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SYNTHESIS OF SQUALAMINE. A STEROIDAL ANTIBIOTIC FROM THE SHARK

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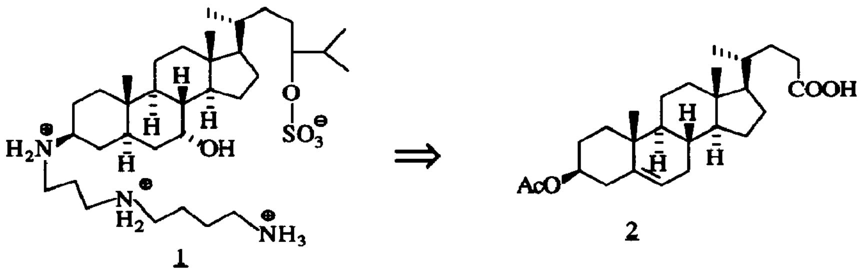
Summary: The title compound was synthesized from 3β-acetoxy-5-cholenic acid (2) in 17 steps.

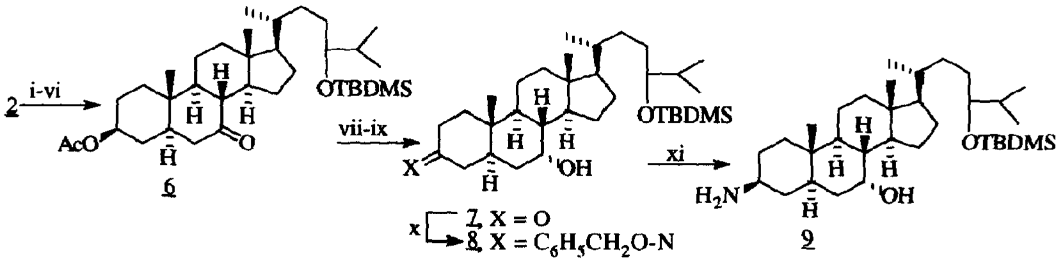
Recently a novel polyaminosterol sulfate named squalamine (1) was isolated from tissues of the dogfish shark *Squalus acanthias*.[1] This compound exhibits potent antimicrobial activity against Gram-negative and Gram-positive bacteria. It is fungicidal and induces osmotic lysis of protozoa. Sharks are predatory scavengers and yet they show remarkable resistance to bacterial and viral infection as well as an array of toxic chemicals which would kill most mammals. It is possible that squalamine (1) is a systemic antimicrobial in sharks and their remarkable resistance to infections is related to this compound.

The structure of the squalamine, 3β-N-1-{N[3-(4-aminobutyl)]-1,3-diaminopropane}-7α,24ζ-dihydroxy-5α-cholestane 24-sulfate (1) was determined by ^{1}H and ^{13}C NMR and FAB mass spectrometry.[1] We report now a synthesis which confirms the proposed structure. *A priori*, inspection of the structure of 1 reveals a similarity to the steroidal bile acids in the sense that the 7α-hydroxyl group is present, but the *trans*-AB ring system of 1 is related to the cholesteryl series. While the 3β-amino group is familiar in aminosterols as in chonemorphine or conessine, the spermidino group attached to any sterol has not been encountered. 3β-Acetoxy-5-cholenic acid (2) is an ideal starting material for the synthesis of squalamine (1) because positions C_3, C_5, C_7 (*via* allylic oxidation) and C_{24} are appropriately disposed for introduction of the key functionality in 1.

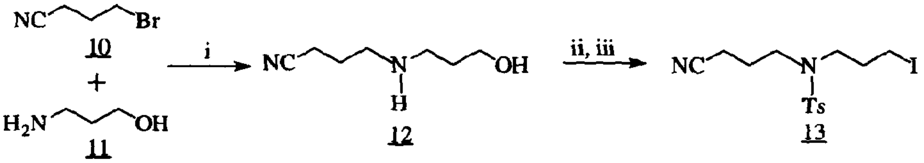


1 2

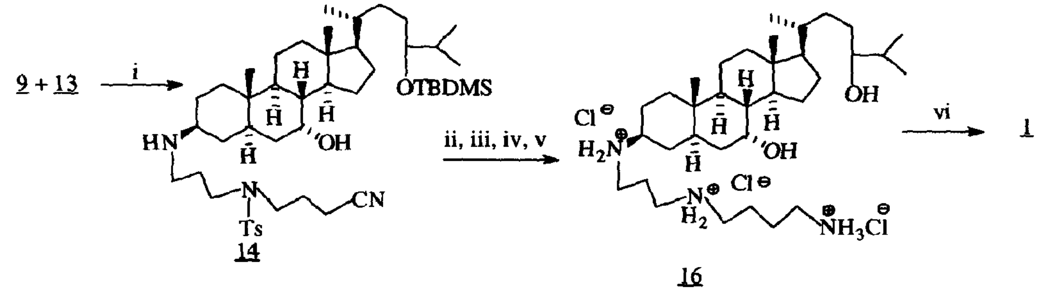
Reaction of the acid chloride from 2 with isopropylcadmium bromide in benzene (generated *in situ* from isopropylmagnesium bromide and cadmium bromide)[2] at room temperature for 1 h afforded the corresponding 24-ketone (3)[3] in 60% yield. Reduction of the 24-ketone with calcium borohydride in THF (generated *in situ* from sodium borohydride and calcium chloride in THF)[4] and protection of the thus formed 24-hydroxyl group with *tert*-butyldimethylsilyl chloride afforded 3β-acetoxy-24-*tert*-butyldimethylsiloxy-5-cholestene (4).



Reagents: i, $(COCl)_2$, CH_2Cl_2, reflux, 2 h, quant.; ii, $(CH_3)_2CHCdBr$, C_6H_6, RT, 1 h, 60%; iii, $Ca(BH_4)_2$, THF, RT, 5 h, 80%; iv, TBDMSCl, imidazole, CH_2Cl_2, RT, 16 h, 90%; v, $Cr(CO)_6$, t-BuOOH, CH_3CN, reflux, 12 h, 46%; vi, Li, liq. NH_3, Et_2O, -78°C, 10 min, 81%; vii, K-selectride, THF, -50°C, 5 h, 80%; viii, NaCN, MeOH, reflux, 8 h, 88%; ix, $(t\text{-}BuO)_3Al$, cyclohexanone, toluene, 120°C, 20 h, 59%; x, $C_6H_5CH_2O\text{-}NH_2{\bullet}HCl$, C_5H_5N, C_2H_5OH, reflux, 16 h, 97%; xi, $LiAlH_4$, Et_2O, reflux, 16 h, 98%.



Reagents: i, K_2CO_3, CH_3CN, reflux, 20 h, 100%; ii, TsCl, Et_3N, DMAP, CH_2Cl_2, 0°C to RT, 16 h, 82%; iii, NaI, acetone, reflux, 16 h, 89%.



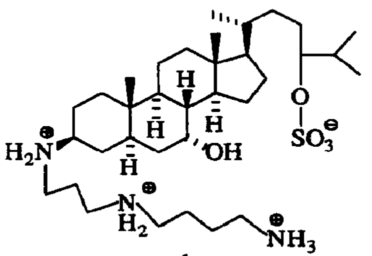
Reagents: i, K_2CO_3, CH_3CN, reflux, 16 h; ii, $C_6H_5CH_2OCOCl$, NaOH, THF, 0° to RT, 4 h, 70%; iii, Na, liq. NH_3, THF, -78°C to RT, 18 h, 91%; iv, $LiAlH_4$, Et_2O, reflux, 6 h, 93%; v, HCl, EtOH, RT, 3h, 98%; vi, $C_5H_5N{\bullet}SO_3$, C_5H_5N, 75°C, 2 h, 10%.

Allylic oxidation of 4 with chromium hexacarbonyl and *tert*-butyl hydroperoxide[5] in refluxing acetonitrile gave the desired 7-keto compound (5) in 46% yield after chromatography. Birch reduction[6] of 5 with lithium in liquid ammonia at -78°C for 10 min gave the A/B *trans* fused steroid compound (6) in 81% yield. Stereoselective reduction[7] of the 7-oxo group of 6 with K-selectride in THF at -50°C gave the 7α-hydroxy compound, and subsequent deacetylation (NaCN, MeOH, reflux) of the 3β-acetoxy group followed by Oppenauer oxidation[8] (aluminum-tri-*tert*-butoxide and cyclohexanone in toluene at 120°C) of the 3β-hydroxyl group afforded the desired 3-keto compound 7.[8a] Compound 7 was converted to the corresponding 3-

benzyloxyimino derivative (8) which on stereoselective reduction with lithium aluminium hydride afforded (9) which was converted to the N-carbobenzyloxy derivative ($C_6H_5CH_2OCOCl$, NaOH, THF) and purified by chromatography then decarbobenzyloxylated to yield pure 9. The spermidine side-chain was made *via* mono-N-alkylation of 11 with 10 to yield 12 which was converted as shown to 13.[9]

Mono-N-alkylation of 9 with 13 gave 14 which was converted to the N-carbobenzyloxy derivative ($C_6H_5CH_2OCOCl$, NaOH, THF) and purified by flash chromatography. Simultaneous reductive cleavage of the N-tosyl and the N-carbobenzyloxy groups (Na, liq. NH_3, THF, -78°C)[10] followed by lithium aluminum hydride reduction of the nitrile gave (15). Final removal of the *tert*-butyldimethylsiloxy group in 15 in the presence of dry hydrogen chloride in anhydrous ethanol[11] at room temperature afforded the des-sulfated squalamine trihydrochloride salt (16). Selective mono-O-sulfation of 24-hydroxyl group in the presence of the 7α-hydroxyl group with the sulfur trioxide-pyridine complex[12] in dry pyridine at 75°C for 2 h gave crude squalamine (1).[13] The crude product was purified by reverse phase column (Waters Sep-pak, C_{18} column) using trifluoroacetic acid-water-acetonitrile solvent system. Squalamine 1 was fully characterized by high-field NMR (600 MHz) and FAB mass spectroscopies. The synthetic squalamine was identical to the natural substance in its physical and biological properties.[1]

No information exists at present regarding the molecular mechanism of the biological action of squalamine. In common with the polyene antibiotics amphotericin B, nystatin, pimaricin, etruscomycin, and filipin, which consist basically of a lipophilic half and a polar half in a large ring, squalamine likewise may be depicted in a similar cyclic form as in 17. This salt bridged cyclic form consists of an upper lipophilic sterol part and the lower part is the polyamino chain.



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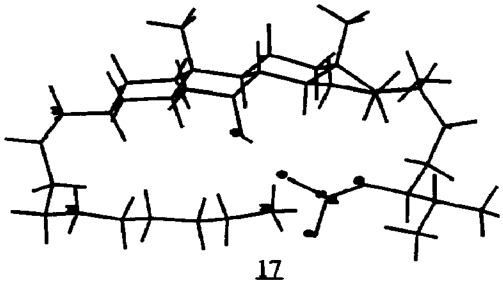

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Membrane lysis could also occur *via* pore formation involving several squalamine molecules in a circular array (half pores-hydrophilic channel) or "barrel stave" channel formation.[14] Alternatively squalamine in a cyclic form could function as an ionophore in common with the large class of ionophoric antibiotics.[15]

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13. **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (1H, m, 24-H), 4.60 (1H, m, 3α-H), and 5.38 (1H, m, C=CH-CH_2); **5**: IR (neat) 1674 (α,β-unsaturated ketone), 1736 ($OCOCH_3$) cm^{-1}; δ 2.04 (3H, s, $OCOCH_3$), 3.38 (1H, m, 24-H), 4.72 (1H, m, 3α-H), and 5.69 (1H, s, C=CH); **6**: IR (neat) 1711 (C=O), 1736 ($OCOCH_3$) cm^{-1}; δ 2.00 (3H, s, $OCOCH_3$), 3.35 (1H, m, 24-H), and 4.66 (1H, m, 3α-H); **7**: δ 3.36 (1H, m, 24-H), and 3.85 (1H, m, 7β-H); CIMS 533 (M^++1, 61%); **8**: δ 2.98, 3.27 (m, syn, and anti oxime next to α-CH_2 of A-ring), 3.37 (1H, m, 24-H), 3.86 (1H, m, 7β-H), 5.06 (2H, br s, $OCH_2C_6H_5$), and 7.26-7.42 (5H, m, C_6H_5); CIMS 638 (M^++1, 100%); **9**: δ 2.69 (1H, ,m, 3α-H), 3.39 (1H, m, 24-H), and 3.84 (1H, m, 7β-H); CIMS 534 (M^++1, 60%); **13**: mp 63-64°C, CIMS 407 (M^++1, 100%); **14**: δ 1.96 (4H, m, 2xCH_2), 2.42 (4H, m, CH_2N, CH_2CN), 2.43 (3H, s, $CH_3C_6H_5$), 2.62 (1H, m, 3α-H), 3.18 (4H, m, 2xCH_2N), 3.38 (1H, m, 24-H), 3.84 (1H, m, 7β-H), 7.32, 7.19 (4H, two d, ArH); CIMS 813 (M^++1, 44%); **15**: δ 2.38-2.78 (9H, m, 4xCH_2N, 3α-H), 3.38 (1H, m, 24-H), and 3.84 (1H, m, 7β-H); CIMS 662 (M^++1, 31%); **16**: (CD_3OD) δ 2.96-3.26 (10H, m, 4 x CH_2N, 3α-H, 24-H), 3.80 (1H, br s, 7β-H); positive FAB-MS 549 (100%); **1**: (CD_3OD) δ 2.94-3.26 (9H, m, 4xCH_2N, 3α-H), 3.80 (1H, br s, 7β-H), and 4.12 (1H, m, 24-H); positive FAB-MS 628.5 (25%), 549 (100%); negative FAB-MS 626.4 (100%).
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