

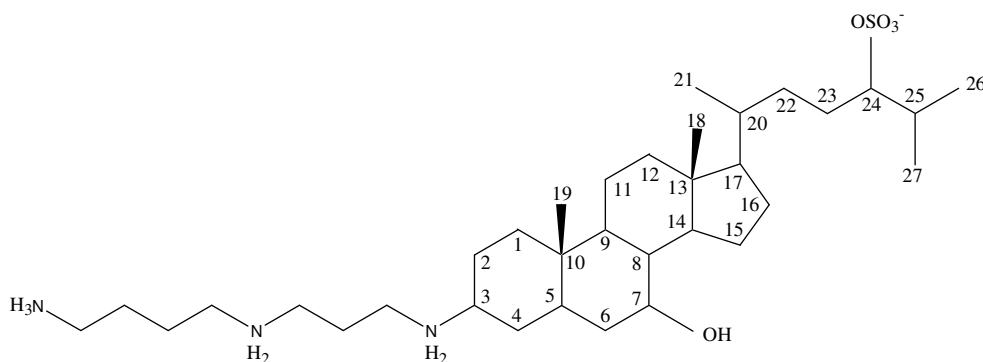
Department of Chemistry

Aimée Peck

Mentor: Ann Shinnar

Biosynthesis of Squalamine in Sharks: Detection of Aminosterols by Mass Spectrometry

Squalamine is an aminosterol with potent, antimicrobial activity.¹ First isolated from the spiny dogfish shark (*Squalus acanthias*), this zwitterionic compound is now produced by synthetic methods for use in clinical trials for the treatment of non-small cell lung cancer, age-related macular degeneration, and prostate cancer.² The structure of squalamine was determined by high resolution NMR and mass spectrometry.¹



Although squalamine has been extracted in abundance from spiny dogfish liver,³ the aminosterol biosynthetic pathway has not been elucidated. Our laboratory is investigating whether squalamine biosynthesis is endogenous in *Squalus acanthias* and other shark species. Our approach involves (a) isolating the major subcellular fractions involved in sterol biosynthesis, (b) extracting zwitterionic compounds⁴ and testing for antimicrobial activity, and (c) analyzing fractions by electrospray ionization mass spectrometry (ESI-MS) to identify compounds. In addition, the fractions are treated with a protease to establish whether the antimicrobial compounds are peptides or proteins.

Antimicrobial activity was observed in the major membranous subcellular fractions, but not in the clear oil fraction of liver. This antimicrobial activity was not diminished when treated with proteinase K, consistent with the known structures of aminosterols. These fractions, with solubility and partitioning properties similar to squalamine, were suitably clean for ESI-MS. ESI-MS analysis provides a fingerprint of the most abundant compounds in this extract and identifies the parent ions of known aminosterols, especially squalamine in *S. acanthias*, as well as in related squaliform species. These studies provide evidence that aminosterol biosynthesis is an endogenous pathway, very active in the liver of certain shark species.

¹ Moore *et al.* "Squalamine: An aminosterol antibiotic from the shark" *Biochemistry* 90 (1993): 1354-1358

² <http://www.genaera.com/clinicaltrials.html>

³ Rao *et al.* "Aminosterols from the Dogfish Shark *Squalus acanthias*" *Journal of Natural Products* 63 (200): 631-635

⁴ This method first developed by Joan Shu (BC '01) and Christine Musich (BC '02), HHMI interns at Barnard College