethyl ether in petroleum ether (40-60°) to yield the desired 4-hydroxy-[U-^{14}C]-benzoic acid methyl ester (40mCi, 234mg, 74% radiochemical yield).

3,5-Dichloro-4-hydroxy-[U-^{14}C]benzoic acid methyl ester (3)

The above 4-hydroxy-[U-^{14}C]-benzoic acid methyl ester (40mCi, 234mg, 1.54mmol) was treated with sulfuryl chloride in dichloromethane (10ml, 1M solution) at room temperature for 18 hours. After this time TLC (CH_2Cl_2) revealed complete conversion to the monochlorinated product (2). The reaction mixture was washed with distilled water and the organic phase dried over anhydrous magnesium sulphate. The solvent was removed by distillation at atmospheric pressure and the residue chromatographed on silica gel in increasing proportions of chloroform in petroleum ether (40-60°) to afford 3-chloro-4-hydroxy-[U-^{14}C]benzoic acid methyl ester (2).

The compound (2) was dissolved in further sulfuryl chloride in dichloromethane (30ml, 1M solution) and heated under reflux for 72 hours. TLC (CH_2Cl_2) revealed apparent quantitative conversion to the desired dichloride (3). The solvent was removed by distillation at atmospheric pressure and the residue chromatographed on silica gel in increasing proportions of chloroform in petroleum ether (40-60^{O}) to afford 3,5-dichloro-4-hydroxy-[U-^{14}C]benzoic acid methyl ester (3), (29mCi, 246mg, 72.5% radiochemical yield).

3,5-Dichloro-4-hydroxy-N-(2-hydroxyethyl)-[U-^{14}C]benzamide (4)

The above methyl ester (3) (29mCi, 246mg, 1.11.mmol) was suspended in ethanolamine (3ml) and heated under reflux on an oil bath at 130^{O}C for 4 hours. After this time, TLC (EtOAc:EtOH, 19:1) revealed conversion to the desired benzamide (4) was complete. Distilled water (15ml) and concentrated hydrochloric acid (5ml) were added to the reaction mixture and this was allowed to cool to room temperature. After the mixture had been allowed to stand for 18 hours, the resulting crystals were removed by filtration and washed with distilled water to afford the desired 3,5-dichloro-4-hydroxy-N-(2-hydroxyethyl)-[U-^{14}C]benzamide (4), (25mCi,-240mg, 86% radiochemical yield).

N-(2-Chloroethyl)-3,5-dichloro-4-hydroxy-[U-^{14}C]benzamide (5)

The above hydroxyethyl benzamide (4) (25mCi, 240mg, 0.96mmol) was dissolved in iso-propyl acetate (5ml, pre-dried over 4A molecular sieves) and treated at room temperature with thionyl chloride (290μl, 3.97mmol) and the reaction mixture left for 18 hours. After this time, TLC ($CHCl_3$: MeOH, 19:1) revealed conversion of the starting material to a faster running component. The solvent was removed in vacuo to afford as a white solid, N-(2-chloroethyl)-3,5-dichloro-4-hydroxy-[U-^{14}C]benzamide (5), (25mCi, 258mg, 100% radiochemical yield). Data on unlabelled material, m.pt. 144-150^{O}C (Found:C, 39.16; H, 3.20; N, 4.90; Cl, 38.21. $C_9H_8Cl_3NO_2$ hemihydrate requires C, 38.95; H, 3.27; N, 5.05; Cl, 38.32%. νmax (KBr) 3250, 3150, 1640, 1540, 1480 cm^{-1}.

5-[5-[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl)-[U-^{14}C]phenoxy]pentyl]-3-methylisoxazole (6)

Potassium carbonate (250mg) was added to a solution of the chloroethyl benzamide (5) (25mCi, 258mg, 0.96mmol) in dimethylformamide (5ml). 5-(5-Bromopentyl)-3-methylisoxazole [4] (260mg, 1.1mmol) was added to the reaction mixture and this was stirred overnight at room temperature. Diethyl ether (10ml) was added and the mixture filtered. The filtrate was evaporated to dryness under reduced pressure to leave a residue which by TLC (Et_2O) contained the desired [^{14}C]WIN 54954. The material was chromatographed through silica gel eluting with diethyl ether to afford crude [^{14}C]WIN 54954. This was finally purified by chromatography on silica gel eluting with toluene: methanol: glacial acetic acid (190: 9: 1) to afford the desired 5-[5-[2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)-[U-^{14}C]phenoxy]pentyl]-3-methylisoxazole, [^{14}C]WIN 54954 (6), (7.4mCi, 109mg) of radiochemical purity ≥98% and specific activity of 26.0 mCi/mmol.

The TLC and IR properties of this material were shown to be identical to those of an authentic sample of WIN 54954.

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