Synthesis of Potential Inhibitors of Squalene Epoxidase

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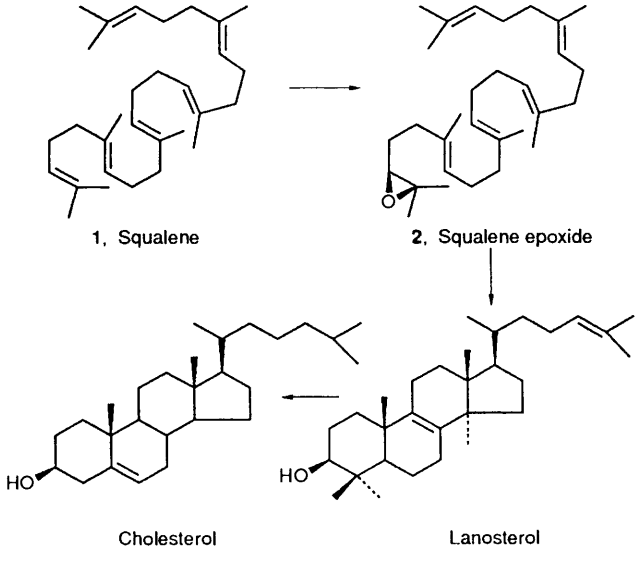
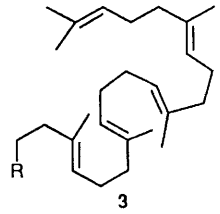
Squalene was degraded to tris-norsqualene aldehyde, and this was converted into 2-fluoromethyl-, 2,2-difluoromethyl-, 2-trimethylsilylmethyl-analogues of squalene, and into 2-cyano-norsqualene.

Squalene epoxidase is a key enzyme in the biosynthesis of sterols and triterpenoids, and is thus an obvious target for inhibition.[1] It catalyses the conversion of squalene **1** into 2,3-epoxysqualene **2**, which then cyclises under the influence of the enzyme epoxysqualene cyclase to yield polycyclic isoprenoids. Little is known about the enzyme, save that its uses a flavin cofactor rather than a cytochrome, and the mechanism of epoxidation is completely unknown.

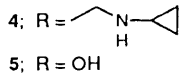


In an effort to throw some light on this process, we have prepared a number of squalene analogues in which the electronic properties of the terminal double bond have been altered. During the course of this work, others[2-4] reported the syntheses of a number of squalene analogues, *e.g.* **3**, **4** and **5**, but all of these compounds lacked the terminal double bond of squalene.

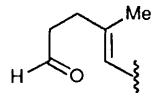


3

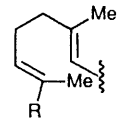
3; R = —≡—

4; R = 

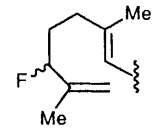
5; R = OH



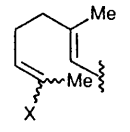
6



7; R = CO_2Et
8; R = CH_2OH
9; R = CH_2F
11; R = CHO
12; R = CF_2F



10



13; X = CN
14; X = CH_2SiMe_3

Selective oxidative cleavage of the terminal double bond of squalene was achieved *via* formation of the epoxide [NBS (*N*-bromosuccinamide) in wet THF (tetrahydrofuran), then K_2CO_3 in MeOH] and subsequent reaction with periodic acid in ether. The overall yield of the aldehyde **6** was *ca.* 35% on the 40 gram scale. Reaction of this aldehyde with ethoxycarbonylethylidene-triphenylphosphorane (dichloromethane at room temp.) provided the ester **7** and thence the alcohol **8** following reduction with $LiAlH_4$ (ether at 0 °C) (overall yield 90%).

Attempted fluorination of the alcohol with diethylaminosulphur trifluoride (DAST) (dichloromethane at −78 °C) produced a mixture of the desired monofluoro product **9** and the rearranged product **10** (ratio 3:7, yield 45%). Modification of the reactivity of DAST through formation of diethylamino(dimethylamino)sulphur difluoride *[5] and reaction of this with the alcohol **8** (trichlorofluoromethane at −78 °C) produced the desired compound **9** as the sole product (45%). This methodology shows considerable promise for the production of the fluorides of sensitive (allylic) alcohols. Finally, oxidation of the alcohol **8** to aldehyde **11** (MnO_2 in dichloromethane, 95%)

* A typical experimental procedure involved addition of DAST (0.55 cm^3, 4.2 mmol) to a solution of dimethylaminotrimethylsilane (0.41 g, 3.5 mmol) in trichlorofluoromethane (10 cm^3) at −78 °C. After 10 min, the solution was allowed to warm to room temp. The mixture was then cooled to −78 °C prior to the addition of 1-hydroxysqualene **8** (0.95 g, 2.3 mmol) dissolved in trichlorofluoromethane (5 cm^3). After 45 min, the reaction mixture was allowed to warm to room temp., and poured into the ice water (20 cm^3). After neutralisation with solid sodium hydrogencarbonate, the product was extracted into dichloromethane.

and reaction with DAST (neat, 40 °C, 48 h) yielded the expected 2,2-difluoromethyl analogue of squalene **12** (45%).

Several further reactions of tris-norsqualene aldehyde **6** were carried out, and the most successful of these involved reaction with cyanoethylidenetriphenylphosphorane (in dichloromethane) to produce the 2-cyanonorsqualene **13** as a mixture of isomers (*E*:*Z* 72:28) in a yield of 90%. Reaction of aldehyde **6** with the ylide from 1-trimethylsilylpropyl-2-phosphonium iodide[6] provided the allylsilanes **14** as a 1:1 mixture of isomers in low yield (*ca.* 20%).

Whilst most of these reactions remain unoptimised, they do, for the first time, allow access to analogues of squalene modified on the terminal methyl group. We anticipated that the fluoro analogues **9** and **12**, and the cyano analogue **13** would possess a terminal double bond with much diminished electron density, thus producing inhibition of the epoxidase if an electrophilic oxidation is involved. Alternatively, the analogue **14** could provide evidence of the existence of an electron-deficient intermediate given the propensity of a silicon atom to stabilise a beta-carbocation. In the event, preliminary *in vitro* studies with crude squalene epoxidase from the yeast *Saccharomyces cerevisiae*[7] demonstrated that all of the compounds possessed inhibitory activity, but were relatively non-potent (millimolar level). We believe that this may be due to lack of penetration of the compounds to the active site(s) of what is apparently a multi-enzyme complex.[7] Future work will thus be directed towards the synthesis of similar analogues of farnesol and geraniol using the methodology described in this communication.

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References

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