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Stereoselective Synthesis of Squalamine Dessulfate

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Abstract: Squalamine dessulfate (24*R*) and the unnatural product squalamine dessulfate (24*S*) have been synthesized from stigmasterol. The key step in establishing the C24 stereochemistry is attachment of the side-chain at C22 using either (2*R*)- or (2*S*)-1,2-epoxy-3-methylbutane to yield the cholesteryl precursors of the epimeric squalamine dessulfates.

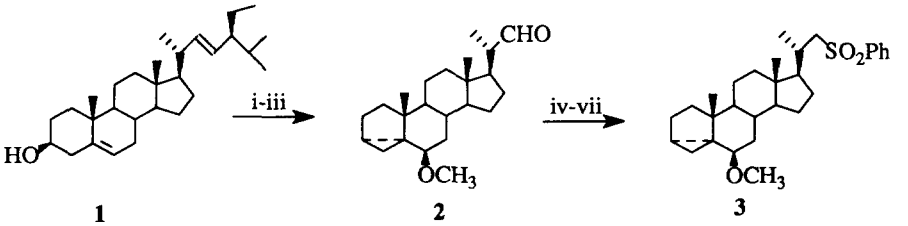
The novel polyaminosteroidal sulfate called squalamine (**11a**) was isolated from tissue of the dogfish shark *Squalus acanthias*. Squalamine (**11a**) is a broad-spectrum antibiotic and it is extremely active against both Gram-negative and Gram-positive bacteria.[1a,b] This host-defense agent is also active *in vitro* against various sexually transmitted disease organisms such as *Nesseria gonorrhoeae*, herpes simplex virus and human immunodeficiency virus.[2]

The structure of 24ξ-squalamine was based on 2D-NMR and high resolution mass spectrometry[1b]. The configurations at all the chiral centers were revealed by these methods with the exception of the C24-sulfated hydroxyl group. A confirmatory synthesis of 24ξ-squalamine yielded both configurations of the C24-hydroxyl group.[3] The stereochemistry of the C24-sulfate monoester remains unknown, and this is a potential impediment to the development of a rationale for the impressive biological activity of the natural product.[4]

We report now the first stereoselective synthesis of 24(*R*) and 24(*S*)-squalamine dessulfate (**10a** and **10b**, respectively)(19 steps, 3.9% overall yield), and the establishment of the C24 stereochemistry of squalamine (**11a**) as *R* by comparison of the two synthetic stereoisomers with the natural squalamine dessulfate.

The logic of the stereoselective synthesis of the C24 *R* and *S* epimers of squalamine dessulfate is centered upon the key 3β, 7α, 24(*R*) or (*S*)-5α-cholestane triacetates (**8a**, **8b**, respectively). Selective stepwise removal of individual acetyl groups, taking advantage of their different hydrolytic rates, enabled sequential introduction of the key functional groups. The first stage was the synthesis of 24(*R*) and 24(*S*)-hydroxycholesterols, **7a** or **7b**, respectively. This was done following a modification[5] of the route reported by

Scheme 1



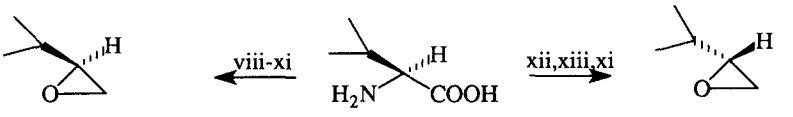
1 **2** **3**

i)TsCl, py, 25°C, 14h, ca.100%; ii)MeOH,KOAc, 4h refl., 80%; iii)O_3, MeOH, -78°C; iv)$NaBH_4$, MeOH, 0°C to 25°C, 85% (2 steps); v)CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0°C, 2h; vi)NaI, Me_2CO, 17h refl., 90% (2 steps); vii)$PhSO_2Na$, DMF, 25°C, 32h, 91%. Ts= pCH_3-C_6H_4-SO_2-; py= pyridine; Ac= CH_3CO-; Ph= C_6H_5-; DMF= N,N-dimethylformamide.

Ourisson et al.[6], combined with a procedure established by Koppenhoefer and Schurig[7] which allowed the use of L-valine as a precursor for both the 24(*R*) and 24(*S*) side chain.

Stigmasterol (**1**) was converted to i-steroid aldehyde **2**, which was reduced *in situ* and thence converted to the C22 phenylsulfone **3** (Scheme 1). Next, the two enantiomeric epoxides, (2*S*)-1,2-epoxy-3-methylbutane (**4**) and (2*R*)-1,2-epoxy-3-methylbutane (**5**), were synthesized from (*S*)-(+)-valine (**6**) (Scheme 2).

Scheme 2

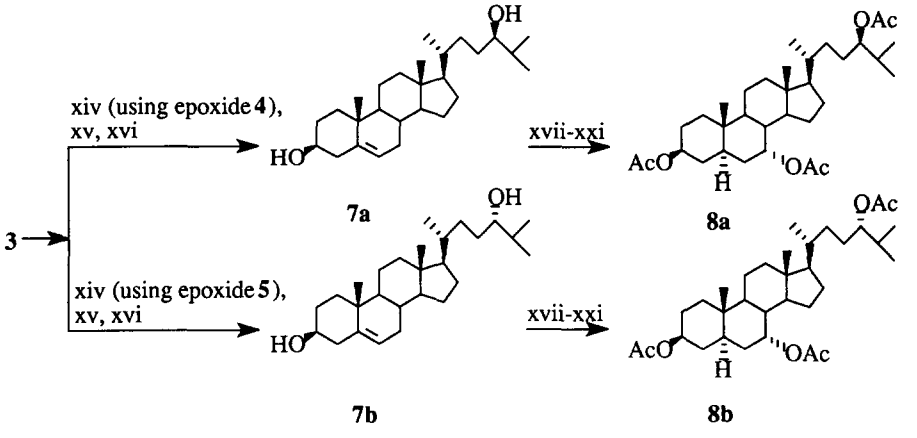


4 **6** **5**

viii)$NaNO_2$, 1N H_2SO_4, 0°C to 25°C, 2h, 60%; ix)$LiAlH_4$, Et_2O, 25°C, 14h, 53%; x)TsCl, py, 25°C, 14h, 99%; xi)KOH, Δ, 75%; xii)$NaNO_2$, 5N HCl, 0°C to 25°C, 5h, 65%; xiii)$LiAlH_4$, Et_2O, 15 min. refl., 75%.

Enantiomeric epoxides **4** and **5** were condensed individually (Scheme 3) with the anion derived from **3** to yield, ultimately, 24(*R*)- and 24(*S*)-hydroxycholesterol (**7a** and **7b**), respectively. Allylic oxidation of the corresponding diacetates, followed by reduction of the enones[8] established the 5α-cholestane stereochemistry. Stereoselective reduction of the 7-keto compounds gave exclusively the 7α stereochemistry.[9] Acetylation gave the key triacetates **8a** or **8b**.

Scheme 3



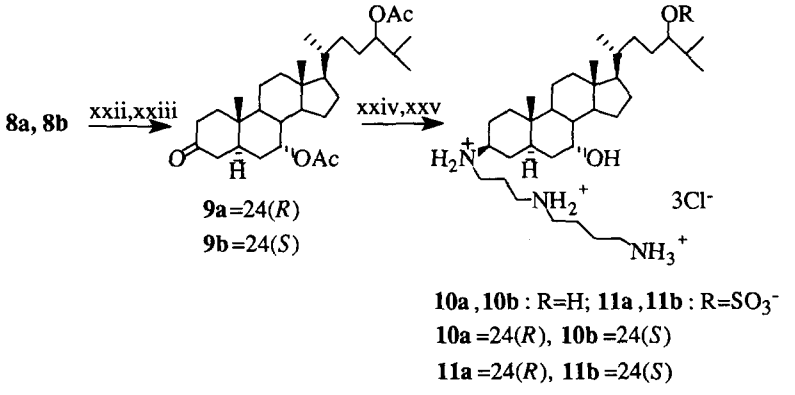
xiv)nBuLi, epoxide **4** or **5**, -78°C, 2h, 90%; xv)Li, NH_3(l), -78°C, 30 min.,80%; xvi)TsOH, dioxane-H_2O 7:3, 80°C, 1h, 95%; xvii)Ac_2O, py, 25°C, 14h, 95%; xviii)CrO_3, DMP, CH_2Cl_2, -20°C, 24h, 75%; xix)Li, NH_3(l), -78°C, 10 min, 80%; xx)KB[$CH(CH_3)C_2H_5]_3H$, THF, -50°C, 6h, 75%; xxi)Ac_2O, DMAP, CH_2Cl_2, 25°C, 14h, 85%. nBu= $CH_3(CH_2)_3$-; DMP= 3,5-dimethylpyrazole; THF= tetrahydrofuran; DMAP= 4-dimethylaminopyridine

Taking advantage of the reactivity differences among the three secondary hydroxyl groups in **8a** and **8b**, we performed a selective deacetylation at C3 with sodium cyanide in methanol, followed by Jones oxidation[10a,b] (Scheme 4), to get 7α, 24(*R*) or 24(*S*)-5α-cholestane-3-one diacetates **9a** or **9b**. The configurations at chiral centers C5, C7, and C24 were revealed by an X-ray structure for **9a** (Fig. 1).

The diprotected spermidine side-chain, N-(3-aminopropyl)-N,N'-di(*tert*-butoxycarbonyl)-1,4-diaminobutane was prepared according to the literature procedure starting from 1,4-diaminobutane and

acrylonitrile.[11] Reductive amination[12a,b] of 3-keto compounds **9a** and **9b** using diprotected spermidine in the presence of sodium cyanoborohydride in methanol, followed by treatment with dry hydrogen chloride in anhydrous methanol at room temperature[13] afforded squalamine dessulfate trihydrochloride salts **10a** ($[\alpha]^{23}_D$=10°, methanol, c=1)[14] and **10b** ($[\alpha]^{23}_D$=ca. 0°, methanol, c=1), respectively, in 50% yield after purification (Scheme 4).

Scheme 4



xxii)NaCN, MeOH, 25°C, 48h, 85%; xxiii)CrO_3, H_2SO_4, H_2O, 75%; xxiv)tBocNH$(CH_2)_4$N(tBoc)$(CH_2)_3NH_2$, $NaBH_3CN$, 14h, 25°C, 80%; xxv)HCl, MeOH, 25°C, 14h, 50%. tBoc= $(CH_3)_3$C-O-CO- .

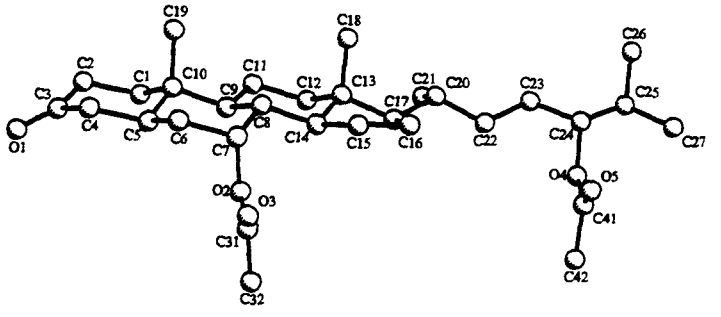


Fig.1 : X-ray structure for 7α,24(*R*), 5α-cholestan-3-one-7,24-diol-7,24-diacetate (**9a**)

The ^{13}C-NMR chemical shift of a C24 carbon atom bearing a hydroxyl group has been used for the assignment of configuration in the case of the bile alcohol 5β-ranol[15]. The chemical shift difference $\Delta\delta=\delta S - \delta R$ of ca. 0.40 ppm was considered diagnostic for the *R* versus *S* configuration at C24 in a series of steroids[16]. Table 1 reveals a difference of 0.38 ppm in the case of the natural product and the unnatural synthetic 24 (*S*) compound.

The configuration at C24 of squalamine dessulfate is established by the ^{13}C NMR data; squalamine dessulfate is 3β-N-1-[N(3-[4-aminobutyl])-1,3-diaminopropane]-7α,24(*R*)-dihydroxy-5α-cholestane trihydrochloride (**10a**), and the corresponding natural product squalamine is 3β-N-1-[N(3-[4-aminobutyl])-1,3-diaminopropane]-7α,24(*R*)-dihydroxy-5α-cholestane, 24-sulfate trihydrochloride (**11a**).

Table 1 - Comparison of ^{13}C NMR Data[a] for **10b**, **10a**, and Natural Squalamine Dessulfate

Position of C atom	^{13}C NMR, δ[ppm]		
	10b(24(*S*)-)	**10a**(24(*R*)-)	natural
C17	57.48	57.60	57.62
C20	37.35	37.03	37.04
C21	19.31	19.16	19.16
C22	33.54	33.33	33.35
C23	31.59	31.47	31.47
C24	78.12	77.74	77.77
C25	34.45	34.85	34.88
C26[b]	17.51	17.97	17.98
C27[b]	19.52	19.41	19.41

[a] ^{13}C NMR spectra were measured in deuterated methanol. [b]These resonances are interchangeable.

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4. This point of stereochemistry could be resolved by an X-ray structure, but squalamine resisted crystallization. The dessulfated squalamine proved to be equally non-crystalline. The tris-trifluoroacetyl spermidino analog was crystalline (m.p. 112°C), but the crystals were inadequate for diffraction.
5. (a) The reaction of the 6β-methoxy-22-iodo-3α,5α-cyclo-23,24-bisnorcholane with sodium phenylsulfinate was carried out at 25°C, instead of 125°C, to avoid the formation of the product of cycloreversion-elimination, which in the original version[6] formed ca. 20% of the crude product. (b) Both (*R*)- and (*S*)-1,2-epoxy-3-methylbutanes were preformed and distilled prior to the coupling reaction, to ensure a 15% higher overall yield and an up to 4 times more efficient usage of the valine derivatives.
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