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TRISNORSQUALENE METHYL HYDROXYLAMINE: A POTENT DUAL INHIBITOR OF MAMMALIAN SQUALENE EPOXIDASE AND OXIDOSQUALENE CYCLASE

Gregory M. Anstead,[1] Hung-Kuei Lin,[2] and Glenn D. Prestwich*
Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400

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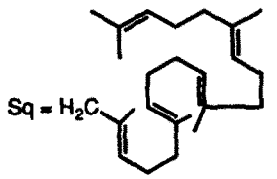
Abstract. Squalene epoxidase and oxidosqualene cyclase, two sequential enzymes in the sterol biosynthesis pathway, are inhibited effectively by trisnorsqualene methyl hydroxylamine.

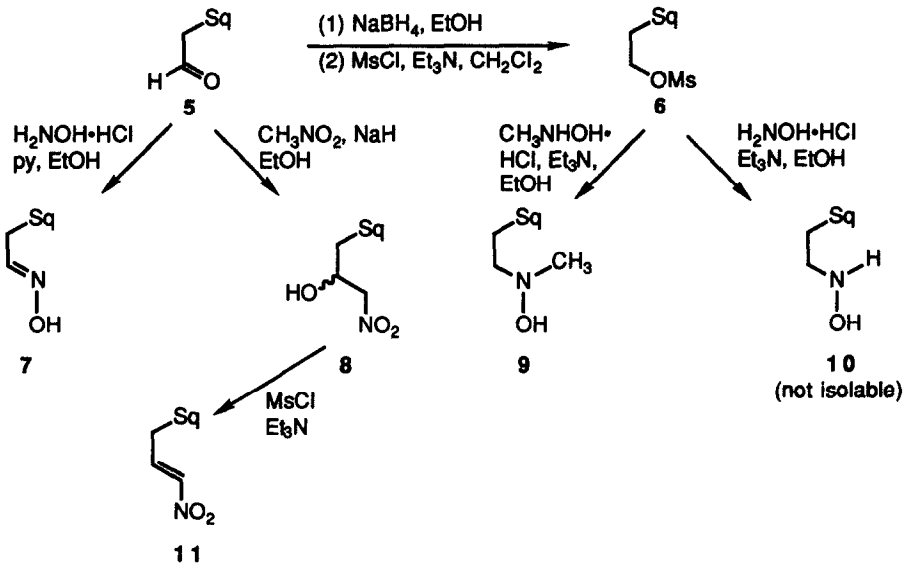
Squalene epoxidase (SE) and oxidosqualene cyclase (OSC) catalyze sequential committed steps of the sterol biosynthesis pathway and thus have been the focus of efforts to develop hypocholesterolemic, herbicidal, and antifungal agents.[3] Recently, trisnorsqualene alcohol **1** (TNSA)[4] and -cyclopropylamine **2**[5] have been reported as effective inhibitors of SE. A number of trisnorsqualene amines and amine oxides (e.g., **3** and **4**) have been investigated for inhibition of OSC.[6] As an extension of these studies, we prepared four squalenoid analogs bearing terminal N-O functionality: trisnorsqualene oxime **7**, hydroxy nitro bisnorsqualene **8**, trisnorsqualene methyl hydroxylamine **9**, and nitro bisnorsqualene **11** (Scheme I). The trisnorsqualene methyl hydroxylamine was found to be a potent combined inhibitor of SE and OSC.

1, R = OH
2, R = NH-cyclopropyl
3, R = $N(CH_3)_2$
4, R = $N(O)(CH_3)_2$



Synthesis. Our syntheses began with the known trisnorsqualene aldehyde **5**.[7] This aldehyde was converted to oxime **7** with hydroxylamine. Reduction of aldehyde **5** to its corresponding alcohol,[4] mesylation, and displacement with methyl hydroxylamine[8] afforded trisnorsqualene methyl hydroxylamine **9**. Similarly, mesylate **6** was reacted with hydroxylamine; however, we were unable to isolate the desired trisnorsqualene hydroxylamine **10**. This is probably due to the facile cyclization/oxidation of 5-alkenyl hydroxylamines reported by House *et al.*[9] Furthermore, hydroxylamines are known to initiate the autoxidation of squalene.[10]

Treatment of trisnorsqualene aldehyde with nitromethane anion provided hydroxy nitro bisnorsqualene **8**. Mesylation of **8** and elimination gave nitro bisnorsqualene **11**.[11]

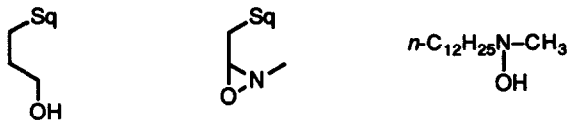


Inhibition of SE and OSC. The inhibitory potencies of compounds **7-9** and **11**, expressed as the concentrations at which a 50% decrease in the activity of either SE or OSC was observed, are shown in Table I. The inhibition assays were conducted with detergent-solubilized enzymes from pig liver microsomes,[12] using [^{14}C]squalene as the substrate, and the assay method previously described.[4]

Table I. IC_{50} Values for Squalene Analogs **7-9** and **11** Using Pig Liver SE and OSC.

	IC_{50} (μM)	
Compound	SE	SE + OSC
trisnorsqualene oxime **7**	No inhibition	No inhibition
hydroxy nitro bisnorsqualene **8**	270	200
trisnorsqualene methyl hydroxylamine **9**	13	5
nitrobisnorsqualene **11**	>400	>400

Trisnorsqualene methyl hydroxylamine **9** (TNS-MHA) was found to be a potent inhibitor of both SE and OSC. In one sense, TNS-MHA resembles TNSA (**1**)[4] and bisnorsqualene alcohol **12**,[13] both of which inhibited SE with an IC_{50} value of 4 μM.[13] It has been hypothesized that these two alcohols may exert their inhibitory effect on SE by mimicking the transition state of epoxide formation.[13] Likewise, TNS-MHA has a hydroxyl group that is spatially positioned in a manner similar to that of bisnorsqualene alcohol **12**. However, unlike TNS-MHA, neither of the two squalenoid alcohols **1** and **12** exhibited inhibition of OSC.



12 13 14

TNS-MHA also resembles the trisnorsqualene amine inhibitors of OSC (e.g., **3**).[6] In this comparison, TNS-MHA has an electron-withdrawing group (OH) attached to the nitrogen, whereas the amines have a nitrogen substituted with donor groups. This difference has a substantial effect on the basicity of the nitrogen atom; e.g., the pK_b of trimethylamine is 4.28, whereas that of hydroxylamine is 7.97.[14] The proposed mechanism of action of the trisnorsqualene amine inhibitors is that the protonated amine simulates the carbocationic species in an S_N1-like epoxide opening in the cyclization of oxidosqualene by OSC.[3b] Thus, the basicity of the amine would appear to be crucial. However, TNS-MHA is at least 1000-fold less basic than these amines, and thus would not be expected to act similarly.

With its N-O functionality, TNS-MHA is analogous to the stable amine oxide (e.g., **4**)[6,15] and the labile oxaziridine (**13**)[15] inhibitors of OSC. However, the amine oxides are zwitterionic and the oxaziridines are even less basic than the hydroxylamines.[16] Furthermore, the oxaziridines are configurationally stable at the nitrogen center, whereas in hydroxylamines the nitrogen can invert rapidly.[16] Thus, despite the superficial similarity of the hydroxylamine to the amine, amine oxide, and oxaziridine, their chemical nature is significantly different, and therefore the mechanisms of inhibition of OSC may be different as well.

To prove that the inhibition of OSC and SE by TNS-MHA is not merely a nonspecific interaction of the hydroxylamine group with the enzymes (e.g., electron donation to a radical center[8]), the C_{12} methyl hydroxylamine **14** was evaluated in the enzyme assays. This compound was prepared from the mesylate of *n*-dodecyl alcohol by displacement with methyl hydroxylamine. Compound **14** had an $IC_{50} > 400$ μM for both SE and SE + OSC, indicating that the trisnorsqualene backbone is essential for the activity exhibited by TNS-MHA. This behavior differs from that of the tertiary amine class of OSC inhibitors, in which such simple hydrocarbon chain analogs retain inhibitory action.[6]

Despite its close structural analogy to TNS-MHA, and to bisnorsqualene alcohol **12**, oxime **9** was not active against either enzyme. Hydroxy nitro bisnorsqualene **8** was a very poor inhibitor compared to TNSA. This is consistent with earlier observations that steric bulk appended distal to the hydroxyl group in squalenoid alcohols has a detrimental effect on the inhibitory activity against SE.[5] Nitro bisnorsqualene **11** could potentially function as an electrophilic affinity label for SE or OSC. However, this compound showed no inhibition. It has been suggested that polarization of the terminus in squalenoids abolishes the inhibition of SE and OSC,[17] although the terminal difluoro analog of squalene is an inhibitor of SE.[3c]

Conclusion. Trisnorsqualene methyl hydroxylamine (**9**) is a potent inhibitor of both SE and OSC. This compound resembles bisnorsqualene alcohol **12**,[13] which may explain its inhibition of SE. However, the chemical nature of the nitrogen atom in **9** is different from other amine inhibitors of OSC,

suggesting a novel mode of interaction of this enzyme. Despite the many compounds that have now been developed as inhibitors of SE and OSC,[3-5] few compounds are known that act on both enzymes.[18]

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References and Notes

1. Present address: College of Medicine, University of Illinois, Urbana, IL 61801.
2. Present address: Dept. of Chemistry, University of Washington, Seattle, WA 98195.
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