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

Xiao-yi Xiao and Glenn D. Prestwich*

Department of Chemistry, State University of New York Stony Brook, New York 11794-3400

Received July 26, 1991

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**.

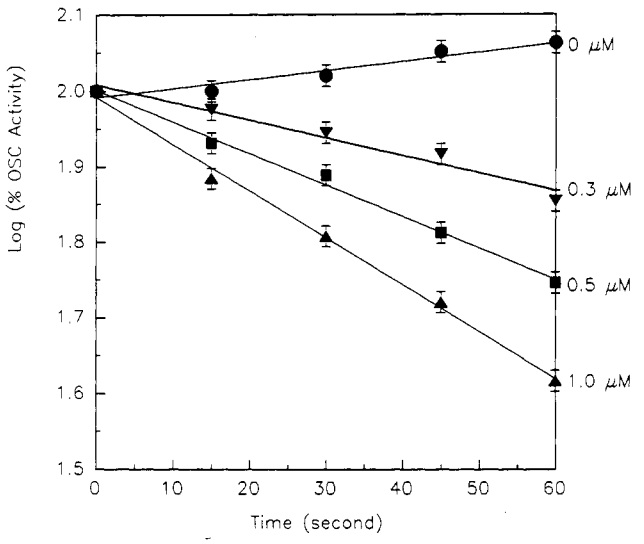


Figure 1. Time dependency of inactivation of pig liver microsomal OSC by 29-MOS (**1a**).

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

(1) (a) Stork, G.; Burgstahler, A. W. *J. Am. Chem. Soc.* **1955**, *77*, 5068-5077. (b) Stadler, P. G.; Eschenmoser, A.; Shenz, H.; Stork, G. *Helv. Chim. Acta* **1957**, *40*, 2191-2198. (c) Johnson, W. S. *Acc. Chem. Res.* **1968**, *1*, 1-8. (d) Johnson, W. S. *Bioorg. Chem.* **1976**, *5*, 51-98. (e) van Tamelen, E. E. *Acc. Chem. Res.* **1975**, *8*, 152-160.

(2) van Tamelen, E. E. *J. Am. Chem. Soc.* **1982**, *104*, 6480-6481,

(3) Cattel, L.; Ceruti, M.; Balliano, G.; Viola, F.; Grosa, G.; Schuber, F. *Steroids* **1989**, *53*, 363-391.

(4) Popjak, G.; Meenan, A.; Nes, W. D. *Proc. R. Soc. London, B* **1987**, *232*, 273-287.

(5) Corey, E. J.; Ortiz de Montellano, P. R.; Lin, K.; Dean, P. D. G. *J. Am. Chem. Soc.* **1967**, *89*, 2797-2799.

(6) (a) Nelson, J. A.; Czarny, M. R.; Spencer, T. A.; Limanek, J. S.; McCrae, K. R.; Chang, T.-Y. *J. Am. Chem. Soc.* **1978**, *100*, 4900-4902. (b) Chang, T.-Y.; Schiavoni, E. S.; McCrae, K. R.; Nelson, J. A.; Spencer, T. A. *J. Biol. Chem.* **1979**, *254*, 11258-11264. (c) Raveendranath, P. C.; Newcomb, L. F.; Ray, N. C.; Clark, D. S.; Spencer, T. A. *Synth. Commun.* **1990**, *20*, 2723-2733.

(7) Ceruti, M.; Delprino, L.; Cattel, L.; Bouvier-Navé, P.; Duriatti, A.; Schuber, F.; Benveniste, P. *J. Chem. Soc., Chem. Commun.* **1985**, 1054-1055.

(8) Duriatti, A.; Bouvier-Navé, P.; Benveniste, P.; Schuber, F.; Delprino, L.; Balliano, G.; Cattel, L. *Biochem. Pharmacol.* **1985**, *34*, 2765-2777.

(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.

(10) Ceruti, M.; Viola, F.; Dosio, F.; Cattel, L.; Bouvier-Navé, P.; Ugliengo, P. *J. Chem. Soc., Perkin Trans. 1* **1988**, 461-469.

(11) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405-4407.

(12) (a) Xiao, X-y.; Prestwich, G. D. *Synth. Commun.* **1990**, *20*, 3125-3130; (b) Bai, M.; Xiao, X-y.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 227-232.

(13) Xiao, X-y.; Prestwich, G. D. *J. Labelled Compd. Radiopharm.* **1991**, *29*, 883-890.

(14) Sen, S. E.; Prestwich, G. D. *J. Med. Chem.* **1989**, *32*, 2152-2158.

(15) Bujons, J.; Guajardo, R.; Kyler, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 604-606.

(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.

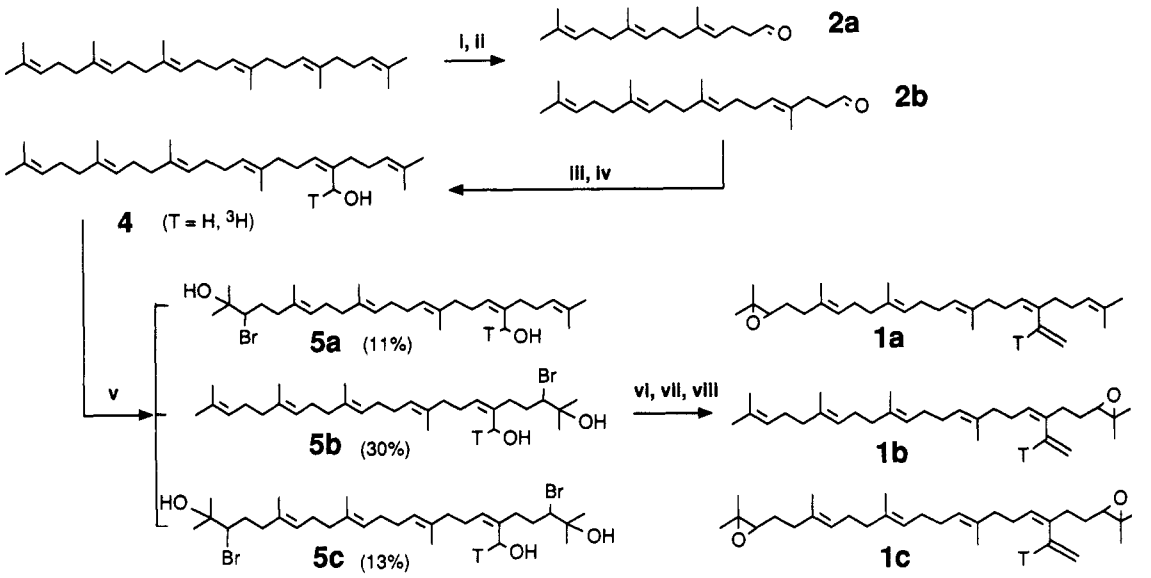
(17) For yeast OSC, IC_{50} values of 1.5 μM (**1a**), 19 μM (**1b**), and 11 μM (**1c**) were observed.

(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).

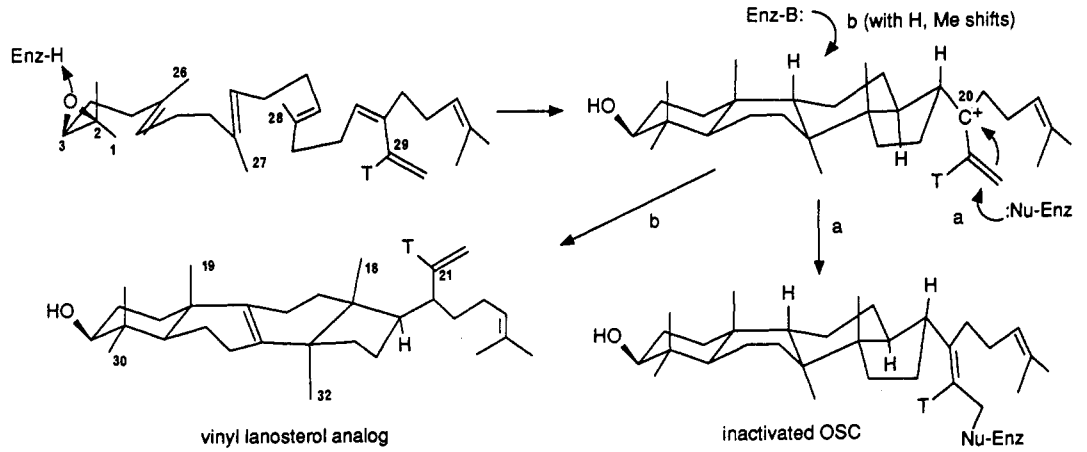
0002-7863/91/1513-9673$02.50/0

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.

Acknowledgment. We thank the NSF (CHE-8809588), the Center for Biotechnology, the New York State Foundation for Science and Technology, Kirin Brewery, Inc., and Sandoz Research Institute for financial support during different stages of this project. Pig liver microsomes were generously provided by M. Bai (Stony Brook).

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.

(21) (a) Johnson, W. S.; Lindell, S. D.; Steele, J. *J. Am. Chem. Soc.* **1987**, *109*, 5852–5853. (b) Johnson, W. S.; Telfer, S. J.; Cheng, S.; Schubert, U. *J. Am. Chem. Soc.* **1987**, *109*, 2517–2518.

(22) Corey, E. J.; Lin, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1969**, *91*, 2132–2134.

(23) Nashed, N. T.; Michaud, D. P.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1986**, *261*, 2510–2513.

(24) Xiao, X-y.; Prestwich, G. D. *Tetrahedron Lett.* **1990**, *31*, 2097–2100.

(25) Medina, J. C.; Guajardo, R.; Kyler, K. S. *J. Am. Chem. Soc.* **1989**, *111*, 2310–2311.

(26) K. S. Kyler, personal communication.