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A concise synthesis of β-sitosterol and other phytosterols

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ABSTRACT

A convenient synthesis of sidechain-modified phytosterols is achieved via a temporary masking of the stigmasterol 5,6-alkene as an epoxide. Following performance of the desired modification, the alkene is regenerated through a mild deoxygenation. The approach is applied to the syntheses of β-sitosterol and campesterol acetate, and suggests a facile route to the (*Z*)-isomers of Δ^{22-23} phytosterols.

1. Introduction

Phytosterols and their derivatives are widely applied in the food and cosmetic industries, and have recently received a great deal of attention as nutraceutical additives [1–3]. Phytosterols have also attracted attention as inhibitors of sarcoplasmic reticulum calcium ATPase and potassium ion channels [4,5]. As part of a collaboration investigating the structural influences on uptake and processing of sterol esters [6], we required semipreparative amounts of β-sitosterol. However, β-sitosterol is commercially available in preparative amounts only as mixtures with other phytosterols, including stigmasterol, campesterol, and/or brassicasterol (Fig. 1); reported separations are relatively laborious [7,8].

Two routes have been reported for the synthesis of β-sitosterol from stigmasterol, which is available in pure form. The first, selective hydrogenation of the sidechain Δ^{22-23} alkene [9], was found to produce β-sitosterol contaminated with varying amounts of recovered stigmasterol as well as the fully saturated stigmastanol [10]. The second approach, which has been applied to the synthesis of sitosterol and related sterols (Fig. 2), circumvents the need for selective hydrogenation by protecting the Δ^{5-6} alkene as a cyclopropyl carbinyl ether [11,12]. Following hydrogenation of the Δ^{22-23} double bond, solvolysis of the cyclopropane reintroduces both the C_3-alcohol and the Δ^{5-6} alkene. Although we found the latter approach very useful as a means of obtaining very pure samples of β-sitosterol, semipreparative applications were challenging in terms of removal of sterol methyl ether byproducts.

We now report a new strategy for the synthesis of sidechain-modified phytosterols based upon protection of the Δ^{5-6} alkene as an epoxide. The approach is illustrated with syntheses of β-sitosterol and campesterol acetate.

2. Experimental

2.1. General experimental procedures

AlI_3 and $Cu(MnO_4)_2$ were prepared by literature procedures [13,14]. All other reagents and solvents were used as supplied commercially, except CH_2Cl_2 (CaH_2) and THF (Na, Ph_2CO) which were distilled from the indicated reagent under an atmosphere of N_2. Melting points are uncorrected. Unless noted, NMR spectra were acquired at 400 MHz (^1H) or 100 MHz (^{13}C) in $CDCl_3$; individual peaks are reported as: multiplicity, integration, coupling constant in Hz. IR spectra were recorded as neat films on a ZnSe crystal with selected absorbances reported in cm^{-1}. Mass spectroscopy was conducted at the Nebraska Center for Mass Spectrometry.

2.2. Stigmasterol acetate (**2**)

Stigmasterol acetate was prepared as a white solid (97%, mp 138–140 °C) by a variant of the procedure of Wang et al. [15]. Other physical and spectral data were identical to literature values.

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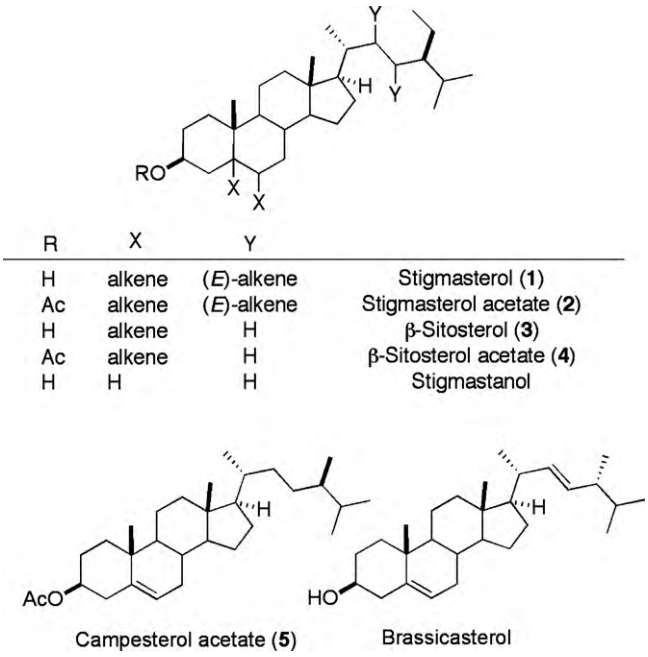

Fig. 1. Structural relationship of phytosterols.

2.3. 5α,6α- and 5β,6β-Epoxides of stigmasterol acetate (**6a**, **6b**)

A mixture of $KMnO_4$ (10 g, 60 mmol) and $CuSO_4{\cdot}5H_2O$ (5.0 g, 20 mmol) was finely ground in a mortar and pestle [14]. Water (0.5 mL) was added, and the slightly wet mixture was transferred to the reaction flask. To the stirred suspension of this mixture in 25 mL CH_2Cl_2 was added stigmasterol acetate (**2**, 2.12 g, 4.51 mmol), followed by *t*-BuOH (2.5 mL). The reaction was heated to reflux for 1 h and cooled to room temperature. The reaction mixture was filtered through a silica pad, which was washed with ether. The residue obtained after concentration was recrystallized from CH_3OH to give a white solid (1.59 g, 75%) with mp 125–126 °C. NMR data indicated the product was a 1:6 mixture of the α- (**6a**) and β- (**6b**) epoxides of stigmasterol acetate [14]. Repeating this reaction with 2.23 g of stigmasterol acetate afforded 1.80 g (78%) of a 1:6 mixture of **6a** and **6b**.

Approach to 5α,6α- and 5β,6β-epoxides of stigmasterol acetate (**6a**, **6b**) via peracid epoxidation: to a 0 °C solution of **2** (0.308 g, 0.66 mmol) in CH_2Cl_2 (20 mL) was added mCPBA (0.170 g, 0.76 mmol). After 4 h at 0 °C, the reaction was diluted with sat. aq. K_2CO_3 (80 mL) and the aqueous layer was extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (hexane/EtOAc, 90:10) to afford 0.257 g (83%) of a white solid which was a 2.6:1 mixture of α- (**6a**) and β-isomers (**6b**) according to ^1H NMR.

2.4. 5α,6α- and 5β,6β-Epoxy sitosterol acetate (**7a**, **7b**)

To a solution of the 1:6 mixture of **6a** and **6b** (1.35 g, 3.0 mmol) in EtOAc (150 mL) was added 10% Pd/C (0.32 g), and the stirred reaction mixture was placed under an atmosphere of H_2 (balloon) for 12 h. The reaction mixture was filtered through a Celite pad, and the filtrate evaporated to furnish white solid (1.28 g, 96%, mp 113–114 °C) as a 1:6 mixture of epoxides **7a** and **7b** [8]. Repeating this reaction on 1.76 g of **6a**/**6b** afforded 1.75 g (99%) of a 1:6 mixture of **7a** and **7b**.

2.5. β-Sitosterol acetate (**4**)

The 1:6 mixture of epoxides **7a** and **7b** (470 mg, 1.0 mmol) was dissolved in 2:1 CH_3CN/CH_2Cl_2 (30 mL). Aluminum triiodide was added (610 mg, 1.5 mmol) and the resulting mixture was stirred at room temperature for 10 min. The reaction was quenched with aq. 10% $Na_2S_2O_3$ (100 mL) and the resulting mixture was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried over Na_2SO_4, and the residue from the concentrated filtrate was purified by flash chromatography (hexane/EtOAc, 95:5) to give 360 mg (80%) of **4** as a white solid: mp 111–112 °C, $[\alpha]_D = -34.5$ ($CHCl_3$, $c = 1.0$). Other physical data were identical to values reported in the literature [11]. Repeating this reaction on 1.70 g of **7a**/**7b** afforded 1.40 g (85%) of **4**.

2.6. β-Sitosterol (**3**)

To a solution of β-sitosterol acetate (**4**, 240 mg, 0.47 mmol) in 1:1 CH_3OH:CH_2Cl_2 (30 mL) was added K_2CO_3 (140 mg, 1.01 mmol). The reaction mixture was stirred at room temperature for 12 h and then concentrated under vacuum. The residue was extracted with 30 mL CH_2Cl_2. The organic layer was washed with 30 mL water and dried over Na_2SO_4. The filtered organic layer was concentrated and the residue was purified through flash chromatography (hexane/EtOAc, 80:20) to give 220 mg (93%) of β-sitosterol **3** as a white solid. Mp 134–135 °C, $[\alpha]_D = -37.0$ ($CHCl_3$, $c = 1.0$). Elemental analysis calculated for $C_{29}H_{50}O$: C 83.60, H 11.96; found: 83.99, 12.15. Other spectral properties were identical to values reported in the literature [11]. Repeating this reaction on 1.35 g of **4** afforded 1.20 g (98%) of beta sitosterol (**3**).

2.7. (*S*)-2,3-Dimethylbutan-1-ol (**8**)

(*S*)-2,3-Dimethylbutan-1-ol **8** was prepared as a colorless liquid (overall yield 60%, $[\alpha]_D = 4.4$, $CHCl_3$, $c = 1.0$) by the procedure of Tietze, affording a product with spectral data identical to literature values [16].

2.8. (*S*)-2-(2,3-Dimethylbutylthio)benzothiazole (**9**)

To a mixture of dimethylbutanol **8** (102 mg, 1.00 mmol), 2-mercaptobenzothiazole (183 mg, 1.10 mmol) and PPh_3 (288 mg, 1.10 mmol) in freshly distilled THF (4 mL) was added diisopropyl

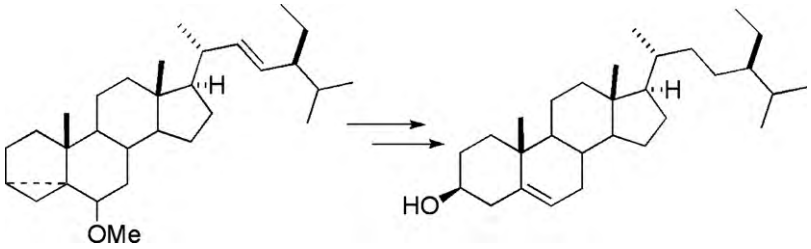

Fig. 2. Selective saturation of cyclopropyl carbinyl ether.