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Investigation on the synthesis of 25-hydroxycholesterol

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ABSTRACT

A very efficient and environmentally benign method has been developed for the synthesis of 25-hydroxycholesterol. The reaction was performed in THF–water (4:1, v/v) using NBS as the brominating agent, followed by the easy reduction of C–Br with lithium aluminum hydride in THF, to yield the final product corresponding to a Markovnikov's rule. Excellent yields and regioselectivity have been obtained.

1. Introduction

Vitamin D_3 is an important biological regulator of calcium and phosphorus metabolism [1]. It is now established that the parent vitamin D_3 is sequentially metabolized in various tissues to the steroid hormone 1,25-$(OH)_2$-D_3 which exerts the highest biological activity of all vitamin D_3 metabolites. This hormonal derivative stimulates the intestinal absorption of calcium and phosphorus, and the mobilization of bone calcium through a target organ receptor mediated mechanism [2]. A common characteristic feature of these metabolites is the C-25 hydroxy group. Thus, the introduction of 25-hydroxy group into an appropriate substrate would be a key step in the synthesis of these compounds.

Using *in situ* generated ethyl(trifluoromethyl)dioxirane (ETDO), a facile synthesis was developed by Ogawa et al. [3] for 25-hydroxycholesterol, as well as its 3-sulfate (overall yield of the sulfate, 24%) and 24-oxocholesterol (16%), starting from cholesterol. However, long linear synthetic route and low yields are major hitches. Unlike cholesterol, the conventional starting material for preparing certain steroids (for example 25-hydroxycholesterol), desmosterol already contains a reactive side chain (Δ^{24}). Desmosterol plays an important role, as a labile intermediate, in the biosynthesis of cholesterol in animals. It was included in a filtrate of recrystallization of crude lanolin which was made from lanolin alcohol obtained by saponification of wool grease, a washing waste of wool, and the content of desmosterol reached 10–25% [4,5].

The reaction of mercuric acetate with desmosterol leads to the addition on the double bond of the groups –OH on one side, and –HgOAc on the other. It can be followed by the easy reduction of the C–Hg bond with sodium borohydride in sodium hydroxide/water, to yield the 25-hydroxycholesterol corresponding to a Markovnikov addition of water on the double bond. The nuclear Δ^5 double bond, which is quite reactive towards most electrophilic reagents, was left untouched. This remarkable selectivity has been confirmed in 1992, but this study has not been extended. Mercuric acetate is an environmental problem, obviously because of the poisonous nature of the reagent and of the products of the reaction. Care must be taken, even when working with small amounts, during the reaction and for the disposal of the residues [6].

The vicinal functionalization of carbon–carbon double bond is a powerful synthetic tool for organic chemists. In particular, selective introduction of two different functional groups, such as hydroxyl and halogen, has attracted sustained attention in organic synthesis [7]. Halohydrins are usually prepared *via* the ring opening of epoxides using hydrogen halides or metal halides. These procedures are associated with the formation of byproducts such as *vic*-dihalides and 1,2-diols. Meanwhile, these procedures require prior synthesis of epoxide. Apart from this, there are two general approaches for heterolytic addition of water and halogen to an olefinic bond. One involves the usage of molecular halogen, $TsNBr_2$, [8] N-halosaccharin [9] or N-halosuccinimide [10–23] for halogenation, and the other employs metal halide along with an oxidizing agent [24,25]. Cheap and available N-halosuccinimide, in particular N-bromosuccinimide (NBS) and N-chlorosuccinimide (NCS), are the better choice of halogen sources over other hazardous reagents for such transformations.

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From these points of view we have undertaken the syntheses of the 25-hydroxycholesterol using inexpensive reagents and available steroid starting compound. We were able to develop a facile synthesis of naturally occurring oxysterols, 25-hydroxycholesterol (**1**), from desmosterol (**2**) by using N-halosuccinimide *via* halohydrin reaction. Then, the reductive of halides is achieved by lithium aluminum hydride ($LiAlH_4$) in THF (Scheme 1). To the best of our knowledge, there are no examples describing the formation of 25-hydroxycholesterol *via* halohydrin reaction.

2. Experimental

Melting points were determined using WRR melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on Bruker AV-400 spectrometer (Bruker Corporation, America) at working frequencies 400 and 100 MHz. respectively in $CDCl_3$ And with TMS as the internal standard. Chemical shifts are expressed in ppm downfield from TMS and observed coupling constants (*J*) are given in Hertz (Hz). Starting materials and reagents were commercially purchased and used without further purification. The progress of the reactions was monitored by thin-layer chromatography (TLC) Analytical thin-layer chromatography (TLC) was conducted using silica gel plates (200 μm) containing a fluorescent indicator (silica gel 60 F_{254}). Detection was performed by spraying with molybdophosphoric acid (5%) at 120 °C Column chromatography was performed using silica gel, 200–300 mesh, and elution was performed with *n*-hexane/ethyl acetate.

2.1. General procedure for the synthesis of desmosterol acetate 3

To a solution of the desmosterol (20 g, 0.05 mol) in hexane (150 mL), DMAP (200 mg) and acetic anhydride (10 g, 0.1 mol) were added, after stirring at 50 °C in 3 h (TLC control, TLC solvents: *n*-hexane/EtOAc (8:1, v/v)), the reaction mixture was successively washed with water, HCl solution (5%wt.) and saturated $NaHCO_3$ solution. Desmosterol acetate (18.85 g, 85.0%) was obtained by evaporating in a vacuum and recrystallization in EtOH.

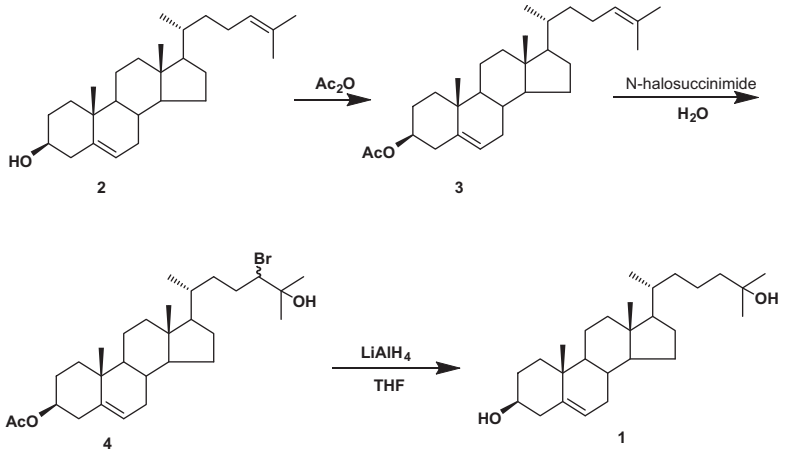
3 [26]: mp: 89.1–90.1 °C (lit. Mp: 91–92 °C) ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (*d*, *J* = 4.0 Hz, 1H, 6-CH), 5.10 (*t*, *J* = 6.4 Hz, 1H, 24-CH), 4.60 (*m*, 1H, 3-CH), 1.61 (*s*, 3H, 26-CH_3), 1.53 (*s*, 3H, 27-CH_3), 1.01 (*s*, 3H, 19-CH_3), 0.86 (*d*, *J* = 6.5 Hz, 3H, 21-CH_3), 0.69 (*s*, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.52 (C-18), 18.31 (C-21), 19.29 (C-19), 19.97 (C-23), 21.68 (C-11), 22.12 (–CO<u>C</u>H_3), 24.95 (C-27 and C-28), 25.37 (C-15), 26.40 (C-16), 28.42 (C-2), 32.52 (C-7 and C-8), 36.27 (C-20), 36.70 (C-22), 37.23 (C-10), 37.64 (C-1), 38.77 (C-12), 40.36 (C-4), 42.98 (C-13), 50.66 (C-9), 56.69 (C-17), 57.31 (C-14), 74.64 (C-3), 123.31 (C-6), 125.88 (C-24), 131.59 (C-25), 140.29 (C-5), 171.23 (–<u>C</u>OCH_3).

2.2. General procedure for the synthesis of bromohydrins 4

To a well-stirred solution of desmosterol acetate **3** (0.427 g, 1 mmol) in THF–water (4:1) (50 mL), NBS (0.213 g, 1.2 mmol) was added, and the reaction mixture was allowed to stir at −10 °C. Progress of the reaction was monitored by TLC (TLC solvents: *n*-hexane/EtOAc (8:1, v/v)). After 2 h, 10% aqueous sodium thiosulfate was added to destroy the excess NBS. The reaction mixture was extracted with dichloromethane (3 × 20 mL) and successively washed with saturated $NaHCO_3$ solution (20 mL × 2) and saturated NaCl solution (20 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. Purification of the crude product by column chromatography on silica gel (200–300 mesh) with a mixture of *n*-hexane/EtOAc (8:1, v/v) as an eluent to give bromohydrins **4** (0.44 g, 85%).

In a large scale, to a well-stirred solution of desmosterol acetate **3** (4.27 g, 10 mmol) in THF–water (4:1) (300 mL), NBS (2.13 g, 12 mmol) was added, and the reaction mixture was allowed to stir at −10 °C. Progress of the reaction was monitored by TLC (TLC solvents: *n*-hexane/EtOAc (8:1, v/v)). After 4 h, 10% aqueous sodium thiosulfate was added to destroy the excess NBS. The reaction mixture was extracted with dichloromethane (3 × 300 mL) and successively washed with saturated $NaHCO_3$ solution (200 mL × 2) and saturated NaCl solution (200 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. Purification of the crude product by column chromatography on silica gel (200–300 mesh) with a mixture of *n*-hexane/EtOAc (8:1, v/v) as an eluent to give bromohydrins **4** (4.2 g, 80.8%).

4: mp: 148.7–149.9 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (*d*, *J* = 4.0 Hz, 1H, 6-CH), 4.60 (*m*, 1H, 25-OH), 2.68 (*m*. 1H, 24-CH), 1.31 (*s*, 3H, 26-CH_3), 1.27 (*s*, 3H, 27-CH_3), 1.01 (*s*, 3H, 19-CH_3), 0.94 (*d*, *J* = 6.5 Hz, 3H, 21-CH_3), 0.69 (*s*, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.76 (C-18), 18.56 (C-21), 19.20 (C-19), 20.90 (C-11), 21.34 (–CO<u>C</u>H_3), 25.30 (C-27 and C-28), 25.58 (C-15), 27.65 (C-16), 28.12 (C-2), 31.73 (C-23), 32.25 (C-7 and C-8), 35.55 (C-20), 36.47 (C-22), 36.87 (C-10), 38.00 (C-1), 39.60 (C-12), 42.23 (C-4), 49.87 (C-13), 55.80 (C-9), 56.56 (C-17), 58.05 (C-14), 64.71 (C-24), 64.84 (C-25), 73.87 (C-3), 122.50 (C-6), 139.53 (C-5), 171.23 (–<u>C</u>OCH_3).



Scheme 1. Synthesis of 25-hydroxycholesterol (**1**) with desmosterol (**2**) as starting compound.