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SYNTHESIS OF SCH 42427 LABELLED WITH ^{14}C IN TWO DIFFERENT POSITIONS.

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SUMMARY

[^{14}C]-Sch 42427 was synthesized by two different methods, resulting in incorporation of the label in different parts of the molecule. In the first synthesis [benzyl-α-^{14}C]-Sch 42427 was synthesized in overall 1.7% yield via a 6 step procedure, using [^{14}C]-trimethyl sulphoxonium iodide as the source of label. The second synthesis gave rise to [SO_2-$^{14}CH_3$]-Sch 42427, which was prepared via a 2 step procedure from [^{14}C]-sodium thiomethoxide. Radiochemical purities were greater than 99% for both batches, and analysis by chiral hplc failed to detect any of the undesired S,S enantiomer.

Key Words: Synthesis of [^{14}C]-Sch 42427, antifungal.

INTRODUCTION

Sch 42427 (Figure 1) is the R,R isomer of Sch 39304, a racemic oral triazole antifungal agent, licensed from Sumitomo Corporation (SM 8668). Sch 39304 has been shown to possess a high degree of specificity for the cytochrome P-450 linked mono-oxygenase component of C14 lanosterol demethylase enzyme, and has an elimination half life some 3-4 times longer than the earlier azole anti-fungal agents (1,2).

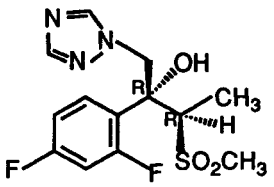

Figure 1: Sch 42427

Metabolism studies using [^{14}C]-Sch 39304 in cynomolgus monkeys and man have shown the drug to be little metabolized (<5%) (3-7).

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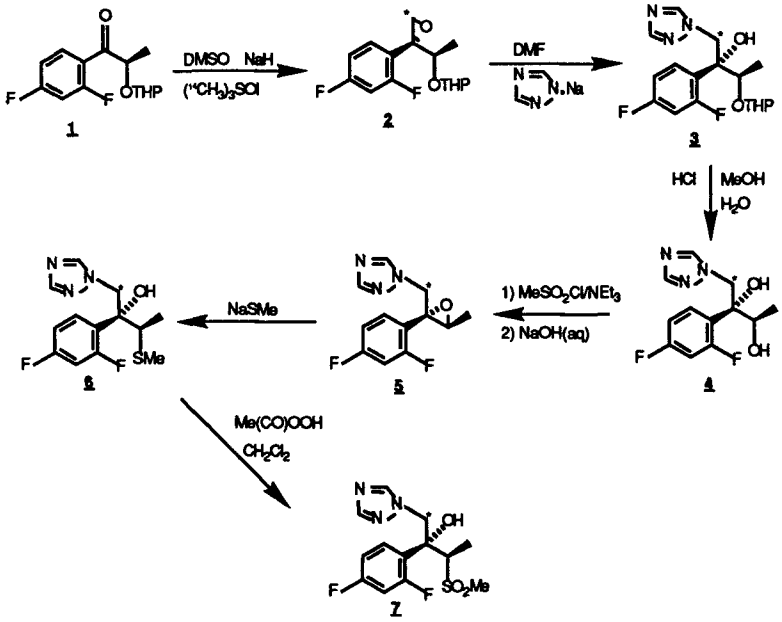
However, given the current trend towards the development of enantiomerically pure drugs, development efforts were shifted to the active enantiomer, Sch 42427. [^{14}C]-Sch 42427 was synthesized in order to study its absorption, distribution, metabolism and excretion.

RESULTS AND DISCUSSION

Scheme 1 shows the reaction sequence employed in the synthesis of [benzyl-α-^{14}C]-Sch 42427, which is a modification of the Sumitomo [^{14}C]-Sch 39304 synthesis (8,9). The disadvantage of this process is that only one third of the ^{14}C can be incorporated into epoxide (2).

SCHEME 1



Some work was performed to investigate the possibility of using other epoxidating agents. Among those considered were diphenylmethyl sulphonium iodide, diphenylmethyl sulphoxonium iodide and methyltriphenyl arsonium iodide. Diphenylmethyl sulphonium iodide was unsuccessful as attempts to make it using $^{14}CH_3I$, gave yields of under 5%. This is thought to be due to the fact that the ylide made from diphenylmethyl sulphonium iodide is thermally and aerobically unstable (10). Similarly unsuccessful was the attempt to prepare diphenylmethyl sulphoxonium iodide using methyl iodide.

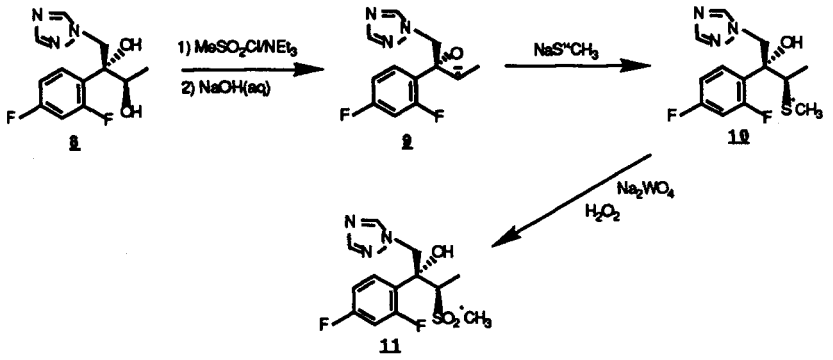
Methyltriphenyl arsonium iodide was prepared from methyl iodide in 80% yield. However when the formed ylide was reacted with ketone (1), using a variety of reaction conditions, only small amounts

of the desired epoxide (2) was obtained. Hence in the interest of time, it was decided to use [^{14}C]-trimethyl sulphoxonium iodide, and accept that the theoretical radiochemical yield for this step would only be 33%.

Thus [^{14}C]-trimethyl sulphoxonium iodide was added to ketone (1), giving the epoxide in 24% radiochemical yield. This step gave rise to required R configuration around Carbon 2. Addition of sodium 1,2,4 triazole to carbon 1, followed by removal of the THP protecting group, led to the formation of the R,R diol (4), which was purifed by silica gel chromatography. The R,S epoxide (5) was formed via the intramolecular displacement of the mesylate, and this was converted to the R,R thioether derivative (6) with a ten fold excess of sodium thiomethoxide. [^{14}C]-Sch 42427 was then formed by oxidising the thioether group to sulphone with peracetic acid. Finally after silica gel chromatography, filtration (to remove traces of undesired S,S enantiomer as the less soluble racemate) and recrystallisation, 4.7mCi of [^{14}C]-Sch 42427 (99.6% radiochemical purity) was isolated in overall 1.7% radiochemical yield.

The synthesis of [SO_2-$^{14}CH_3$]-Sch 42427 is shown in Scheme 2.

SCHEME 2



This shorter synthesis replaced the earlier one, as metabolic data on the racemate, Sch 39304, indicated that the S-methyl position was metabolically stable (3-7). The first step was to prepare the R,S epoxide (9) from the R,R diol (8) which was accomplished in 65% yield via the intramolecular displacement of the mesylate.

The label was incorporated by the addition of [^{14}C]-sodium thiomethoxide, which regenerated the desired R configuration of Carbon 3. The oxidation to sulphone was carried out in the same reaction vessel, using sodium tungstate and hydrogen peroxide as oxidants. This fully oxidised the sulphide in about 3 hours, according to monitoring by hplc, but gave rise to two major peaks on the hplc instead of the expected one, which had been seen in the tracer level synthesis. It is possible that the quality of the ^{14}C thiomethoxide may be responsible for the lowering of the yield. However after purification by a silica gel chromatography, filtration and recrystallisation, 27mCi of [^{14}C]-Sch 42427 was isolated with a

radiochemical purity in excess of 99%. Analysis by chiral hplc using β-cyclodextrin, failed to detect any of the undesired S,S enantiomer (Figure 2).

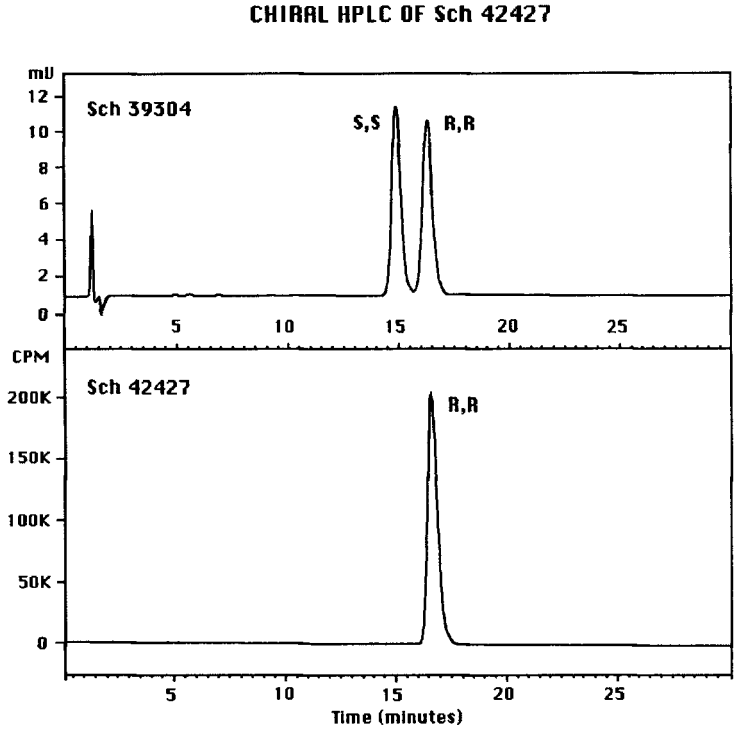


Figure 2

EXPERIMENTAL

Materials

[^{14}C]-trimethyl sulphoxonium iodide and [^{14}C]-sodium thiomethoxide were obtained from Amersham plc and were used without further purification. All reagents and solvents used were reagent grade and were also used without further purification.

Liquid scintillation counting

Quantitation of radioactivity was performed using a Packard 2000CA liquid scintillation analyser, with Scintiverse BD cocktail used throughout.

Thin layer chromatography

Thin layer chromatography was performed using Whatman LK6DF (Silica gel 60) 5x20cm, 0.25mm for normal phase analysis and when reverse phase tlc was performed, Whatman LKC18F 5x20cm, 0.20mm

reverse phase plates were used. The plates were scanned on a Bioscan 1000 linear analyser. The following tlc solvent systems were used:

	Solvent system	Parts by volume
1)	Methylene chloride/methanol	98:2
2)	Methanol/water	70:30
3)	Ethyl acetate	100
4)	Chloroform/methanol	90:10

High performance liquid chromatography

[^{14}C]-Sch 42427 from both synthesis was analysed by hplc for radiochemical and chemical purity. A Waters 600E system controller was used, with a Waters 712 WISP auto-injector. Chemical purity was determined using a Waters 490 programmable multi-wavelength detector and radiochemical purity using a Radiomatic Flow 1 radioflow detector with a Radiomatic Flo-Scint II liquid scintillation cocktail. The following systems were used:-

1). NovaPak C18 (15cm x 3.9mm I.D.) column at 254nm; acetonitrile:water, 11:89, (with 0.75% β-cyclodextrin and 0.25% KH_2PO_4) at 1.5mL/minute.

2). μ-Bondapak C18 (30cm x 3.9mm I.D at 210nm; methanol:water, 30:70 at 1.5mL/minute.

Hplc was also performed during the second synthesis to monitor the progression of the reactions. The system employed Waters 510 pumps controlled with a Waters model 660 solvent programmer. Injections were performed using a WISP 712 auto-injector and detection was achieved using a Waters lambda max 481 LC spectrophotometer. The data was recorded on a Waters 745 data module. A Spherisorb 5μ ODS2 4.6mm x 15cm column was used throughout the analysis.
The following solvent systems were used.

	Solvent system	Parts by volume
3)	Methanol/water	51:49
4)	Methanol/water	35:65
5)	Methanol/water	42:58

Synthesis of [benzyl-α-^{14}C]-Sch 42427

threo-2(R)-(2,4-Difluorophenyl)-3(R)-(2-tetrahydropyranyloxy)-1,2-epoxy-[1-^{14}C]butane (2)

Sodium hydride (400mg, 10mmole) was suspended in dry dimethyl sulphoxide (30mL) and stirred for 40 minutes under nitrogen. To it was added trimethyl sulphoxonium iodide (100mg), with stirring for two minutes, followed by [^{14}C]-trimethyl sulphoxonium iodide

(277mCi, 1060mg) and then the remaining 1047mg of cold trimethyl sulphoxonium iodide, to give a total of 10mmoles added. After stirring for 1 hour and 20 minutes, ketone (1) (2.97g, 11mmole) in anhydrous tetrahydrofuran (12mL) was added and the reaction was allowed to proceed for 3 hours, by which time monitoring by radio-tlc on silica gel in system 1 and on C18 in system 2, showed it had gone to completion. Work up was achieved by pouring the reaction mixture onto 250mL of crushed ice and extracting with methylene chloride (3x40mL). The organic extracts were combined, dried and evaporated to dryness to give the epoxide (2) (67mCi, 24% at 80% radiochemical purity), which was used without further purification in the next step.

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-3(R)-(2-tetrahydropyranyloxy)-2-[1-^{14}C] butanol (3)

Under a nitrogen atmosphere, Epoxide (2), (67mCi, 1.522g, 5.58mmole) and sodium 1,2,4 triazole (2.031g) were stirred in dry dimethylformamide (30mL) at 70°C for 4.5 hours. After dilution with crushed ice, the reaction mixture was extracted with methylene chloride (2x50mL). The organic layers were combined, evaporated and quantitated to give 55mCi, 82% of the alcohol (3), (80% rcp) which was used without further purification in the next step.

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-2-[1-^{14}C] butane-2,3(R)-diol (4)

The crude alcohol (3) was dissolved in methanol (50mL) and to this solution, concentrated HCl (5mL) was added. After stirring for 3 hours at room temperature, the solution was concentrated to a low volume by rotary evaporation and neutralised with 5% sodium carbonate solution. After evaporating to dryness, the crude diol (4) was partitioned between water (46mL) and methylene chloride (50mL). The aqueous layer was extracted with additional methylene chloride (2x50mL) and the organic fractions were combined, dried and evaporated to dryness to yield 51mCi of crude diol (4). The diol was purified by column chromatography using flash grade 40μ silica gel, using ethyl acetate as eluent, to give 43mCi, 78% of diol (4). The radiochemical purity measured by normal phase tlc using solvent system 3, and reverse phase tlc using solvent system 2 was found to be 96%.

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-2,3(S)-epoxy-[1-^{14}C] butane (5)

Under nitrogen, to a solution of the diol (4) (43mCi, 4.47mmole) in dry methylene chloride (20mL) and triethylamine (3.05mL, 21.8mmole), was added dropwise a solution of mesyl chloride (0.85mL, 10.9mmole) in methylene chloride (50mL) at 0°C over a period of 1 hour. After 3 hours, tlc in system 3 showed that the reaction was incomplete, so the reaction was held at -20°C overnight and checked the next morning, by which time it was found to have gone to completion. Sodium hydroxide (15%, 8.5mL) was added dropwise, and the solution was stirred at room temperature for 6

hours. The reaction mixture was extracted with methylene chloride (25mL), which in turn was washed with water (25mL) and filtered through glass wool to give 35.5mCi, 83% of epoxide (5) at a radiochemical purity of 82%.

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-3(R)-thiomethoxy-[1-^{14}C] butane (6)
Under a nitrogen atmosphere, the crude epoxide (5) (35.5mCi, 1.408g, 5.6mmole) was dissolved in methanol (25.5mL). A 15% aqueous solution of sodium thiomethoxide (3.96g, 56mmole) was added and the mixture was stirred at 70°C for 3.5 hours. The reaction was worked up by pouring the mixture into methylene chloride (300mL) and washing with water (100mL). 32.1mCi, 90% of the thioether (6) was obtained, and tlc in system 3 showed the radiochemical purity to be 63%.

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-3(R)-methyl-sulphonyl-[1-^{14}C] butane (7) Sch 42427
The methylene chloride solution containing thioether (6) from the previous stage, was evaporated to dryness under reduced pressure and redissolved in methylene chloride (30mL). After cooling to 0°C in an ice bath, peracetic acid (32%w/w, 3mL, 14.25mmole) was added and the mixture was stirred at 0°C for 2 hours, by which time tlc in system 3 showed the reaction was finished. The reaction was quenched by the addition of a 30%w/v solution of sodium thiosulphate (4g in 13mL) and a 5%w/v solution of potassium carbonate (4.3g in 75mL), while stirring at 0°C. The layers were allowed to separate and were quantitated to show that 28.7 mCi, 90% was present in the organic layer, with 59% of the activity corresponding to the desired product Sch 42427 (7).

Purification of Sch 42427 (7) was achieved by dissolving the crude material in methylene chloride (10mL), filtering to remove any undissolved material and applying the solution to an Analtec 4000μ silica gel rotor for a Chromatotron (model 7924T, Harrison Research Inc) and using ethyl acetate as the eluent. The fractions were analysed for chemical and radiochemical purity using hplc (system 1) and the fractions containing radiochemically pure (7) were pooled and quantitated to give a yield of 10.6mCi, corresponding to a weight of 461mg. The material was crystalised by adding 1.25mCi of (7) dissolved in methanol, prepared from an earlier tracer level synthesis of (7) to the production batch and heating until a solution formed. Activated charcoal was added and the solution heated to reflux, before it was filtered through celite, allowed to cool and slowly evaporated under a stream of nitrogen. The solution was evaporated to a slush and 1mL of methanol was added with stirring, before the material was allowed to crystalise at -15°C, yielding 414mg. This was then suspended in acetone (3mL) and heated to reflux. The solution was kept at room temperature for 16 hours before it was filtered and dried over a stream of nitrogen. The solid was dissolved in ether (19mL) and allowed to crystalise at -15°C.

After removing the mother liquor with a pipette, the crystals were washed with ether (2x1mL) and ethyl acetate (2x1mL), before being dried to constant weight to give 209mg, 4.74mCi, 15% and an overall radiochemical yield from [^{14}C]-methylsulphoxonium iodide of 1.7%. Analysis by hplc on systems 1 and 2, showed a chemical purity of 98.6 % and a radiochemical purity of 99.9% and 99.3%.

Synthesis of [S-^{14}CH$_3$]-Sch 42427

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-2,3(S)-epoxy-butane,(9)

Under a nitrogen atmosphere to a mixture of diol (8) (3.86g, 14.3mmole) and triethylamine (6mL, 42.9mmole) in methylene chloride (27mL) at a temperature of 0°C, a solution of mesyl chloride (1.7mL, 21.5mmole) in methylene chloride (5mL) was added dropwise over one hour, keeping the temperature at 0°C. The reaction was stirred for a further two hours in the ice bath, by which time tlc on silica gel in system 4 showed no diol remained. A 15% aqueous solution of sodium hydroxide (14.1mL, 42.9mmole) was then added dropwise over 30 minutes and the resulting two phase mixture was stirred for 2.5 hours at 0°C. At this point the reaction was checked by tlc (system 4) which indicated the mesylate had been converted into the epoxide (9). The reaction mixture was worked up by adding water to the flask and pouring the contents into a separating funnel. The epoxide (9) was extracted with methylene chloride (3x50mL), and the organic extracts were combined, before being washed with water (2x50mL), dried over anhydrous magnesium sulphate and evaporated to dryness. Analysis by reverse phase hplc using system 3 showed that the epoxide was about 90% pure, with the major impurities being the diol and the mesylate. Recrystallisation from ethyl acetate and hexane raised the purity to 98.5%, and resulted in a yield of 2.34g, 65%. Analysis: Calculated% C: 57.37, H: 4.41, N: 16.73, Found% C: 57.06, H: 4.29, N: 16.51. EI mass spectrometry m/z 251(M), 236, 188, 153, 141, 110, 96(B), 69. CI mass spectrometry m/z 252(M). NMR($CDCL_3$), CH_3 δ1.6ppm(d), CH δ3.25ppm(q), CH_2 δ4.7ppm(m), Ar-H(3) δ6.9-7.3ppm(m), Het-H(2) δ7.9ppm(s), δ8.3ppm(s).

threo-1-(1H-1,2,4-Triazol-1-yl)-2(R)-(2,4-difluorophenyl)-3(R)-[^{14}C-methyl-sulphonyll-butane,(11) Sch 42427

Epoxide (9) (470.6mg, 1.875mmole) was dissolved in methanol (1.97mL) and the resulting solution stirred under nitrogen. To this solution was added [^{14}C]-sodium thiomethoxide(97mCi, 139.7mg, 1.94mmole), and the resulting mixture was stirred at 65°C for 4 hours. Radio tlc (system 4) and hplc (system 1) indicated that the reaction was complete and that mesylate (10) had formed. At this point the pH was adjusted to pH 8.5 with HCl (1M) and 25% aqueous sodium tungstate (52.5µl, 0.04mmole) and 30% hydrogen peroxide (721µl, 7.03mmole) were added. The reaction was stirred at 60°C for

3 hours and analysed by hplc (system 4), which showed that the oxidation was complete, and that about 60% of the material appeared to be desired product. The reaction was quenched by the addition of 5% aqueous sodium thiosulphate solution (2.6mL) and the pH was raised to pH 9 by the addition of a few drops of 15% aqueous sodium hydroxide solution. After removing the methanol under a stream of nitrogen, the formed Sch 42427 (11) was extracted with ethyl acetate(3x25mL). The organic extracts were combined, washed with water(2mL) and quantitated to give 90mCi of crude (11).

This was purified by applying an ethyl acetate solution (10mL) to a column containing flash grade 40μ silica gel (55g). The eluent used was ethyl acetate, and the fractions were monitored by liquid scintillation counting, and those fractions containing significant amounts of activity were analysed by hplc (system 5). The desired fractions containing the cleaned up (11) were pooled, and evaporated to dryness. This was suspended in ethyl acetate (8mL) and the flask was gently warmed until a solution was obtained. After sitting at room temperature for 1 hour, the solution was filtered through glass wool, and the ethyl acetate was removed under nitrogen. Finally the material was crystalised from ethyl acetate/hexane to yield 24mCi, 166mg, corresponding to an overall radiochemical yield of 27%. The specific activity was adjusted by the addition of 508mg, 7.3mCi of low specific activity material and recrystalising the whole batch in ethyl acetate/hexane to yield 592mg, 27.5mCi at a specific activity of 46μCi/mg. Analysis by radio hplc in systems 1 and 2 gave radiochemical purities of 99.6% and 99.3% respectively.

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