A low toxicity method for the separation of lanosterol and dihydrolanosterol from commercial mixtures

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Abstract

We describe an inexpensive, low-toxicity and high-yielding method for the production of pure lanosterol and dihydrolanosterol from the commercially available mixture. Optimum conditions are presented for the one-pot production of the intermediate 24,25 vicinal diol of lanosterol acetate (via either epoxidation or hydroxyhalogenation) which is readily separated from the unreacted dihydrolanosterol acetate. The lanosterol diol can then be converted to pure (>97%) lanosterol. Hypophophorous acid was used for both the conversion of the epoxide to the diol, and as a catalyst for the hydroxyhalogenation by N-halosuccinimides of the olefinic bond.

Keywords: Lanosterol; Dihydrolanosterol; Hydroxyhalogenation; Dihydroxylation; Hypophosphorous acid

1. Introduction

Side chain derivatives of lanosterol (3β-hydroxy-5α-lanosta-8,24-diene, **1a**) have been shown to act as inhibitors of $\Delta^{24(25)}$ sterol methyl transferase, which is an essential enzyme in the sterol biosynthesis pathway of protozoa, fungi and plants [1]. Because of this activity these compounds have potential therapeutic applications. Significant chemopreventive activity of lanosterol against colon carcinogenesis in rat has been reported [2].

Pure lanosterol for bioassay or as a starting material for synthesis is expensive and the usual starting material for synthesis is a commercial "lanosterol" containing approximately 40-50% of dihydrolanosterol **2a**, as the major impurity as well as other steroids as minor impurities. Various methods of obtaining pure lanosterol have been reported in the literature but most are limited in their suitability for scaling-up either by their low yield [3-6] or the toxic or hazardous nature of the reagents involved [7].

We here report a technically simple method for the production of pure lanosterol in high yield without recourse to highly toxic reagents. Pure dihydrolanosterol, which is also of interest as a synthetic starting material [9] is also isolated. The method has evolved from our earlier development of techniques for efficient synthesis of epimerically pure side-chain derivatives of lanosterol [8].

2. Experimental

2.1. General

Lanosterol 50-60% was purchased from Sigma and used without any further purification. Hypophosphorous acid (50 wt.% in water) and all other reagents were purchased from Aldrich and used as such. Column chromatography was carried out on Merck silica gel (70-250 mesh) using the eluants indicated. TLC was carried out using Augram® Sil G/UV_{254} plates. All melting points were obtained on a Reichert-Jung micro-melting point determination apparatus and are uncorrected. Optical rotations were measured on an Optical Activity Ltd. automatic polarimeter.

Gas chromatography with flame ionisation detection (GC-FID) was carried out using a Hewlett-Packard Ultra 2 (25m x 0.33mm) crosslinked phenyl methyl silicone fused silica capillary column. Conditions used were 55°C (1 min), 30°C min^{-1} to 200°C, 4°C min^{-1} to 320°C.

Gas Chromatography with mass spectrometry was carried out using a Hewlett Packard HP 1 cross-linked methyl silicone fused silica capillary column interfaced with a Hewlett-Packard 5970 mass spectrometer (70eV). Conditions used were 200°C (1 min), 30°C min^{-1} to 260°C, 3°C min^{-1} to 295°C.

^{1}H and ^{13}C NMR spectra were obtained using a Bruker ADV DRX 400 MHZ spectrometer, in chloroform-*d* and referenced to chloroform.

*2.2. Acetylation of mixed lanosterols (**1a**+**2a**)*

This was carried out using commercial lanosterol (80g, 0,187 mol, 50-60% pure) as previously described [8] to give 3β-acetoxy-5α-lanost-8,24-diene (**1b**) and 3β-acetoxy-5α-lanost-8-ene (**2b**) as a colourless solid (72 g), mp 125-129°C.

*2.3. 24(R,S)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene (**4**)*

Compound **4** was prepared by two different routes - epoxidation and hydroxyhalogenation (hydroxy-bromide or hydroxy-iodide)

*2.3.1. Epoxide route to **4***

To a solution of the mixed lanosterol acetates **1b** and **2b** (20 g) (**1b** = 12.3 g, **2b** = 7.7 g by GC) in dichloromethane (600 mL) a mixture of *m*-chloroperbenzoic acid (70%) (5.5 g) and sodium hydrogen carbonate (2.2g, 0.05 mol) was added in following manner: half added at room

temperature over 3h and the remainder at 0°C (ice bath), also over 3 h. The mixture was stirred vigorously (1 h) and left in a refrigerator overnight. The reaction mixture was diluted with 2-propanol (500 mL), hypophosphorous acid (20 mL) and water (200 mL). Dichloromethane was removed by distillation at atmospheric pressure, an additional amount of hypophosphorous acid (10 mL) added and the mixture heated under reflux (2.5 h). The reaction mixture was poured into water (1.2 L) and the solid filtered and washed with water. Purification by short column chromatography (CH_2Cl_2) yielded dihydrolanosterol acetate, **2b** (7.3g, 95% recovery), m.p. 120-121°C (acetone-methanol, lit.[7] 120-122°C). NMR spectra were identical with the literature [12]. Further elution with ethyl acetate afforded a stereoisomeric mixture of the diol, 24(R,S)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene, **4 ,** as colourless needles (10.7 g, 82%), m.p. 173-176°C (lit. [10] 166-169°C or [11] 183-185°C; the melting point depends on proportions of R and S epimers [8]). Anal. Calcd for $C_{32}H_{54}O_4$: C: 76.44, H: 10.83. Found C: 76.52, H: 10.84. NMR spectra were assigned by comparison with NMR data for the individual R and S epimers [8] and gave: ^{1}H NMR δ 0.98 (3H, s, 19-CH_3), 0.67 (3H s, 18-CH_3), 1.18 (3H, s, 26-CH_3), 1.13 (3H, s, 27-CH_3), 2,02 (3H, s, 3β-$OCOCH_3$), 1.10-1.36 (2H,m, 23-CH_2), 3.32 [1H, d, 24-CH (R-24 hydroxy) *J*=6.1 & 6.1 Hz], 3.26 [1H, d, 24-CH (S-24 hydroxy) *J*=2.0 & 10.0 Hz; ^{13}C NMR δ (R,S): C-16 (28.59, 28.48), C-17 (50.91, 50.83), C-20 (36.60, 37.06), C-21 (18.86, 19.14), C-22 (33.45, 33.90), C-23 (28.73, 29.03), C-24 (79.05, 79.22), C-25 (73.45, 73.50), C-26 (26.85, 26.82), C-27 (23.61, 23.53).

*2.3.2. Bromohydroxy route to **4**.*

To a solution of lanosterol acetates **1b** and **2b** (10 g) (**1b** = 6.15 g, **2b** = 3.85 g by GC) in acetone (900 mL), water (15 mL) and hypophosphorous acid [2.7 mL (50% in water)] was added *N*-bromosuccinimide (3.04 g, 0.017 mol). The reaction mixture was stirred at room

temperature for 5 min., $NaHCO_3$ (3 g) was added and the mixture concentrated under vacuum. The residue was dissolved in 2-propanol (300 mL), water (100 mL) and hypophosphorous acid (7.2 mL, 50% in water). More $NaHCO_3$ (4.5 g) was added, and the reaction mixture was refluxed (4 h), diluted with water, filtered and washed until neutral. Separation by flash column chromatography (dichloromethane) yielded dihydrolanosterol acetate, **2b** , (3.3 g, 85%), m.p. 119-120°C (twice from acetone; lit.[7] 120-122°C). NMR spectra were as reported [12]. Further elution with ethyl acetate afforded a stereoisomeric mixture of the diol, 24(R,S)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene, **4 ,** as colourless needles (5.8 g, 88%), m.p. 172-175°C (Lit. [10] 166-169°C or [11] 183-185°C, depending on epimeric composition).

*2.3.3. Iodohydroxy route to **4**.*

The reaction was carried out as described in 2.3.2 with *N*-iodosuccinimide in place of *N*-bromosuccinimide to give **2b** , (3.3 g, 81%), m.p. 119-120°C (twice from acetone) and **4** (5.6 g, 86%) m.p. 170-174°C.

2.4. *Conversion of 24(R,S)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene, **4**, to lanosterol acetate, **1b**.*

To a solution of 24(*R,S*)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene (**4**, 10 g, 0.0199 mol) in dichloromethane (150 mL) was added *N,N*-dimethylformamide dimethylacetal (13.2 mL, 0.597 mol) and the mixture was refluxed (2.5 h). The reaction mixture was cooled, acetic anhydride (20 mL) was added and the dichloromethane was distilled under reduced pressure. An additional amount of acetic anhydride (100 mL) was added and the mixture containing acetal **6** was refluxed at 130°C (3.5 h), cooled, poured into ice-water and the solid filtered and washed until neutral to yield a light brown powder. The crude product after chromatography through

silica gel (eluent dichloromethane) afforded lanosterol acetate, **1b**, (8.3 g, 90%) as a white powder (97% purity by GC), m.p. 129.5-131.5°C $[\alpha]_D$ = + 58.6 (c 1.16, $CHCl_3$). [Lit [6,7] m.p.=129-130°C, $[\alpha]_D$ = + 57.1° (c 1.1, $CHCl_3$) or m.p. = 129-130°C, $[\alpha]_D$ = +59.0° (c 1.0 $CHCl_3$). NMR spectra matched those in the literature [13]. Purification by recrystallisation at this stage resulted in poor recovery of material, so the crude product was used for the next stage.

2.5.*Conversion of lanosterol and dihydrolanosterol acetates (**1b** and **2b**) to the corresponding hydroxy derivatives (**1a** and **1b**)*

Lanosterol acetate (**1b**, 5 g) was hydrolysed with 10% potassium hydroxide in ethanol (150 mL) at 60°C for 2 h. The resulting mixture was poured into ice water and after standing 6-7 h was collected by filtration, dried and recrystallised from methanol-benzene to yield 3β-hydroxy-5α-lanosta-8,24-diene (**1a**, 4.2 g, 92%), m.p. 139-140°C, (Lit. [6] 140°C). NMR was identical with literature [13]. GC-MS analysis indicated a purity of 98% lanosterol with the major impurity being agnosterol (2%).

Similar treatment of dihydrolanosterol acetate (**2b**) afforded 3β-hydroxy-5α-lanosta-8-ene (**2a**, 4.25 g, 93%), m.p. 144-145°C, (Lit. [9] 144-144.5°C). NMR was identical with literature [13]. GC-MS indicated 98.6% dihydrolanosterol and 1.4% of agnosterol.

3. Results and Discussion

Lanosterol has been purified by three different routes, all of which have in common the formation of 24(*R,S*)-3β-acetoxy-24,25-dihydroxy-5α-lanost-8-ene, **4**, **Scheme 1**. In the first stage, **Scheme 1**, the epimeric 24,25-epoxylanosterol acetate **3**, or 24-halo-25-hydroxylanosterol

acetates, **5a**,**5b**, were formed as a precursor to the diols, **4**. Separation from **2b** was effected by short column chromatography at the diol stage (a significant difference between the solubility of **2b** and **4** in organic solvents makes this process very easy for large scale production). The mixture of epimeric diols was then converted *via* the *N,N*-dimethylacetal, **6**, to lanosterol acetate, **1a**.

In the first method, epoxidation [7, 8] of acetylated commercial lanosterol (mixture of **1b** and **2b** in the ratio 61.5:38.5) with *m*-chloroperbenzoic acid afforded a mixture of the epimeric 24,25-epoxylanosterol acetates, **3**, which were not isolated. Upon refluxing in 2-propanol and aqueous hypophosphorous acid the epoxide, **3**, afforded the mixed epimers of 24,25-dihydroxylanosterol acetate, **4**. 2-Propanol was chosen as the organic solvent to afford miscibility with water without competing as a nucleophile and thereby forming unwanted ethers.

Alternatively, treatment of acetylated commercial lanosterol (**1b** and **2b**) with *N*-bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS) in aqueous acetone in the presence of a catalytic amount of hypophosphorous acid afforded a mixture of the corresponding 24(*R,S*)-halo-25-hydroxylanosterol acetates **5a** and **5b** (c.f. [8]). These were converted directly to the mixed epimeric diol, **4**, by refluxing in 2-propanol with aqueous hypophosphorous acid and its sodium salt, which was generated *in situ* by addition of $NaHCO_3$ to the reaction mixture.

Hypophosphorous acid (phosphinic acid, H_3PO_2) is cheap and readily available, and its residues are environmentally benign. The remarkable features of the effect of the hypophosphorous acid are the rapidity of the hydroxyhalogenation reaction, which is complete in 5 minutes and the high yield of diols obtained, which indicates a very high yield in the preceding hydroxyhalogenation step.

The diol, **4**, was readily converted to lanosterol acetate, **1b**, *via* the *N,N*-dimethylacetals, **6**, [14]. Assay of **1b** before crystallisation indicated about 97% purity (by GC). Hydrolysis of the

acetate **1b** generated lanosterol with >98% purity, with agnosterol being the main remaining contaminant.

The overall four-step process provides pure lanosterol and dihydrolanosterol from the commercial mixture, with better than 90% yields at each stage. This new method of separation compares favourably with the yield obtained by Rodewald and Jagodzinski [7] using environmentally unacceptable mercury (II) acetate and $LiAlH_4$ and is a significant improvement on other yields quoted in the literature [3-6].

We believe that one pot dihydroxylation reaction of the 24,25-double bond of lanosterol through hydroxyhalides could be successfully applied to other olefins as well.

Conclusion

A simple, inexpensive and low toxicity route is described for the purification of lanosterol from mixtures of lanosterol and dihydrolanosterol. The method makes use of two different routes to a common intermediate 24,25-dihydroxylanosterol acetate, which is then readily converted to lanosterol. High yields are made possible by the use of hypophosphorous acid as a reagent for the ring-opening of the intermediate epoxides, or as a catalyst for the hydroxyhalogenation.

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