SYNTHESIS OF ^{2}H, ^{3}H AND ^{14}C LABELLED SCH 40120

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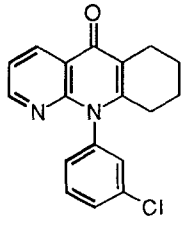
SUMMARY

^{2}H and ^{3}H labelled Sch 40120 were prepared by Pt catalysed exchange with isotopic water. D7-Sch 40120 was obtained in two exchanges in 53% yield and ^{3}H-Sch 40120 was prepared by a single exchange at a specific activity of 19.8 Ci/mmole. ^{14}C-Sch 40120 was prepared in 4 steps from ^{14}C-*m*-chloro aniline in overall 16% radiochemical yield.

Key Words: Sch 40120, tritium, deuterium, carbon-14, synthesis.

INTRODUCTION

Sch 40120 1, an inhibitor of the 5-lipoxygenation of arachidonic acid in vitro, and modulator of the immune system, is currently in clinical trials as an anti-psoriatic agent (1,2). In order to support the development of Sch 40120, labelled Sch 40120 was required.



Sch 40120 1

Three labelled forms of Sch 40120 were ultimately synthesized; ^{14}C for ADME studies, high specific activity ^{3}H for protein binding and ^{2}H for use as a standard in a mass spectrometry based bioanalytical assay. The paper describes the synthesis of each labelled form.

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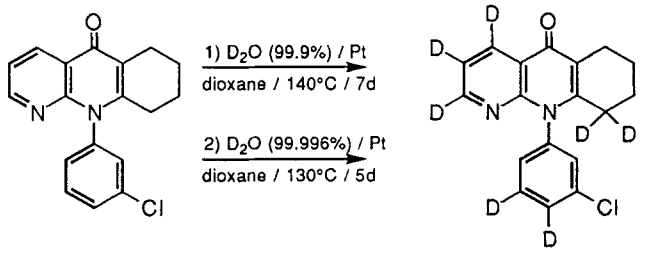
RESULTS AND DISCUSSION

Scheme 1 shows the reaction sequence for the synthesis of ^{2}H-Sch 40120 **2**.



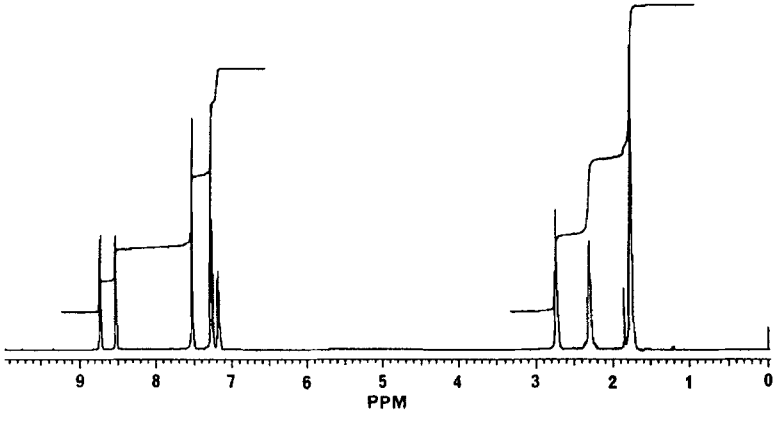
1 **2**

Scheme 1

^{2}H-Sch 40120 was required for use as an internal standard to validate a mass spectrometry based bioanalytical assay. An enrichment of at least three deuterium atoms per molecule was required to prevent interferences from the ^{13}C and Cl isotope cluster in unlabelled Sch 40120. Preliminary small scale experiments showed heterogeneous Pt catalysed exchange (3-5) with deuterated water would achieve this goal. Two deuterium exchange reactions were performed in order to convert all the unlabelled Sch 40120 to a multiply labelled species. The first exchange employed 99.9% D_2O to exchange the majority of the label into the molecule and the second used 99.996% D_2O to eliminate all traces of unlabelled Sch 40120. In the second exchange, the additional precautions of preparing the Pt in D_2O with sodium borodeuteride, and subsequently washing the catalyst with D_2O and deuteroacetone were taken.

Analysis by ^{1}H-NMR (Figures 1a, 1b) and EI mass spectrometry (Figure 2) indicated an enrichment of 7 deuterium atoms with less than 1% residual unlabelled Sch 40120 remaining. The sites of labelling are shown in Scheme 1.

Figure 1a: ^{1}H NMR of ^{1}H-Sch 40120, **1**.



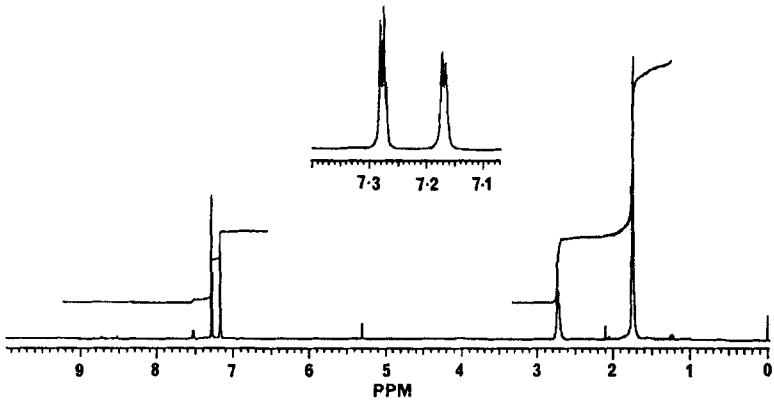


Figure 1b: ^{1}H NMR of ^{2}H-Sch 40120, **2**.

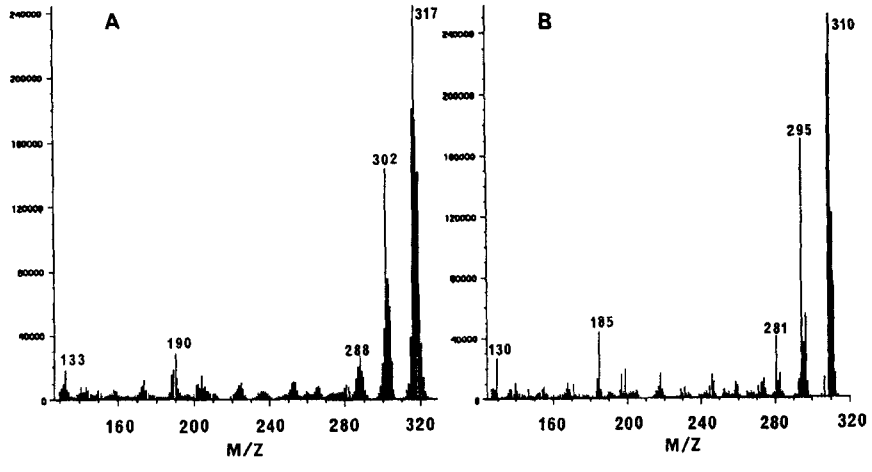
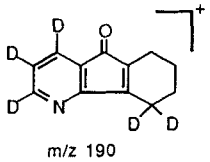


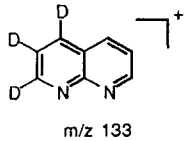
Figure 2: EI Mass Spectrum of (A) ^{2}H-Sch 40120, (B) ^{1}H-Sch 40120.

The three deuterium atoms in the pyridine ring and the two aliphatic positions were determined from the ^{1}H-NMR and from the ions m/z 190 and m/z 133 in the EI mass spectrum as shown in Figure 3. The precise location of the aliphatic sites was fixed by ^{1}H NMR (δ2.30 ppm).



m/z 190

Appears as m/z 185 in unlabelled Sch 40120



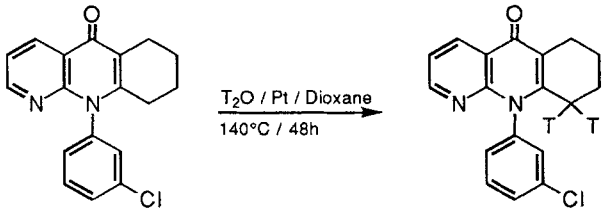
m/z 133

Appears as m/z 130 in unlabelled Sch 40120

Figure 3

The two remaining deuterium atoms in the aniline ring, were fixed by NMR NOE experiments.

^{3}H-Sch 40120 **3** was labelled in a similar manner to ^{2}H-Sch 40120 **2**, using heterogeneous Pt catalysed exchange with high specific activity tritiated water.



1 **3**

Scheme 2

The conditions differed from those used in the deuterium exchanges, as a shorter reaction time was used to minimise the radiolysis of Sch 40120, and a higher co-solvent to isotopic water ratio was used.

Analysis by ^{3}H-NMR (6) (Figure 4) showed a different pattern of incorporation to ^{2}H-Sch 40120.

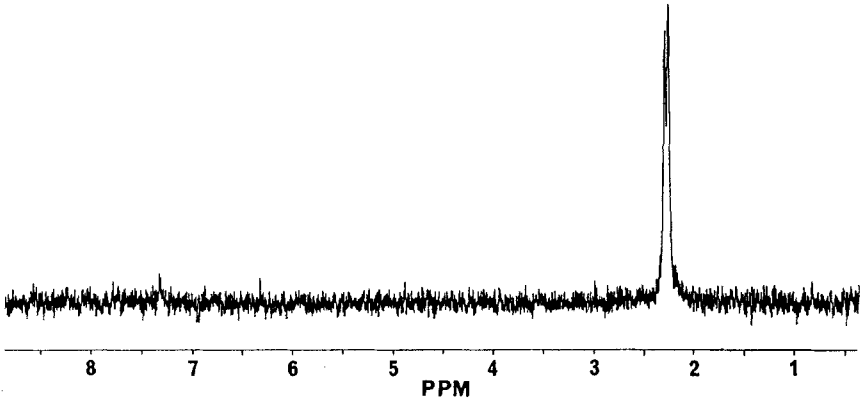
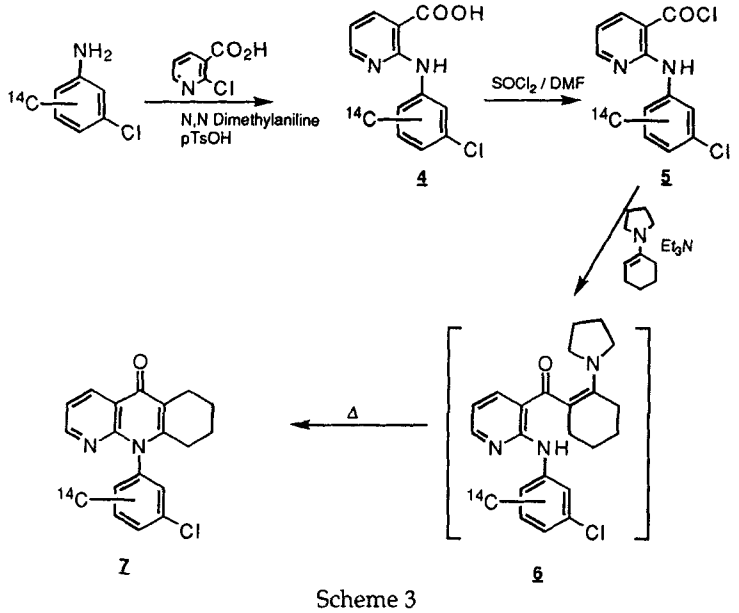


Figure 4: 320 MHz ^{3}H NMR of ^{3}H-Sch 40120 **3** with proton decoupling.

All the label was located in the aliphatic portion of the molecule, and none in the aromatic areas. This clearly establishes that the aliphatic site is kinetically the most reactive.

The reaction scheme for the synthesis of ^{14}C-Sch 40120 7 is shown in Scheme 3:



Scheme 3

The synthesis was based on the route developed by Schering-Plough Research Institute, Chemical Research Department (7,8) by using ^{14}C-*m*-chloroaniline to introduce the label. The reaction of ^{14}C-*m*-chloro aniline with 2-chloro nicotinic acid proceeded in 75% yield by tlc, with the remaining 25% unreacted aniline, which could be recovered and used in subsequent reactions. The next step in the synthesis involved conversion to the acid chloride, which was done by reaction with 10 equivalents of thionyl chloride to drive the reaction to completion. The formed acid chloride was then reacted with 1-(1-pyrrolidino-1-cyclohexene) to form Intermediate 6, which was cyclized in the same pot to ^{14}C-Sch 40120 7 by heating to 80°C, in about 40% yield.

EXPERIMENTAL

Materials

[^{14}C]-*m*-chloroaniline was purchased from Amersham plc and D_2O (99.996%) was purchased from Isotec Inc and were used without further purification. All reagents and solvents were reagent grade and were also used without further purification.

Liquid Scintillation Counting

Quantitation of radioactivity was performed using a Packard 2200CA liquid scintillation analyser, with Scintiverse BD cocktail used throughout.

Thin layer Chromatography

Thin layer chromatography was performed using Whatman LK6DF (silica gel 60) 5 x 20cm, 0.25mm plates. The plates were scanned on a Bioscan 1000 linear analyser. The following solvent systems were used:

1. methylene chloride: methanol (95: 5).

2. isopropanol: methylene chloride: acetic acid (4: 96: 0.2).

High Performance Liquid Chromatography
[^{3}H] and [^{14}C]-Sch 40120 were analysed by hplc for radiochemical and chemical purity. [^{2}H]-Sch 40120 was analysed for chemical purity by hplc. A Waters 600E system controller was used with a Waters 712 WISP auto-injector. Chemical purity was determined using a Waters 490 programmable multiwavelength detector and radiochemical purity using a Radiomatic Flow 1 radioflow detector with Radiomatic Flo-Scint III liquid scintillation cocktail. The following systems were used:

1. Waters Novapak C18 column, 15cm x 4.0mm I.D., 254nm, methanol: water: glacial acetic acid (60:40:1), at 0.6 ml/min. for about 15 minutes, followed by a gradient to acetonitrile.

2. YMC PVA-Silica column, 15cm x 4.6mm I.D., 254nm, isooctane: ethanol (90: 10) at 1 ml/min. followed by a gradient to ethanol.

Synthesis of ^{2}H-Sch 40120 2

Exchange 1
Platinum oxide (200 mg) was weighed into two 250 mL conical flasks, and water (100 mL) was added into each flask. Sodium borohydride (1g) was carefully added to each flask over 15 minutes, with periodic stirring. The flasks were then warmed to 70°C until effervescence had ceased (about 30 minutes). The water was decanted from each flask, and the platinum metal was washed twice with water, and three times with acetone. The platinum from each flask was transfered in acetone to two 5 mL 'reactivials', and the acetone was removed under a stream of nitrogen. Sch 40120 **1** (250 mg) was then weighed into each vial and dioxane (1 mL) and D_2O (99.9%, 2 mL) was added to each. The vials were crimp sealed and heated at 140°C for 1 week.

The contents of both vials were transferred to a separating funnel containing ethyl acetate (50 mL). The ethyl acetate layer was washed successively with sodium bicarbonate (50 g/L, 5 mL), hydrochloric acid (0.1M, 5 mL), and water (5 mL). The ethyl acetate solution was dried over anhydrous sodium sulphate, filtered and evaporated to dryness.

A yield of 526mg of crude ^{2}H-Sch 40120 was obtained.

Exchange 2
This was performed as described for 'Exchange 1', with the exceptions that platinum oxide was reduced with sodium borodeuteride (99.8%) in D_2O (99.9%). The freshly prepared Pt was washed with D_2O and dried with deuteracetone (99.8%). The crude ^{2}H-Sch 40120 from 'Exchange 1' was added to each vial containing the dried Pt, followed by dioxane and D_2O (99.996%). After sealing the vials were heated at 130°C for 5 days.

Both vials were worked up in the same way as described for 'Exchange 1'.

The crude ^{2}H-Sch 40120 **2** was purified by preparative hplc using a Whatman 25 cm x 22.4 mm I.D. 10μ silica gel column, with a 75: 25 methylene chloride: t-butyl

methyl ether mobile phase at a flow of 12.5 mL/minute. Detection was at 254 nm. The hplc purified product was finally purified by recrystallisation from isopropanol (4 mL) A yield of 282 mg was obtained (55%). The sample was analysed by EI mass spectrometry and ^{1}H-NMR. EI mass spectrometry unlabelled Sch 40120 **1** : m/z 310 (M), 295, 281, 185, 130. EI Mass Spectrometry ^{2}H-Sch 40120 **2**: m/z 317 (M), 302, 288, 190, 133. ^{1}H-NMR ^{1}H-Sch 40120 **1** Ar-H δ8.72ppm (dd 1H), Ar-H δ8.52 ppm (dd, 1H), Ar-H δ7.55 ppm (m 2H), Ar-H δ7.28 ppm (m 2H), Ar-H δ7.17ppm (m 1H), CH_2 δ2.75ppm (m 2H), CH_2 δ2.30 ppm (m 2H) and CH_2 δ1.75ppm (m 4H). ^{1}H-NMR of ^{2}H-Sch 40120 **2** Ar-H δ7.28 ppm (d 1H), Ar-H δ7.17 ppm (d 1H), CH_2 δ2.75 ppm (m 2H) and CH_2 δ1.75ppm (m 4H).

Synthesis of ^{3}H-Sch 40120 3

Platinum dioxide (25.9 mg) was weighed into a 50 mL flask and water (10 mL) was added with stirring. Sodium borohydride (104.4 mg) was added over 10 minutes. After the effervescence has ceased, the flask was heated to 70°C for 30 minutes. The water was decanted off and the platinum metal was washed with water (2 x 10 mL), and then acetone (2 x 10 mL). The acetone was decanted off and the platinum metal was transferred to a reaction tube in a little acetone, which was then gently blown to dryness.

Sch 40120 **1** (10.9 mg) was added to the tube in dioxane (100 µL), and tritiated water (20 Ci) was distilled in. The tube was sealed, wrapped in foil and heated to 140°C for 48 hours.

After cooling the tube was frozen in liquid nitrogen and opened. The contents were pumped to dryness, and the solid was taken up in ethanol (3 x 2 mL), and filtered through a Millex-SR filter to remove the catalyst. The filtrate was rotary evaporated to dryness to remove labile tritium. The residue was dissolved in ethanol (5 mL) and the labile removing process was repeated twice more.

A yield of 952.6 mCi was obtained. The Radiochemical purity by radio tlc (system 1) was 26%.

100 mCi of crude ^{3}H-Sch 40120 was purified by hplc on a YMC 25 cm x 9.4 mm PVA silica column with a 92: 8 methylene chloride: *t*-butyl methyl ether mobile phase at 4 mL/ minute. Detection was at 254 nm.

17 mCi at a specific activity of 19.8 Ci/mmole was obtained. The radiochemical purity as determined by radio hplc (system 1) and radio tlc plate scanning (system 1) was 98.5%. ^{3}H-NMR of ^{3}H-Sch 40120, δ2.29 ppm, δ2.25ppm.

Synthesis of ^{14}C-Sch 40120 7

^{14}C-2-(*m*-chloroanilino)-nicotinic acid, **4**
2-chloronicotinic acid (170 mg, 1.07 mmoles), ^{14}C-*m*-chloroaniline (75 mCi, 1.07 mmoles), N, N-dimethyl aniline (136 µl, 1.07 mmoles) and *p*-toluenesulphonic acid (16 mg) were suspended in water (2 mL). The mixture was refluxed for 8 hours, before it was cooled to room temperature and stirred at this temperature overnight. The resulting suspension of **4** was extracted with ethyl acetate (3 x 5

mL). The ethyl acetate solution was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. Analysis by tlc in system 2 showed a radiochemical purity of 75%. Compound 4 was used directly in the next step.

^{14}C-2-(*m*-chloroanilino)-nicotinic acid chloride, 5

1 drop of DMF, followed by thionyl chloride (780 µl, 10.7 mmoles) was added to compound 4. The mixture was stirred for 1 hour. The excess thionyl chloride was evaporated under a stream of nitrogen. Benzene (3 mL) was added, and then removed by evaporation under vacuum. The residue containing compound 5 was used directly in the next step.

^{14}C-10-(*m*-chlorophenyl)-6,8,9,10,-tetrahydrobenzo [b] [1,8] naphthyridin-5(7H)-one, ^{14}C-Sch 40120, 7

Compound 5 was suspended in toluene (3 mL) and cooled to -5°C under argon. Triethylamine (195 µl, 1.4 mmoles) was added. The suspension was cooled to -10°C and 1-(1-pyrrolidino-1-cyclohexene) (225 µl, 1.4 mmoles) was added dropwise. The reaction mixture was maintained between -5°C and +5°C over 2 hours and then allowed to warm to room temperature over a 30 minute period. Intermediate compound 6 was then heated to 80°C for 4 hours to form ^{14}C-Sch 40120 7, and the mixture was allowed to cool to room temperature.

The resulting crude compound 7 was dissolved with ethyl acetate (ca. 10 mL in portions) and washed with 5 mL each of 1M hydrochloric acid, 1M sodium carbonate and brine. The ethyl acetate layer was then dried over anhydrous sodium sulphate, filtered and evaporated to dryness.

Crude ^{14}C-Sch 40120 was cleaned up by silica gel chromatography using 95: 5 methylene chloride: methanol as eluent. Further purification was performed by silica gel chromatography using 25: 75 *t*-butyl methyl ether: benzene as eluent. Finally after adjustment of the specific activity with unlabelled Sch 40120 and recrystallisation from isopropanol, 12mCi of ^{14}C-Sch 40120 7 was isolated (16% overall radiochemical yield) with a specific activity of 100 µCi/mg. The radiochemical purity as determined by hplc analysis in systems 1 and 2 was 99.5%.

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