Synthesis of (3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[4-^{3}H]carbazolepropanoic acid

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Summary

(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[4-^{3}H]carbazolepropanoic acid ([^{3}H]BAY u 3405) (5) was synthesized by catalytic reduction of (3R)-3-(4-fluorophenylsulfonamido)-4-oxo-1,2,3,4-tetrahydro-9-carbazolepropanoic acid (4) with tritium. The precursor (4) was prepared by esterification and following oxidation of BAY u 3405 with 2,3-dichloro-5,6-dicyano-p-benzoquinone. ^{3}H NMR analysis of the final product showed the formation of [4α-^{3}H]BAY u 3405 and [4ß-^{3}H]BAY u 3405 in a ratio of 1:1.

Keywords: tritiation, catalytic reduction, ^{3}H NMR, thromboxane antagonist

Introduction

(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoic acid (BAY u 3405) is a thromboxane A_2-receptor-antagonist [1]. The tritium labelled compound was required for receptor binding studies. This report describes our approach to the chemical synthesis and characterization of [^{3}H]BAY u 3405 (5).

Results and Discussion

(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[4-^{3}H]carbazolepropanoic acid (5) ([^{3}H]BAY u 3405) was prepared in the following way: BAY u 3405 (1) was esterified

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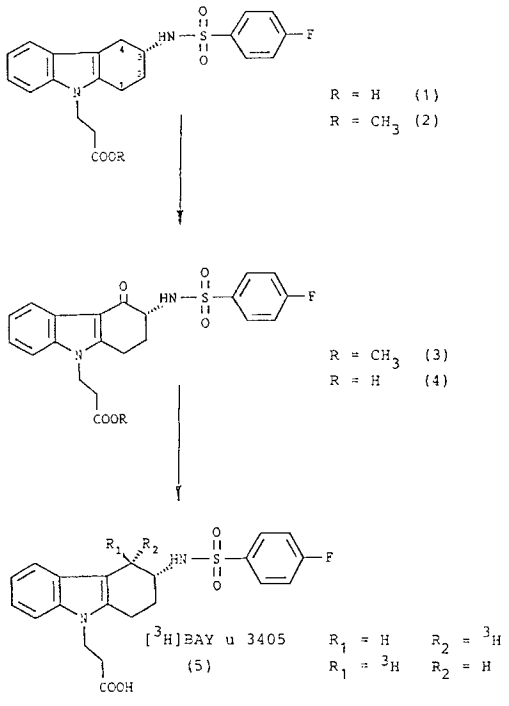
Scheme 1



by diazomethane yielding (2). Subsequently, (2) was oxidized with 2,3-dichloro-5,6-dicyano-p-benzoquinone to give compound (3). The oxidized product (3) was hydrolyzed by sodium hydroxide to yield the precursor (4) for the tritium labelling. Catalytic hydrogenation of (4) using palladium on charcoal as the hydrogenation catalyst and tritium afforded the desired [^{3}H]BAY u 3405 (5). In preliminary experiments using deuterium it was not possible to obtain a complete degree of conversion by varying the reaction conditions. Therefore, the reaction mixture had to be separated by HPLC after the tritiation. Before the purification, the labile tritium in the sulfonamide and carboxyl group was removed by dissolving the compound in ethanol and freeze-drying. This procedure was repeated several times until the condensate contained no more tritium. The specific activity was 7.9 Ci/mmol (292.3 GBq/mmol). This value is very low in comparison with the specific activity of 58.2 Ci/mmol (2.15 TBq/mmol) theoretically being possible. There are several reasons that the specific activity is lower than expected. The two labile hydrogen positions (sulfonamide and

carboxyl group) are rapidly exchanged by tritium. This has the effect of reducing the specific activity of the hydrogenating gas mixture and consequently the specific activity of the final product decreases [2]. On the other hand traces of moisture in the solvent cause the same effect.

A tritium NMR spectrum was run of the [^{3}H]BAY u 3405 (5) for the determination of the position and distribution of the tritium in the labelled drug. This technique has proven to be a very powerful tool in the analysis of tritiated drugs [3]. The two diastereotopic protons in the 4R and 4S position show a different chemical shift in the ^{1}H NMR spectrum. Four signals of an AB-system should be expected in a broad-band ^{1}H decoupled ^{3}H NMR spectrum of ditritiated BAY u 3405. But only two singlets at 2.446 ppm and 2.673 ppm were observed in the ^{3}H NMR spectrum of [^{3}H]BAY u 3405 (5) (see fig. 1) prepared by the described approach. This pattern can only result from a monotritiated [^{3}H]BAY u 3405 (5), one part labelled in the 4α position and the other part labelled in the 4ß position. Integration of the NMR signals shows a ratio 4α-^{3}H to 4ß-^{3}H of 1:1 (see fig. 1). According to the unexpected distribution of tritium, it can be supposed that the enantiomeric alcohols (4α)-OH and (4ß)-OH were chiefly formed and reacted finally to the [^{3}H]BAY u 3405 (5).

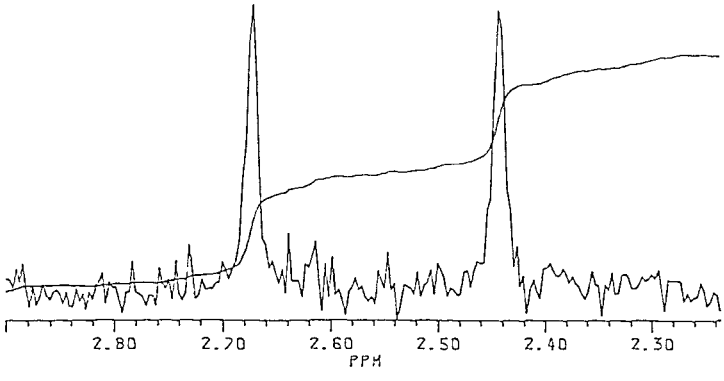


Fig. 1 Broad-band ^{1}H decoupled ^{3}H NMR spectrum of [^{3}H]BAY u 3405 (5)

Experimental Section

The tritiation reaction was carried out at Amersham International plc, Cardiff, U.K., by tritium labelling service. ^{3}H NMR

spectra with broad band ^1H decoupling were recorded on a Bruker AM 300 spectrometer operated at 320.136 MHz. Chemical shifts are referred to "ghost" tetramethylsilane, the value of which was obtained by multiplying the ^1H frequency of tetramethylsilane by 1.06663975.

For the analytical HPLC a Varian-Chromatograph model 5060 (Varian, Darmstadt, FRG) equipped with a radioactivity detector Ramona 4 (Raytest, Straubenhardt, FRG) was used. Radioactivity of liquid samples was measured on a LS-counter PW4700 (Philips, Netherlands).

Methyl-(3R)-3-(4-fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoate (2)

An etherial diazomethane solution was dropped to 2.0 g (4.8 mmol) BAY u 3405 (1) dissolved in 2 ml ether and 1 ml methanol until the yellow colour remained. The reaction mixture was stirred for 15 minutes and evaporated under reduced pressure to obtain 2.06 g (yield 100 %) of methyl-(3R)-3-(4-fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoate (2).

Methyl-(3R)-3-(4-fluorophenylsulfonamido)-4-oxo-1,2,3,4-tetrahydro-9-carbazolepropanoate (3)

2.06 g (8.7 mmol) 2.3-Dichloro-5,6-dicyano-p-benzoquinone dissolved in 36 ml tetrahydrofuran and 4 ml water were dropwise added under ice cooling and nitrogen atmosphere to a solution of 2.06 g (4.8 mmol) methylester (2) in 90 ml tetrahydrofuran and 10 ml water. The reaction mixture was stirred for 8 hours at room temperature and then diluted with 240 ml water. Overnight a white substance precipitated. The precipitate was filtered off and recrystallized from 2-propanol yielding 1641 mg (3.7 mmol) methyl-(3R)-3-(4-fluorophenylsulfonamido)-4-oxo-1,2,3,4-tetrahydro-9-carbazolepropanoate (3) (yield: 77 %), melting point 208 °C under decomposition. ^1H NMR (DMSO-d_6) δ (ppm) = 2.05 (m, 1H); 2.25 (m, 1H); 2.83 (t, 2H); 3.15 (m, 2H); 3.57 (S, 3H); 4.05 (m, 1H); 4.42 (t, 2H); 7.15 - 7.30 (m, 2H); 7.40 (m, 2H), 7.57 (m, 1H); 7.90 - 8.05 (m, 4H).

(3R)-3-(4-Fluorophenylsulfonamido)-4-oxo-1,2,3,4-tetrahydro-9-carbazolepropanoic acid (4)

1641 mg (3.7 mmol) methylester (3) were stirred with 80 ml ethanol and 40 ml 1 M sodium hydroxide solution for 2 hours at room temperature. The pH was adjusted to 1.0 with 1 M hydrochloric acid. The reaction mixture was diluted with 300 ml water and extracted 3 times with 50 ml ether. The ether was removed in vacuo to obtain 1018 mg (2.4 mmol) (3R)-3-(4-fluorophenylsulfonamido)-4-oxo-1,2,3,4-tetrahydro-9-carbazolepropanoic acid (4) (yield: 64 %), purity: approx. 99 % by TLC (silica gel plates, solvent: dichloromethane/ methanol 7:1, R_f = 0.22).

(3R)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-[4-^{3}H]carbazolepropanoic acid, [^{3}H]BAY u 3405, (5)

20 mg (46.5 µmol) precursor (4) in 0.5 ml dry tetrahydrofuran and 75 mg palladium on charcoal (10 % Pd) were stirred magnetically in a 25 ml vessel under a 50 Ci tritium gas atmosphere for 18 hours at room temperature. After reabsorption of tritium gas, the catalyst was separated by filtration and carefully washed twice with 2 ml ethanol. The filtrates were combined and lyophilized. The labile tritium was removed by repeated dissolving in 2 ml ethanol followed by lyophilisation. The product was dissolved in 4 ml acetonitrile/water 1:1 and purified by HPLC (column Lichrosorb^R RP18, 7 µm, 250 x 10 mm, Merck, Darmstadt FRG, eluent acetonitrile/water 1:1, flow rate 5 ml/min; detection UV 225 nm) yielding 140 mCi (5.18 GBq) of pure [^{3}H]BAY u 3405 (> 97 % by HPLC). [^{3}H]BAY u 3405 (7.9 Ci/mmol, 292,3 GBq/mmol): ^{3}H NMR (DMSO-d_6) δ 2.446 (s, CHT) and 2.673 (s, CTH).

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