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# Synthesis of an isomeric mixture (24RS,25RS) of sodium scymnol sulfate

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ABSTRACT

This is the first reported multistep synthesis of the shark bile sterol sodium scymnol sulfate epimeric at the C-24 hydroxyl and C-27 sulfate positions. The starting cholic acid was protected as the tetrahydropyran ether (THP) derivative, reduced to the C-24 alcohol and oxidized to the protected aldehyde. This aldehyde was then coupled with methyl 3-hydroxypropionate using 2 equiv. of lithium diethylamide at −65 °C to produce methyl (24RS,25RS)-24,27-dihydroxy-3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-oate. After protecting the 24 and 27 hydroxyls as the THP derivatives, this fully protected ester was then reduced to the monoalcohol. The monoalcohol was sulfated using the sulfur trioxide–triethylamine complex in dimethylformamide. The protective THP groups were removed with methanolic HCl and the sulfate was converted to the sodium salt with sodium ethoxide in methanol. This general synthetic scheme has application to produce a range of monosulfated sterols.



## 1. Introduction

The natural sodium scymnol sulfate has been purified by our group from shark bile by preparative HPLC for use in commercial skin care products [1]. We have investigated many of the wide-ranging biological properties of scymnol and its sulfate including its potent antioxidant [2,3] and hepatoprotective activities [4,5]. Ishida et al. have also investigated its protective properties against vascular endothelial cell injury [6–8].

The sodium scymnol sulfate isolated from shark bile contains the 24R alcohol substituent and either or both of the 27R and 27S sulfate esters [9] (see Fig. 1). Thus, as a further progression in the potential replacement of the natural product with a synthetic product, we designed the synthetic scheme shown in Fig. 2 to allow monosulfation at the C-27 position. This scheme leads to the (24RS,25RS) epimeric mixture of alcohols whereas the natural product comprises the (24R,24R) and/or (24R,25S) epimers. Our group had synthesized epimeric unsulfated (24RS) scymnol [10] previously. (24R) scymnol was synthesized subsequently by Adhikari et al. [11]. However a sulfated form of scymnol had not been previously synthesized.

## 2. Experimental

### 2.1. General

$^{1}$H NMR were carried out on samples purified from the crude reaction products by silica gel column chromatography using Bruker 300 and 400 MHz Avance Spectrometers using deuterochloroform (or other indicated solvents) as the reference or an internal deuterium lock. The multiplicity of the signals is indicated as s = singlets, m = multiplet and br = broad. $^{13}$C NMR spectra were recorded on an Avance 400 MHz instrument using an internal deuterium lock and

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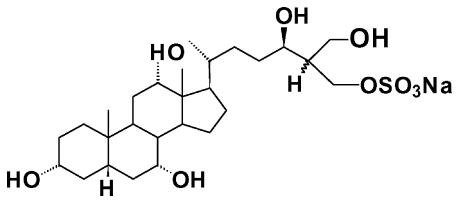


**Fig. 1 – (24R,25RS) sodium scymnol sulfate.**

proton decoupling. The chemical shifts are given as $\delta$ values (ppm) and referenced to tetramethylsilane for deuterochloroform and 2,2,3,3-d(4)-3-(trimethylsilyl)propionic acid sodium salt (TSP) for deuterium oxide ($D_2O$) and deuteromethanol ($CD_3OD$) as solvent. High resolution mass spectroscopy was carried out using a Bruker BioApex II FTICR electrospray in positive ion mode using ammonium acetate in acetonitrile and water mixture. Microanalysis was performed by the University of Otago, Dunedin New Zealand. Analytical TLC was carried out using precoated Merck Kieselgel 60 $F_{254}$ silica gel aluminium plates from E. Merck (Darmstadt, Germany). Visualization was performed by spraying with phosphomolybdic acid (5%, w/v) and sulfuric acid in glacial acetic acid (7.5%, w/v) followed by heating for 5 min at 110 °C. Column chromatography was performed with Merck Kieselgel 60 (230–400 mesh). Melting points are uncorrected. Reagents were purified by standard techniques. Tetrahydrofuran (THF) and diethyl ether (ether) were dried with sodium using benzophenone ketyl as the indicator. Petroleum spirits refers to the fraction boiling in the range 40–60 °C. The 3,4-dihydro-2*H*-pyran (DHP) was freshly distilled before use. All reagents were analytical grade and purchased from regular commercial sources. Cholic acid (3α,7α,12α-5β-cholan-24-oic acid) ≥99% was obtained from Fluka BioChemika (Buchs, Switzerland). The sulfur trioxide–triethylamine complex was prepared by the method of Tserng and Klein [12].

## 2.2. *Synthesis*

### 2.2.1. *Tetrahydropyran-2-yl-3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-oate (compound* **2***, Fig. 2)*

A mixture of cholic acid (16.42 g, 40 mmol) and *p*-toluenesulfonic acid (40 mg) in DCM (100 ml) was magnetically stirred for 10 min at 22 °C and then 3,4-dihydro-2*H*-pyran (17.5 g, 208 mmol, 1.3 equiv.) added in stages over 30 min. An exotherm raised the temperature to 39.5 °C and the insoluble cholic acid dissolved during the reaction. The reaction returned to 22 °C and after a further 3 h of stirring the crude product **2** was concentrated in a rotary evaporator under vacuum to yield a colourless oily residue (37.1 g) that was used in the next reaction after 1.5 h; $R_f$ 0.5 (ethyl acetate/petroleum spirits = 1/3). The product on standing yielded a second main spot at $R_f$ 0.15—presumably due to equilibration between the eight possible tetrahydropyran (THP) acetals. $^1$H NMR ($CDCl_3$) 0.63–0.75 (3H, m, H-18), 0.86–1.07 (6H, m, H-19, H-21), 3.32–4.22 (11H, m, H-3β, H-7β, H-12β, 4$CH_2O$), 4.54–5.01 (4H, m, 4OCHO). Positive ion high resolution MS: calculated for $C_{44}H_{72}O_9Na$ [$M + Na^+$]: 767.5074. Found: 767.5077.

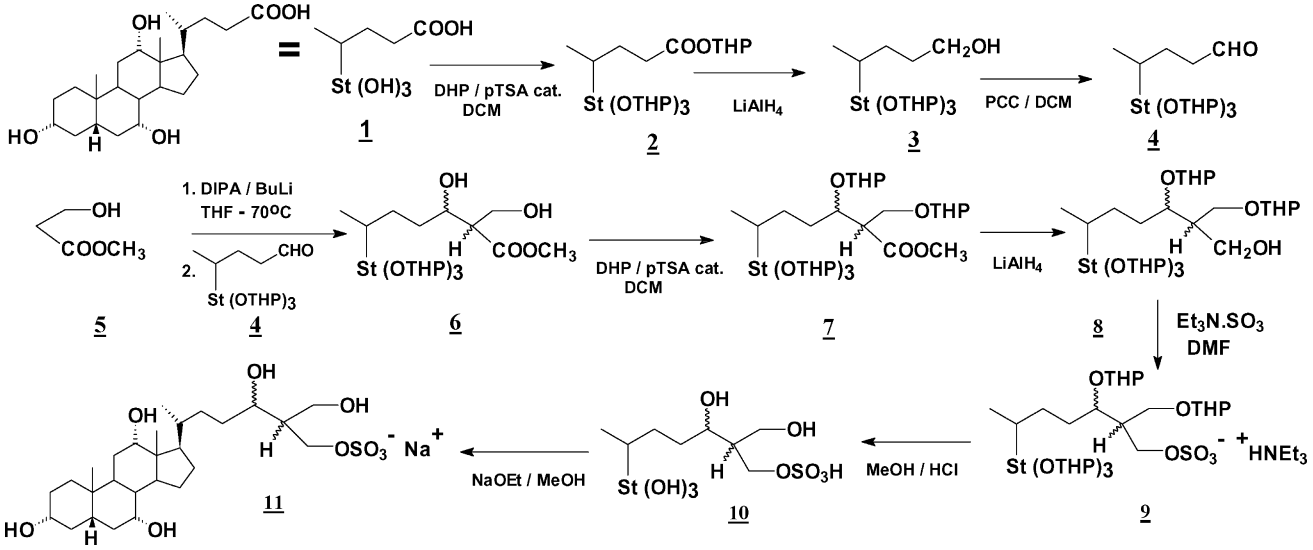


**Fig. 2 – (1) Cholic acid; (2) tetrahydropyran-2-yl-3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-oate; (3) 3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-ol; (4) 3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-al; (5) methyl 3-hydroxypropionate; (6) methyl 24,26-dihydroxy-3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-oate; (7) methyl 3α,7α,12α,24,26-penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-oate; (8) 3α,7α,12α,24,26-penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-ol; (9) (24RS,25RS)-3α,7α,12α,24,26-penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-27-yl triethylammonium sulfate; (10) (24RS,25RS)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sulfonic acid; (11) (24RS,25RS)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate.**

2.2.2. *3α,7α,12α,Tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-ol (compound* **3**, *Fig. 2)*

A dry flask was charged with dry ether (120 ml) and dry THF (25 ml) containing crude compound **2** (37.1 g). The mixture at 22 °C was mechanically stirred under dry nitrogen and $LiAlH_4$ (50 ml, 1 M in ether) added over 10 min. During this time the spontaneous reflux was moderated with room temperature water bath cooling. After further reflux heating of 1.5 h the resulting clear solution was cooled and ethyl acetate (17.5 ml) was added over 7 min. Reflux heating was recommenced for a further 15 min, after which water (3.5 ml) was added drop-wise. The reaction was cooled to room temperature and the resulting precipitate was stirred for another 30 min. The reaction mixture was filtered through a bed of Celite and washed with ether (300 ml). The crude product was concentrated in a rotary evaporator under vacuum, with toluene (6 ml) being added towards the end to azeotrope out residual water. The crude title compound **3** was isolated as a colourless oil (27.2 g). $R_f$ 0.4 (ethyl acetate/petroleum spirits = 4/6); $^1$H NMR ($CDCl_3$) 0.62–0.75 (3H, m, H-18), 0.84–1.05 (6H, m, H-19, H-21), 3.33–4.23 (11H, m, H-3β, H-7β, H-12β, 2H-24, $3CH_2O$), 4.55–4.97 (3H, m, OCHO).

2.2.3. *3α,7α,12α,Tris[(tetrahydropyran-2-yl)oxy]-5β-cholan-24-al (compound* **4**, *Fig. 2)*

A mixture of of pyridinium chlorochromate (15.09 g, 70 mmol) and anhydrous sodium acetate (1.15 g, 14 mmol) in DCM (80 ml) was mechanically stirred at 22 °C while crude oil **3** (27.2 g) in DCM (10 ml) was added in one lot. After an initial exotherm, the reaction returned to 22 °C and after 3.5 h dry ether (80 ml) was added, stirring continued for another 30 min and finally the solvent was decanted. The resulting tarry residue was triturated with dry ether (2× 20 ml) and the solvent washes decanted. The reaction mixture was concentrated in a rotary evaporator under vacuum to yield the crude product (31.4 g). Purification of the crude product by column chromatography with silica gel using stepwise elution with ether in petroleum spirits increasing from 5 to 50%, yielded a colourless oily residue of compound **4** (11.12 g, 17.24 mmol) after rotary evaporation under vacuum. This product represented a yield of 46.6% from cholic acid. $R_f$ 0.5 (ethyl acetate/petroleum spirits = 1/3); $^1$H NMR ($CDCl_3$) 0.61–0.72 (3H, m, H-18), 0.81–1.05 (6H, m, H-19, H-21), 3.34–4.20 (9H, m, H-3β, H-7β, H-12β, $3CH_2O$), 4.59–4.81 (3H, m, OCHO), 9.75 (1H, s, H-24).

2.2.4. *Methyl-(24RS,25RS)-24,27-dihydroxy-3α,7α,12α,tris[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-oate (compound* **6**, *Fig. 2)*

A solution of diisopropylamine (3.83 g, 37.8 mmol) in dry THF (100 ml) was mechanically stirred under dry nitrogen. After cooling to −65 °C in dry ice/ethanol, 1.6 M butyl lithium (23.7 ml, 37.8 mmol) in hexane was added over 3 min. The reaction was warmed to −20 °C and held for 20 min before recooling to −65 °C. Methyl-3-hydroxypropionate **5** (1.975 g, 1.82 mmol) in THF (3 ml) was added over 12 min. The reaction was warmed to −20 °C and held for 10 min before recooling to −65 °C. Toluene (5 ml) was added to the protected aldehyde **4** (11.12 g, 17.2 mmol) and distilled in a rotary evaporator under vacuum in order to remove any residual water or DCM, which would react with lithium diethylamide. The protected aldehyde **4** in THF (12 ml) was added to the lithiated methyl-3-hydroxypropionate solution over a period of 30 min at −65 °C. After 2 h the reaction mixture was removed from the cooling bath and ether (55 ml) and 20% aqueous ammonium chloride (35 ml) were added. The mixture was warmed to room temperature and the organic layer was separated. The aqueous layer was washed with ether (60 and 30 ml) and the combined organic layer and ether extracts were dried ($Na_2SO_4$). The solution was rotary evaporated under vacuum to give 15.9 g of the crude product. Purification of the crude product by silica column chromatography using a stepwise elution with DCM/petroleum spirits (3/1) and increasing in stages to 100% DCM gave the title compound **6**. A second chromatography was required on some impure fractions. Following rotary evaporation under vacuum, the title compound **6** (4.515 g, 6.027 mmol) was isolated as a colourless oil. This corresponded to a yield of 35% from purified aldehyde **4** and 16.3% from cholic acid. $R_f$ 0.25 (ether/DCM = 3/2); $^1$H NMR ($CDCl_3$) 0.60–0.73 (3H, m, H-18), 0.82–1.04 (6H, m, H-19, H-21), 2.51–2.60 and 2.63–2.70 (1H, dm, HC–COO), 3.35–3.56 (H-3β, 3OCHax), 3.56–3.67 (1H, br, H-7β), 3.67–3.81 (4H, H-12β, $CH_3$) 3.81–4.20 (6H, m, 3OCHeq, $CH_2OH$, CHOH), 4.57–4.83 (3H, m, OCHO). MS: calculated for $C_{43}H_{76}O_{10}N$ $[M+NH_4^+]$: 766.5464. Found: 766.5501.

2.2.5. *Methyl-(24RS,25RS)-3α,7α,12α,24,27-penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-oate (compound* **7**, *Fig. 2)*

Compound **6** (7.46 g, 8.13 mmol) and *p*-toluenesulfonic acid (45 mg) in DCM (70 ml) was magnetically stirred while DHP (2.04 g, 24.2 mmol) was added over 5 min resulting in a 5 °C exotherm. The reaction was allowed to proceed for a further 3 h after which the reaction mixture was concentrated in a rotary evaporator under vacuum to give the title compound **7** (9.69 g) as a crude colourless oil. $R_f$ 0.75 (ether/DCM = 1/4); $^1$H NMR ($CDCl_3$) 0.60–0.73 (3H, m, H-18), 0.82–1.04 (6H, m, H-19, H-21), 2.20–2.40 (1H, m, H-25), 3.36–3.58 (6H, m, H-3β, 5OCHax), 3.58–3.66 (1H, m H-7β), 3.66–3.80 (4H, m, H-12β, $CH_3$), 3.80–4.18 (8H, CHOTHP, $CH_2$OTHP, 5OCHeq), 4.54–4.84 (5H, m, OCHO). MS: calculated for $C_{53}H_{92}O_{12}N$ $[M+NH_4^+]$: 934.6614. Found: 934.6590.

2.2.6. *(24RS,25RS)-3α,7α,12α,24,27-Penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-26-ol (compound* **8**, *Fig. 2)*

Crude fully protected ester **7** (9.69 g, 8.13 mmol) in dry ether (150 ml) was mechanically stirred under dry nitrogen and $LiAlH_4$ (12.9 ml of 1 M in ether) was added over 3 min and the reaction mixture exotherm resulted in mild reflux. After a further 2 h reflux the solution was cooled to room temperature and ethyl acetate (5 ml) was added over a 2-min period. The reaction mixture was refluxed for a further 10 min, and cooled again to room temperature after which water (1.3 ml) was added drop-wise and a precipitate formed. The reaction mixture was stirred for a further 30 min and filtered through a bed of Celite, washing with ether (200 ml). The combined

ether solution was concentrated in a rotary evaporator under vacuum to give the crude title compound **8** (9.4 g). Purification by silica column chromatography using a stepwise elution with DCM/petroleum spirits (1/1–0/1) immediately followed by ether/petroleum spirits (15/75 and 1/3) gave pure **8** (6.33 g, 6.90 mmol) as a colourless oil. This represented a yield of 77.6% based on compound **6**. $R_f$ 0.3 (ether/DCM = 3/7); $^1$H NMR ($CDCl_3$) 0.60–0.73 (3H, m, H-18), 0.81–1.08 (6H, m, H-19, H-21), 3.34–3.58 (H-3β, 5OCHax), 3.58–3.69 (1H br, H-7β), 3.69–3.81 (3H H-12β, $CH_2OH$), 3.81–4.18 (8H, m, CHOTHP, $CH_2OTHP$, 5OCHeq), 4.44–4.85 (5H, m, OCHO). MS: calculated for $C_{52}H_{92}O_{11}N$ $[M + NH_4^+]$: 906.6665. Found: 906.6625.

### 2.2.7. *(24RS,25RS)-3α,7α,12α,24,26-Penta[(tetrahydropyran-2-yl)oxy]-5β-cholestan-27-yl triethylammonium sulfate (compound* **9**, *Fig. 2)*

Compound **8** (4.40 g, 4.80 mmol) in dry dimethylformamide (DMF) (15 ml) was warmed to obtain solution. The solution was cooled to room temperature and sulfur trioxide–triethylamine complex (1.28 g, 7.06 mmol, 1.47 equiv.) was added and magnetically stirred for 3.5 h after which time methanol (1.5 ml) was added and the reaction allowed to proceed with stirring for a further 30 min to react excess reagent. The solution was concentrated in a rotary evaporator under vacuum to remove DMF and the resulting crude oil of title compound **9** (6.35 g) was used immediately in the next step. An attempt to purify this compound in a previous preparation led to partial deprotection as indicated by a reduction of the acetal protons by NMR analysis. It is proposed that the THP-protected triethylammonium alkyl sulfate **9** acted as a mild acidic catalyst during chromatography with methanol and DCM causing partial deprotection, whereas an NMR of the crude compound **9** showed the required five acetal protons indicating no deprotection. MS on the crude product: found mass of 986.6160 which corresponded to $[M - Et_3N + NH_4^+]$ calc. 986.6239; $R_f$ 0.75 ($BuOH/CH_3COOH/H_2O$ = 8/1/1).

### 2.2.8. *(24RS,25RS)-3α,7α,12α,24,26-Pentahydroxy-5β-cholestan-27-yl sodium sulfate (compound* **11**, *Fig. 2)*

*2.2.8.1. Deprotection to (24RS,25RS)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sulfonic acid (compound* **10**, *Fig. 1).* The crude compound **9** (6.35 g) was dissolved in methanol (100 ml) and stirred magnetically at room temperature. One molar HCl was freshly prepared from 8.3 ml of 38% aqueous HCl and made up to 100 ml with methanol. Addition of the 1 M HCl solution (17 ml, 17 mmol) was completed over a 5-min period. The reaction was allowed to proceed at room temperature and the deprotection was carefully monitored by TLC. The deprotection was complete after 3 h and showed one main spot on TLC at $R_f$ 0.4 ($BuOH/CH_3COOH/H_2O$ = 8/1/1). The resulting deprotected sulfonic acid compound **10** as shown in Fig. 2 was then converted at room temperature to the sodium salt by the addition of sodium ethoxide (1.80 g, 26.4 mmol) in methanol (15 ml) that was added over a 2-min reaction period with constant stirring. A small drop of the reaction mixture added to a drop of water indicated a pH 10 had been reached. Gaseous $CO_2$ was bubbled through the reaction mixture for 15 min and this resulted in a drop to pH 8. The reaction mixture was concentrated in a rotary evaporator under vacuum

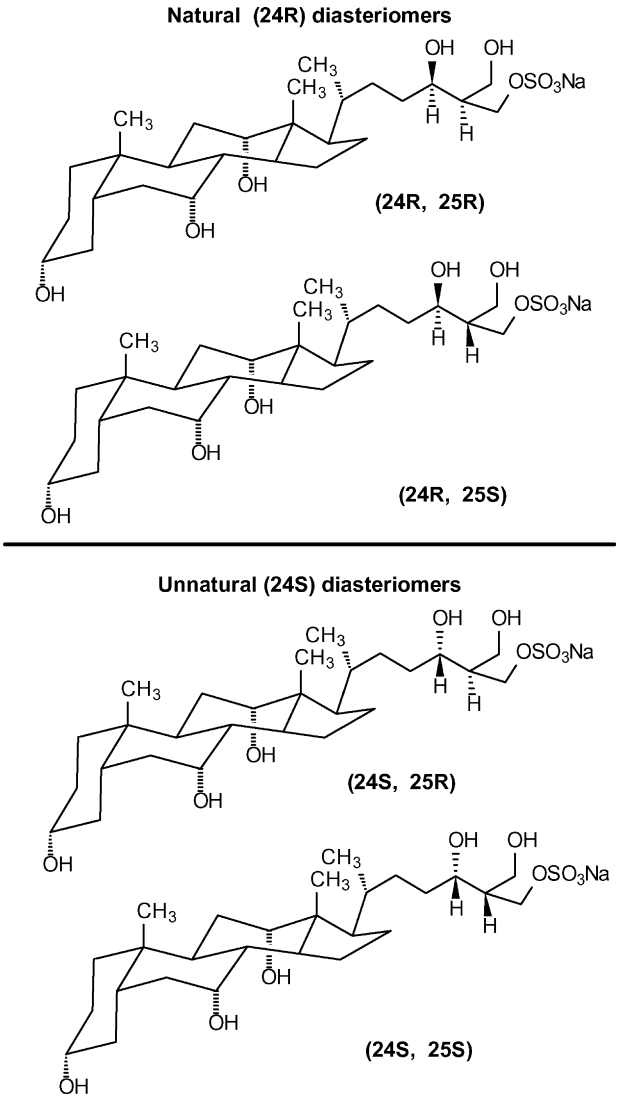


**Fig. 3 – The four stereoisomers for the synthetic compound 11 of Fig. 2.**

to a low volume and silica gel (25 ml) was added. Further evaporation of methanol gave a dry powder, which was added to the top of a large chromatographic column of silica gel in methanol (5%, v/v) in DCM. Fractions were collected while increasing the methanol in a stepwise manner from 5 to 10, 15 to 20, 25 and finally 30 and 50% in DCM. The title compound **11** (2.13 g, 3.74 mmol) was collected and the yield was 78% based on the purified protected monoalcohol compound **8**. $R_f$ 0.3 ($BuOH/CH_3COOH/H_2O$ = 8/1/1); calculated for $C_{27}H_{47}O_9SNa$: C, 56.8; H, 8.3; S, 5.6; Na, 4.0. Found: C, 55.6; H, 8.3; S, 5.6; Na, 3.9; $^1$H NMR ($CD_3OD$) 0.71 (3H, s, H-18), 0.92 (3H, m, H-19), 1.0–1.06 (3H, m, H-21), 2.18–2.36 (2H, m, H-4 and 9), 3.27–3.43 (m, H-3β + $CH_3OH$), 3.43–3.66 (3H, m, H-24, H-26), 3.79 (1H, m, H-7β,) 3.97 (1H, m, H-12β), 4.07–4.17 (1.5H, m, H-27), 4.17–4.22 (0.5H, m, H-27); $^{13}$C NMR ($CD_3OD$) 15.28 (C18), 20.07, 20.19 (C21), 25.36 (C19), 26.10 (C15), 29.24 (C9), 30.46 (C16), 30.86 (C11), 32.15, 32.77, 32.96 (C23), 33.21 (C2), 34.46, 34.68 (C22), 37.00 (C6), 37.41 (C10), 38.13 (C1), 38.75 (C20), 41.24 (C4), 42.53 (C8), 44.23 (C5), 47.99, 48.36 (C25), 48.66 (C13), 49.04 (C14),

49.31 (C17), 61.75, 62.44, 62.52 (C26), 68.94, 69.80, 69.96 (C27), 70.88 (C7), 73.06, 73.29, 73.54 (C24), 74.34 (C3), 75.75 (C12); MS: calculated for $C_{27}H_{52}O_9NS$ $[M^- + NH_4^+ + H^+]$: 566.3339. Found: 566.3357; mp: 173 °C and after rechromatography 185–186 °C.

## 3. Results and discussion

The protective group chosen for this synthesis was the THP group rather than the silyl ether for the following reasons. It is more amenable to commercial synthesis, and can be removed under mild conditions without causing desulfation. However, the NMR of the protected intermediates is more difficult to interpret due to the chirality of each THP group.

This first reported synthesis of sodium scymnol sulfate commences with cholic acid. This reacted with DHP and the resulting THP protected THP cholate ester **2** was reduced with lithium aluminium hydride and worked up by addition of a small amount of water to produce a precipitate that was filtered off. The ester had been stripped of the DCM solvent before reduction. Investigation of an alternative reduction with Red Al® in DCM resulted in problematic emulsions and was not pursued further. The alcohol **3** was oxidized to the aldehyde **4** with pyridinium chlorochromate in the presence of anhydrous sodium acetate [13] and purified by column chromatography. Methyl-3-hydroxypropionate (**5**) was prepared from 3-hydroxypropionitrile and methanolic HCl containing a little water [14]. The pure aldehyde **4** was coupled using 2 equiv. of lithium diethylamide in THF at −65 °C according to the method of Sunazuka et al. [15]. The reaction produced compound **6** which was chromatographed and then protected as the THP derivative **7**. The methyl ester group at C-26 was reduced to the monohydroxyl compound **8**. The triethylamine salt of the C-27 monosulfate **9** was prepared by reaction of the monohydroxyl compound **8** and $Et_3N{\cdot}SO_3$ in DMF according to the method of Tserng and Klein [12]. After stripping the DMF under high vacuum, the THP protective groups were removed in methanolic HCl to produce compound **10**. Care was taken to prevent desulfation by excessive methanolic HCl treatment [16]. Sodium ethoxide was dissolved in methanol and gradually added to the compound **10** reaction mixture, changing the pH to the range of 9–10. The pH was then adjusted to between pH 7 and 8 and the crude mixture was stripped of solvent, absorbed onto a portion of silica gel and chromatographed to yield pure (24*RS*,25*RS*) sodium scymnol sulfate **11**. This was characterised using $^{13}C$ and $^{1}H$ NMR and compared with the two [24*R*,25*R*] and [24*R*,25*S*] sterioisomers of natural sodium scymnol sulfate using the results of Ishida et al. [9]. Our $^{13}C$ NMR results comparison is summarised in Table 1. Extra chemical shifts were observed for the unnatural isomers (24*S*,25*R*) and (24*S*,25*S*) produced in our product (24*RS*,25*RS*) sodium scymnol sulfate **11** at positions 21, 23, 26 and 27 as shown in Fig. 4. Fig. 3 shows the steric orientations at the 24 and 25 positions for the four sterioisomers of our synthetic compound **11**.

We believe that the observed protective effect of scymnol is due to its free radical inhibitory effect in biological systems [2]. If this is so, the 24*R* and 24*S* sterioisomers may be equivalent in activity. We have also produced $^{14}C$-radiolabelled 24*RS*,25*RS* sodium scymnol sulfate for use in mouse pharmacokinetic studies [17] using the synthetic method in this paper.

**Table 1 – $^{13}C$ NMR data of the two natural scymnol sulfate and four synthetic stereoisomers**

| Position | Isheda et al. [9] | | Present work |
|---|---|---|---|
| | 24R,25R | 24R,25S | 24R,25R, 24R,25S (24S,25R; 24S,25R) |
| 20 | 38.6 | 38.6 | 38.74 |
| 21 | 19.9 | 19.9 | 20.17, 20.19 |
| 22 | 34.5 | 34.6 | 34.46, 34.67 |
| 23 | 32.9 | 33.1 | 32.15, 32.77, 32.96 |
| 24 | 73.1 | 72.9 | 73.05, 73.27 |
| 25 | 48.3 | 48.5 | 47.98, 48.35 |
| 26 | 61.7 | 62.3 | 61.74, 62.42, 62.51 |
| 27 | 69.7 | 68.9 | 68.93, 69.78, 69.95 |

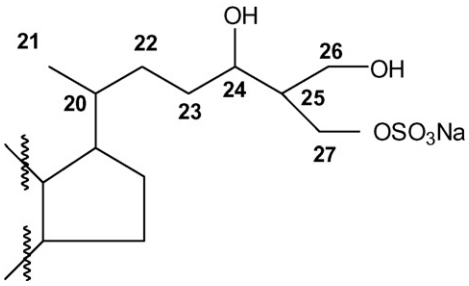


**Fig. 4 – Positions in the sodium scymnol sulfate side-chain.**

The synthetic sodium scymnol sulfate was also confirmed by high resolution MS and microanalysis.

In conclusion, we have successfully completed the synthesis of (24*RS*,25*RS*) sodium scymnol sulfate and the general synthetic scheme presented in this paper may be useful in the synthesis of a range of sterol monosulfated products commencing from various bile acids.

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