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VINYL OXIRANE ANALOG OF (3*S*)-2,3-EPOXYSQUALENE: A SUBSTRATE FOR OXIDOSQUALENE CYCLASES FROM YEAST AND FROM HOG LIVER

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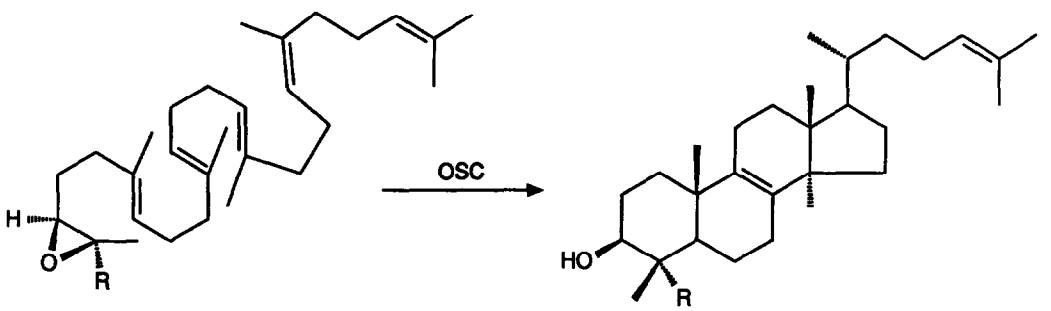
Summary: A vinyl oxirane analog of (3*S*)-2,3-oxidosqualene was synthesized as a potential mechanism-based inactivator of oxidosqualene cyclase. Although modest inhibition was observed, the tritiated substrate was efficiently converted to a new product with no detectable label in the enzyme fraction after ion-exchange separation of enzyme and inhibitor. This product was identified as the 4α-vinyl analog of lanosterol and is produced by both fungal and vertebrate oxidosqualene cyclase.

Oxidosqualene cyclase (OSC, EC 5.4.99.7) catalyzes the conversion of (3*S*)-2,3-epoxysqualene (**1a**) to lanosterol (**2a**) (or cycloartenol) in vertebrates, plants, and fungi. This enzyme also cyclizes the (3*S*)-enantiomer of a variety of polyenic epoxide analogs of squalene within certain narrow steric constraints at the initiating center.[1,2] Most recently, Kyler and co-workers[3] have employed the OSC activity of sonicated baker's yeast suspensions to perform preparative, enantiospecific syntheses of novel sterols, including the production of the 4α-hydroxymethyl analog of lanosterol (**2b**) from precursor **1b**. We now report the synthesis and cyclization of the *trans*-vinyl substituted analog **1c** of (3*S*)-2,3-epoxysqualene, a material which had been prepared as a putative mechanism-based inactivator of OSC.



1a R = CH_3
1b R = CH_2OH
1c R = $CH{=}CH_2$
1d R = $CT{=}CH_2$

2a R = CH_3
2b R = CH_2OH
2c R = $CH{=}CH_2$
2d R = $CT{=}CH_2$

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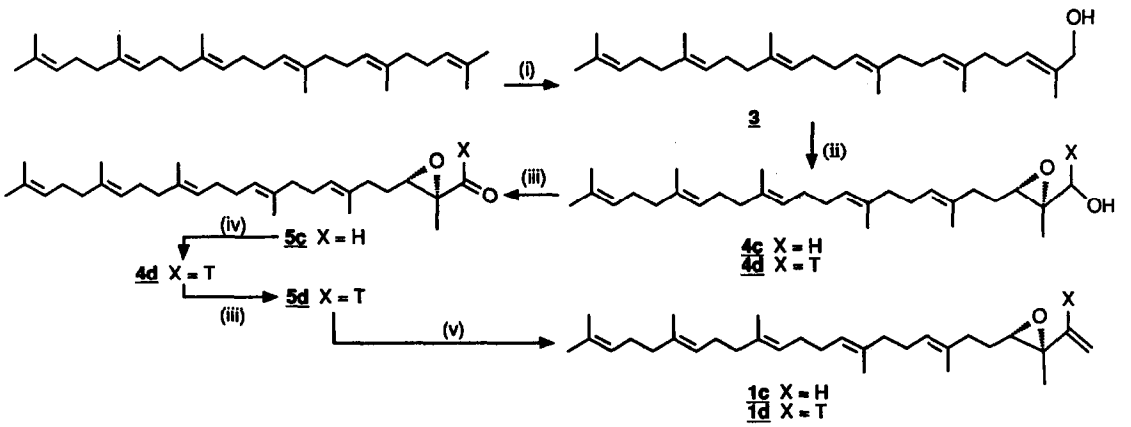
Synthesis of the vinyl oxirane 1c was accomplished by asymmetric epoxidation[4] of the squalene-derived allylic alcohol[5] 3 followed by TPAP oxidation[6] and Wittig olefination.[7] The tritium-labeled vinyl oxirane was prepared by reduction of aldehyde 5c with sodium borotritide followed by TPAP oxidation and Wittig olefination. The labeled material had specific activity 2 Ci/mmol.



(i) SeO_2 , t-BuOOH , CH_2Cl_2 ; (ii) $Ti(i\text{-}OPr)_4$, (+)-DET , t-BuOOH , CH_2Cl_2 ; (iii) CrO_3 , pyridine , CH_2Cl_2 ; (iv) $NaBT_4$, EtOH ; (v) $CH_2{=}P(Ph)_3$, THF

The inhibitory potency and time dependency of inhibition was tested using solubilized OSC from pig liver microsomes as previously described.[8] The IC_{50} value for vinyl oxirane 1c was 200 μM. At this concentration, inhibition reached a constant level within 5 min. When inactivated enzyme was placed on a DEAE anion exchange column, the inhibitor was separated from the enzyme and enzyme activity was fully restored. This was achieved by stepwise elution with 25 mM KCl and then with 200 mM KCl in a 20 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 1 mM DTT, and 0.5% Triton X-100.

The tritium-labeled vinyl oxirane 1d was employed to test for a low level of irreversible inhibition and to determine whether cyclization was occurring in the hog liver microsomes. Thus, after incubation of 200 μM of labeled 1d for 10 min, the mixture was placed on a DEAE column pre-equilibrated with 25 mM KCl buffer (as above). Unreacted inhibitor eluted in the void volume, while enzymatic activity was retained until elution with 200 mM KCl. In several experiments at lower concentrations, no counts could be observed in the enzyme fraction. Even at this high substrate concentration, over 99% of the recovered counts were in the void volume, and less than 0.5% were associated with the OSC-active fraction.

Next, we determined that 75% of the radioactivity was in the substrate 1d and 25% was in a new product eluting near lanosterol on TLC. To obtain an authentic sample of this suspected cyclization product, we employed the yeast cyclization protocols developed by Kyler. Thus, 34 mg of 1c was incubated at room temperature for 70 hr with 34 ml yeast solution (0.1 g baker's yeast / ml, 0.1 M phosphate buffer, pH 7.4, activated by sonication for 1.5 hr at -12 °C). The products were isolated by column chromatography after quenching and saponification with 10% KOH in MeOH followed by ether extraction. Control yeast cyclizations were performed with active enzyme and 2,3-oxidosqualene, boiled enzyme with 1c and with active enzyme with no added substrate. Neither inactive enzyme with 1c nor live enzyme alone produced any detectable new sterol products. Spectral data for the product isolated in 28% yield from yeast cyclization of 1c were completely consistent with the 4α-vinyl lanosterol structure 2c. Comparison of this compound with the hog liver cyclization product (GC, MS) unambiguously defined 2c as the product of the vertebrate OSC as well. This structural assignment was confirmed by the conversion of an authentic sample of 4α-hydroxymethyllanosterol (2b)[3b] to the vinyllanosterol 2c.[9]

Oxidosqualene cyclase can be inhibited by product analogs, such as the *trans*-decalols[10], and by transition state mimics of the incipient cationic charge at C-4 (steroid numbering), notably the tertiary amine N-oxides.[11] Vinyl oxiranes have previously been shown to be competitive, reversible inhibitors of microsomal and cytosolic epoxide hydrases of mouse liver.[12] In rat liver, an epoxide hydrase specific for cholesterol 5,6-epoxide hydration was irreversibly inactivated by an alkenyl oxirane analog, 7-dehydrocholesterol 5,6β-epoxide.[13] In this case, the transient allylic cation was trapped by an active site nucleophile instead of undergoing normal trapping by a water molecule. In contrast to this inhibitory effect of an incipient allylic cation, Johnson and co-workers found increased cyclization when a 3-methyl-2-butenyl substituent was introduced at C-10 (steroid numbering); this effect was attributed to stabilization of the transient cationic species occurring during polycyclization.[14] Apparently, the incipient allylic cation generated by OSC action on the vinyl oxirane 1c facilitates cyclization, rather than irreversibly inhibiting the enzyme catalyzing the epoxide opening.

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9. A sample of 4α-hydroxymethyllanosterol, generously provided by K.S. Kyler, was selectively oxidized to the 3-ketone by the action of $RuCl_2(PPh_3)_3$/benzene[9a], protected as the ketal with ethylene glycol in the presence of PPTS, oxidized to the aldehyde by PDC, methylenated with methyltriphenylphosphonium bromide, deprotected by treatment with wet acetone/PPTS, and epimer-selectively reduced with $LiAlH_4$/ether at room temperature[9b] to afford authentic 4α-vinyllanosterol; this was identical by spectroscopic (^1H NMR and MS) and chromatographic (TLC and DB-1 capillary GC) comparisons with the material obtained in our cyclizations. References: (9a) Tomioka, H., Takai, K., Oshima, K., and Nozaki, H., *Tetrahedron Lett.*, **1981**, *22*, 1605-1608; (9b) Shoppe, C.W. and Summers, G.H.R., *J. Chem. Soc.*, **1950**, 687-689.

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