Registry No. 1 (coordinate entry), 137203-49-7; 1 (salt entry), 137203-52-2; 2 (coordinate entry), 137203-50-0; 2 (salt entry), 137203-53-3; 3 (coordinate entry), 137203-51-1; 3 (salt entry), 137203-54-4.

Supplementary Material Available: Atomic numbering schemes and tables of crystallographic data, atomic positional and thermal parameters, bond lengths and angles, and selected torsion angles for the three mixed-alkali HMDS dimers 1-3 (44 pages). Ordering information is given on any current masthead page.

29-Methylidene-2,3-oxidosqualene: A Potent Mechanism-Based Inactivator of Oxidosqualene Cyclase

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The cyclization of (3*S*)-2,3-oxidosqualene to lanosterol by oxidosqualene cyclase (OSC) (EC 5.4.99.7) has fascinated organic chemists for over 30 years.[1] Substrate studies using crude liver microsomes suggested that partially cyclized cationic species were involved in the enzymatic mechanism.[2] Inhibitors of OSC have been examined with crude solubilized microsomes with OSC activity from plants, fungi, and vertebrates[3] and in cell culture systems.[4] The known OSC inhibitors include (i) substrate mimics (e.g., 2,3-iminosqualene[5]), (ii) product mimics (e.g., the decalols[6]), or (iii) transition-state analogues.[7] The last group includes mimics of the initial acyclic C-2 cation as well as mimics of partially cyclized bicyclic cations.[8] However, as yet, no irreversible inhibitors have been reported. We describe herein the synthesis and biological activity of 29-methylidene-2,3-oxidosqualene (29-MOS, **1a**), the first mechanism-based irreversible inactivator of OSC.

Scheme I summarizes the synthesis of the 26- and 29-methylidene-2,3-oxidosqualenes and the corresponding bis(epoxide).[9] Aldehydes **2a** and **2b**[10] were converted[11] to the unsaturated esters **3a,b** (*Z*:*E* = 44:1) and reduced, and the allylic alcohols were separated to give 26-hydroxysqualene (**4**). The two terminal monobromohydrins **5a** (11%) and **5b** (30%) and the bis(bromohydrin) **5c** (13%) were processed independently by base-induced oxirane formation, allylic oxidation,[12] and olefination to provide the isomeric 29-MOS (**1a**) and 26-MOS (**1b**) and bis(epoxide) **1c**.

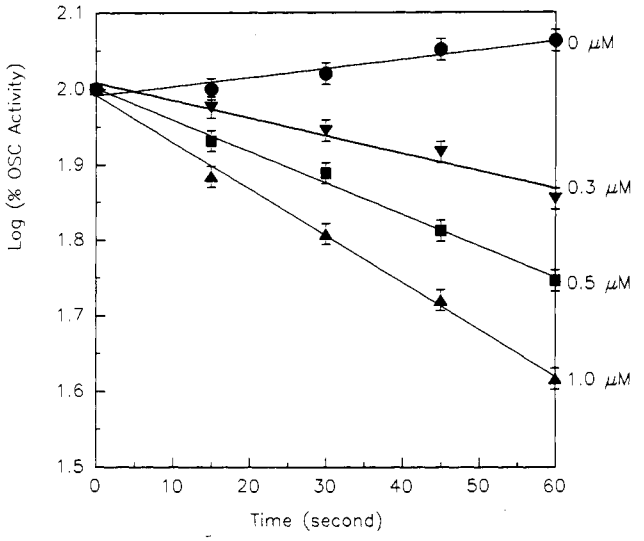

Figure 1. Time dependency of inactivation of pig liver microsomal OSC by 29-MOS (**1a**).

Enzyme assays to measure inhibition of OSC used [^{14}C]-(3*S*)-2,3-oxidosqualene[13] as substrate and either solubilized microsomal protein from pig liver[14] or a sonicated bakers' yeast suspension.[15] Conversion was determined by radio-TLC, and reversibility was determined using DEAE chromatography to separate the enzyme from the inhibitor.[16] The IC_{50} values for inhibition of liver OSC at 20 μM substrate were determined to be 0.5, 78, and 1.6 μM for **1a**, **1b**, and **1c**, respectively.[17] Note that methylidene substitution at the 26-position is 100-fold less potent than at the 29-position, but the 22,23-epoxide only reduces the potency of 29-MOS 3-fold. Most importantly, only the 29-substituted 2,3-epoxide **1a** and the bis(epoxide) **1c** showed irreversible inhibition of OSC.

The inhibition of microsomal OSC by 29-MOS (**1a**) showed an apparent K_I value of 4.4 μM. The time dependence of inhibition at [29-MOS] = 1, 0.5, and 0.3 μM allowed determination of the k_{inact} value of 221 min^{-1} for liver OSC (Figure 1),[18] of the same magnitude as that expected for k_{cat} for oxidosqualene. A partition ratio of 3.8 was calculated for 29-MOS by measuring the decrease in OSC activity at increasing 29-MOS concentrations.

Cyclization reactions of [^{3}H]-29-MOS (**1a**, T = ^{3}H), [^{3}H]-**1b**, and [^{3}H]-**1c** were followed by radio-TLC.[19] Incubation of 0.1 μM [^{3}H]-29-MOS (specific activity = 2.3 Ci/mmol[19]) with pig liver microsomes or with sonicated bakers' yeast suspension gave a new polycyclic product in yields of 30% and 15%, respectively. At [29-MOS] > K_I, complete inactivation precluded isolation of product. On the basis of the regiospecificity of the methylidene substitution for inhibition, we propose that this product is the 21-methylidenelanosterol. Similarly, cyclization of **1b** also gave

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(9) All compounds in Scheme I are racemic mixtures. New compounds have IR, NMR, and mass spectra fully consistent with the indicated structures. Full experimental details are provided in the supplementary material.

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(16) Dialysis is ineffective in removing lipophilic inhibitors from OSC. Reactivation of squalene epoxidase inhibited by trisnorsqualene cyclopropylamine and other inhibitors was accomplished similarly: (a) Sen, S. E.; Prestwich, G. D. *J. Am. Chem. Soc.* **1989**, *111*, 8761-8762. (b) Bai, M.; Prestwich, G. D., submitted manuscript.

(17) For yeast OSC, IC_{50} values of 1.5 μM (**1a**), 19 μM (**1b**), and 11 μM (**1c**) were observed.

(18) Analyses were performed using Lineweaver-Burk and Kitz-Wilson plots. The K_I values for liver OSC were 122 μM (**1b**) and 7.1 μM (**1c**); the k_{inact} value for **1c** was 113 min^{-1}. Assays with **1a** and **1c** with short (10, 30, 45, 60 s) incubation periods were required to obtain these data.

(19) High specific activity [^{3}H]-29-MOS had to be used to detect cyclization at this low concentration. Reduction of **7a** (T = H) with [^{3}H]sodium borohydride to **6a** (T = ^{3}H), oxidation, and methylenation gave 5.4 mCi of [^{3}H]-29-MOS (2.3 Ci/mmol).

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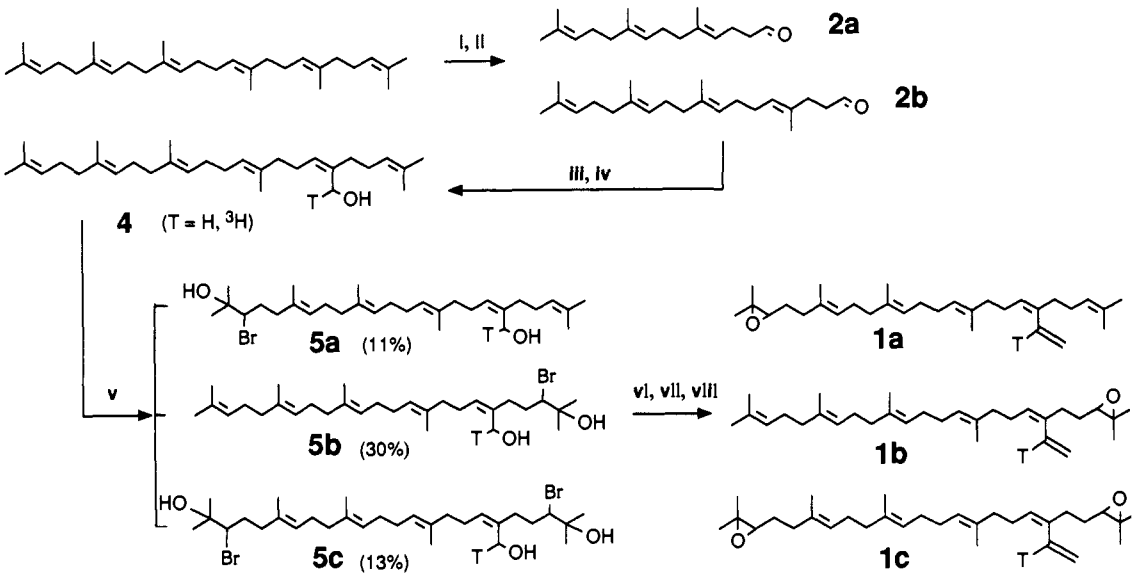
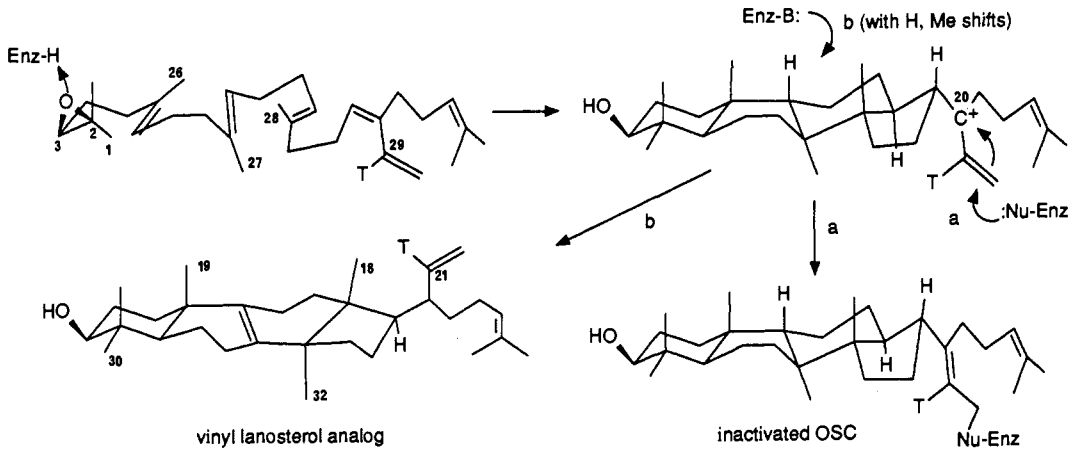
Scheme I. Synthesis of 29-Methylidene-2,3-oxidosqualene[a]



[a] Reagents and conditions: (i) 1.0 equiv of MMPP, THF–H_2O (3:4), 25 °C, 48 h, 26%; (ii) 1.0 equiv of H_5IO_6, THF–H_2O (3:1), 0–20 °C, 10 h, 86%; (iii) $(CF_3CH_2O)_2P(O)CH(CO_2CH_3)CH_2CH_2CH{=}C(CH_3)_2$, $KN(TMS)_2$, 18-crown-6, −78 °C, 2 h, 96%; (iv) $LiAlH_4$, 0 °C, 1 h, 77%; octadecylsilyl-silica gel, gradient 70–100% CH_3CN–H_2O; (v) 1.0 equiv of *N*-bromosuccinimide, THF–H_2O (3:1), 0 °C, 3 h; (vi) K_2CO_3, CH_3OH, 20 °C, 0.5 h, 75–80%; (vii) MnO_2, Na_2CO_3, hexane, 20 °C, 35 h, 60–70%; (viii) $Ph_3P{=}CH_2$, −78 to 0 °C, 1 h, 92–100%.

Scheme II. Proposed Mechanism of Cyclization and OSC Inactivation by 29-MOS (**1a**)



a 21% yield of a tetracyclic product tentatively assigned as 19-methylidenelanosterol.[20]

We postulate that inhibition and cyclization both occur through a common intermediate, as illustrated in Scheme II. Normal cyclization of 29-MOS (**1a**) can occur to give a tertiary C-20 cation, which may undergo the usual hydride and methyl migrations and proton loss to a lanosterol analogue (path b), or it can be trapped by an active-site nucleophile (path a). Allylic stabilization of incipient carbocationic species during polycycle formation has precedence in the biomimetic cyclization of butenyl-substituted polyolefins[21] and by the ability of 20,21-dehydrosqualene to undergo conversion to dehydroprotolanosterol.[22] Irreversible inhibition of cholesterol 5,6β-epoxide hydrase by 7-dehydrocholesterol 5,6β-oxide may also involve an allylic cation.[23] In addition to the C-26 and C-29 substitutions described here, the C-1, C-27, and C-28 methylidene analogues of 2,3-oxidosqualene cyclize to 31-methylidenelanosterol,[24] cyclize with vinyl migration,[25] or fail to cyclize,[26] respectively. The use of [^{3}H]-29-MOS for stoichiometric, covalent modification of the active site and identification of active-site residues in purified liver OSC is in progress.

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Supplementary Material Available: Experimental details for the synthesis and enzyme assays (10 pages). Ordering information is given on any current masthead page.

(20) The cyclization product had the same mobility as lanosterol (R_f = 0.42, 10% EtOAc/hexane, radio-TLC). Moreover, 26-hydroxy- and 29-hydroxy-2,3-squalene epoxide isomers are efficiently cyclized to the 21-hydroxy- and 19-hydroxylanosterol isomers, respectively (Xiao, X-y.; Prestwich, G. D. *Tetrahedron Lett.*, in press). The bis(epoxide) was also a substrate, but the product was not further characterized.

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