Squalene Analogues Containing Isopropylidene Mimics as Potential Inhibitors of Pig Liver Squalene Epoxidase and Oxidosqualene Cyclase

Stephanie E. Sen and Glenn D. Prestwich*

Department of Chemistry, State University of New York, Stony Brook, New York 11794-3400. *Received December 19, 1988*

Several squalene analogues containing 1,1-dihaloalkene, acetylene, allene, diene, and cyclopropane functionalities were synthesized and evaluated as potential inhibitors of pig liver squalene epoxidase and oxidosqualene cyclase. Both monofunctionalized and bisfunctionalized analogues were prepared. Poor inhibition of squalene epoxidase and oxidosqualene cyclase was found for most compounds ($IC_{50} \gg 400$ μM), with the exception of the monofunctionalized alkynol ($IC_{50} = 300$ μM). This alkynol showed mixed-function inhibition with $K_I = 0.95$ mM. Oxidation of the alcohol to the alkynone resulted in loss of epoxidase activity, indicating that the hydroxyl group is necessary for inhibition and that the alkynol is not a proinhibitor. Molecular mechanics calculations indicated that a good inhibitor should possess hydrophobic substituents on an unpolarized, unsaturated system; additionally, the presence of a pro-C-3 hydroxyl group can confer inhibitory potency.

Elevated levels of cholesterol are associated with atherosclerosis. Several drugs have been found to effectively lower blood serum cholesterol levels; however, many have serious side effects. Compactin and its analogues,[1] a variety of oxysterols,[2] and several synthetic agents which inhibit HMG-CoA reductase[3] can perturb the synthesis of important isoprenoid-containing biological precursors such as isopentenyladenine.[4] Triparanol and 20,25-diazacholesterol, which inhibit Δ^{24}-reductase during the final stages of cholesterol biosynthesis, allow the accumulation of desmosterol, thus causing deleterious effects, including myotonic lystrophy.[5]

An attractive approach is the development of drugs which selectively block the conversion of the acyclic polyolefin squalene to the tetracyclic steroid lanosterol. The two enzymes involved in this process are squalene epoxidase (SE) and oxidosqualene cyclase (OSC). Squalene epoxidase (EC 1.14.99.7) catalyzes the conversion of squalene to (3*S*)-2,3-oxidosqualene.[6] In addition to oxygen, squalene epoxidase requires flavin adenine dinucleotide (FAD), cytochrome P-450 reductase, NADPH, and a soluble protein factor for activity.[7] Purified SE does not appear to be a heme-containing enzyme and no metals have been implicated in the active site.[8]

The second enzyme, oxidosqualene cyclase (EC 5.4.99.7), has not been as well characterized, although many substrate analogues have been prepared and subjected to enzymatic cyclization conditions.[9] Unlike squalene epoxidase, oxidosqualene cyclase has not been purified to homogeneity; moreover, no cofactors or protein factors are required for activity.[10] Chemical modification and partial purification of hog liver OSC has provided good evidence that a thiol residue is present at the active site of this enzyme.[11] Vertebrate OSC is believed to bind (3*S*)-2,3-oxidosqualene in a chair–boat–chair conformation and to mediate the sequential formation of four rings through a series of rigidly held carbocationic intermediates.[9,12] The initial cyclization product, protolanosterol, then undergoes several hydride and methyl shifts, followed by proton loss to produce lanosterol.[13]

Several reversible inhibitors of SE and OSC are known, but as yet no effective mechanism-based inactivators have been described.[14] The first reported OSC inhibitor was 2,3-iminosqualene, a simple aza analogue of 2,3-oxidosqualene. This analogue had a micromolar IC_{50} for the pig and rat liver enzymes, a value which is yet to be equalled.[15] The product analogues of the *trans*-decalol group[16] also show modest inhibition of cyclase action. Several quaternary ammonium species[17,18] and trialkylammonium *N*-oxides[19-21] are high-energy intermediate analogues which effectively mimic the ionic "transition state" of epoxide opening.

No selective and general inhibitors of SE are known for the vertebrate enzymes. Several members of a class of allylamines discovered at Sandoz (e.g., terbinafine and naftifine) effectively inhibit SE in a wide spectrum of fungi.[22] The most potent agent, terbinafine, showed a K_I value of 30 nM for *Candida albicans* SE and showed good inhibition of guinea pig SE ($IC_{50} = 6$ μM); however, it was essentially inactive toward rat liver enzymes ($IC_{50} = 93$ μM).[23]

While many mechanism-based inhibitors of the alkene-epoxidizing cytochrome P-450 monoxygenases are known,[24] there are no examples of active site-directed in-

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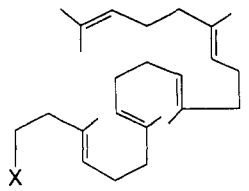
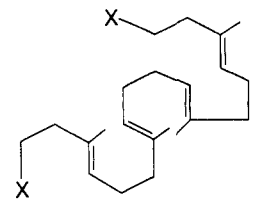
Chart I. Squalene Analogues 1–16



		COMPOUND
X=	$CH{=}CBr_2$	1
	$CH{=}CCl_2$	2
	$C{\equiv}CH$	3
	$CH(OH)C{\equiv}CH$	4
	$CH{=}C{=}CH_2$	5
	CH=◁	6
(*E*)-	$CH{=}C(CH_3)CH{=}CH_2$	7
(*E*)-	$CH{=}C(CH_3)$◁	8
	$CH(O)C{\equiv}CH$	4b



		COMPOUND
X=	$CH{=}CBr_2$	9
	$CH{=}CCl_2$	10
	$C{\equiv}CH$	11
	$CH(OH)C{\equiv}CH$	12
	$CH{=}C{=}CH_2$	13
	CH=◁	14
(*E*)-	$CH{=}C(CH_3)CH{=}CH_2$	15
(*E*)-	$CH{=}C(CH_3)$◁	16

hibitors of flavoprotein monooxygenases.[25,26] This paucity of inhibitors may be attributed, in part, to an inadequate understanding of the catalytic mechanism of this unique non-heme alkene epoxidase.

In this paper, we describe the application of the mechanistic principles used in the development of selective and irreversible cytochrome P-450 inhibitors to prepare potential mechanism-based inhibitors of SE and OSC. While squalene epoxidase appears to be an external flavoprotein monooxygenase, the mode of oxygenation remains unclear. The inherent symmetry of squalene necessitated the investigation of both mono- and bisfunctionalized analogues, i.e., molecules in which the isopropylidene group is replaced with a sterically or electronically perturbed π electron containing system at one or both ends of the polyolefinic chain. These analogues were envisaged as either suicide substrates or prosuicide substrates. That is, each should be capable of inactivating either the epoxidase or the cyclase following catalytic conversion to an activated oxirane species.

Results and Discussion

Design and Synthesis of Squalene Analogues. The molecules which were synthesized for the enzymatic assays are listed in Chart I and include 1,1-dihaloalkenes **1**, **2**, **9**, and **10**, alkynes **3** and **11**, alkynols **4** and **12**, allenes **5** and **13**, cyclopropylidenes **6** and **14**, dienes **7** and **15**, and alkenyl cyclopropanes **8** and **16**. For each electronic or steric modification of the terminal isopropylidene moiety, both the mono- and bisfunctionalized compounds were prepared. Since squalene possesses an internal symmetry imposed by the head-to-head coupling of two farnesyl units, it is possible for the epoxidase active site to "avoid" the modified terminus of a monofunctionalized analogue by simply binding with the unmodified terminus at the monooxygenation site.

In principle, each of these analogues can act as an enzymatically activated inhibitor in two ways: (i) direct irreversible ("suicide") modification of the active site or (ii) irreversible modification of the second enzyme in the cascade ("prosuicide" mode). The intermediates formed by enzymatic oxygenation of some isopropylidene analogues could be sufficiently reactive to bind to SE or OSC active sites. Similarly, modes of activation and irreversible binding to specific cytochrome P-450 enzymes by acetylenic,[29] alkynic,[30] and allenic[30] functionalities have been described. The other compounds may not be reactive enough in their epoxidized form and may require epoxide ring opening by OSC to generate the reactive species. For one of the isopropylidene mimics **1–16** to function as a potent irreversible inhibitor of SE or OSC, it must be accepted as a pseudosubstrate by SE and it must then interact with an available nucleophile at or near the catalytic site.

The extensive studies of the last twenty years by the van Tamelen group clearly established several key structural requirements for acceptance of a substrate by OSC.[9] The presence of a *cis*-methyl group was required; both the proton and ethyl in the C-2 cis position were poorly recognized. However, considerable latitude for cyclization of mimics with substitution in the trans orientation at C-2 of the oxirane ring was found for the vertebrate enzyme[31,32] and the fungal enzyme.[33] Initially, we focused on preserving these geometrical constraints when choosing isopropylidene analogues to prepare as pseudosubstrates for SE.

The analogues were synthesized from one of two routes. First, squalene was converted to the trisnorsqualene aldehyde via selective bromohydrin formation, epoxide ring closure, and periodic acid cleavage as described earlier.[34] Under suitable conditions, the bis-terminal bromohydrins could be obtained and converted to hexanorsqualene dialdehyde. The dibromo- and dichloroalkenes **1** and **2** (and their bisfunctionalized analogues **9** and **10**) were prepared using the Wittig reagent derived from the corresponding carbon tetrahalide and triphenylphosphine.[35] Base-induced eliminations of the dibromoalkenes[35] provided the two alkynes **3** and **11**, while alkynols **4** and **12** were obtained by addition of lithium acetylide to the appropriate aldehydes. The allenes **5** and **13** were prepared by treatment of the corresponding alkynols with $AlCl_3/LiAlH_4$.[36] The cyclopropylidene analogues **6** and **14** were obtained by condensation of the aldehydes with cyclopropylidene triphenylphosphorane.

A second route for synthetic modification involved selective production of the terminal *E* allylic alcohol (Figure 1). Incorporation of the diene functionality in the monofunctionalized analogue was accomplished by SeO_2 oxidation[37] of squalene to give the *E* allylic alcohol with high

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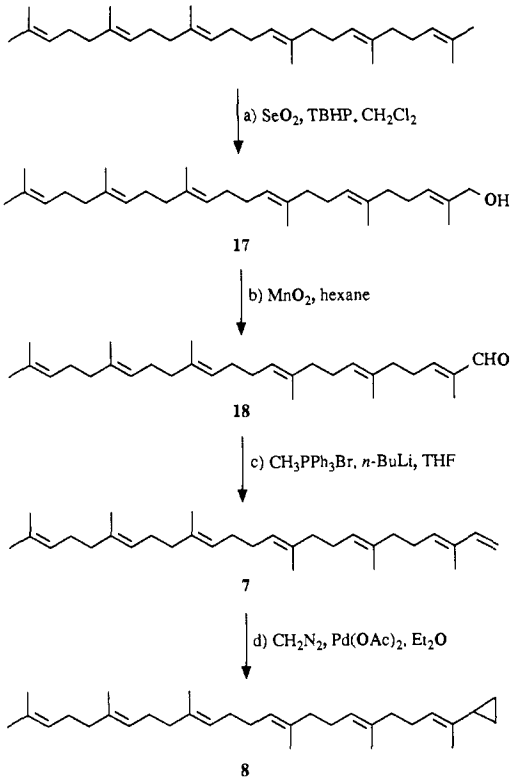

Figure 1.

stereoselectivity but in low (<10%) yield. Oxidation of the allylic alcohol to the *E* enal, followed by methylidenation produced diene analogue **7**. The alkenyl cyclopropane **8** was obtained in near quantitative yield by regioselective cyclopropanation[38] of diene **7** using $Pd(OAc)_2$–diazomethane.

The bisfunctionalized analogues **15** and **16** were more difficult to synthesize. Since the SeO_2 oxidation of squalene could not be optimized to produce a sufficient quantity of the bis-allylic alcohol, another route was chosen (Figure 2). Thus, Wittig–Horner condensation[39] of hexanorsqualene dialdehyde with triethyl-2-phosphonopropionate afforded a mixture of 2*E*,22*E*, 2*Z*,22*E*, and 2*Z*,22*Z* unsaturated diesters in a 70:20:10 ratio. After hydride reduction of the diesters, the crude bis-allylic alcohols could be partially separated by a flash chromatography to remove the unwanted *Z*,*Z* isomer and to increase the *E*,*E*/*Z*,*E* isomeric ratio to 88:12. Next, oxidation of the partially purified bis-allylic alcohols with MnO_2, followed by Wittig coupling and cyclopropanation, produced the crude bis-diene **15** and bis-alkenyl cyclopropane **16** from which the undesired *Z*,*E* isomer could be removed by preparative reverse-phase chromatography.

Studies of Enzyme Inhibition. The inhibitory potencies of analogues **1–16**, expressed as the concentrations at which 50% inhibition of either SE or OSC activity was observed, are presented in Table I. Essentially no inhibition of the turnover of [^{14}C]squalene can be seen for either SE or OSC for the majority of these analogues. The only squalene analogues which show appreciable inhibition are alkynol **4**, monofunctionalized diene **7**, and alkenylcyclopropane **8**.

To explain the apparent lack of interaction of these synthetic analogues with SE, we employed MacroModel

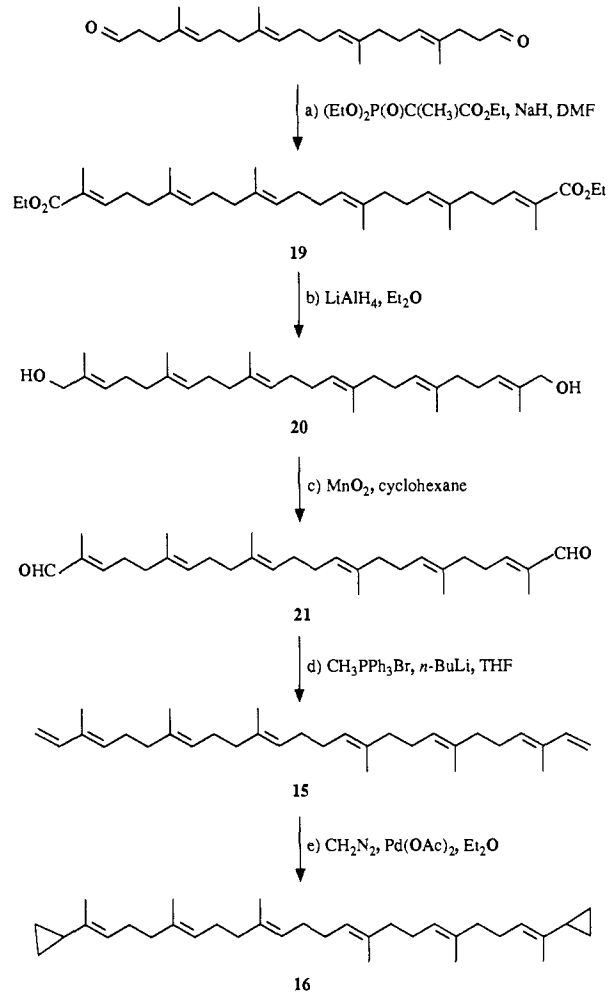

Figure 2.

Table I. IC_{50} and K_I Values for Squalene Analogues 1–16[a]

squalene analogue	IC_{50}, μM (SE, OSC)	K_I, mM (SE)	squalene analogue	IC_{50}, μM (SE, OSC)	K_I, mM (SE)
1	ni, ni		9	ni, ni	
2	ni, ni		10	>400, 400	
3	ni, >400		11	>400, >400	
4	400, 300	0.95	12	>400, >400	1.0
5	ni, ni		13	ni, >400	
6	>400, >400		14	ni, ni	
7	>400, 300		15	ni, >400	
8	>400, 300		16	ni, ni	
			4b	ni	

[a] Analogues with IC_{50} that were >400 μM showed some inhibition at high [I], while compounds with IC_{50} noted at ni showed essentially no inhibitory effect at 400 μM.

(version 1.5)[40] to compare the geometrical, steric, and charge differences of the modified isopropylidene moieties to those of the squalene isopropylidene group. First, hydrophobic regions that mimic the terminal methyl groups seem to be required for activity. Analogues **3**, **5**, **6**, **11**, **13**, and **14**, which do not have terminal hydrophobic groups, show marginal inhibitory effect. Second, the overall size of the squalene analogue and the charge distribution of the reactive π system appear to be important in substrate recognition. Squalene itself does not possess appreciable charge polarization at its double bond; however, analogues **1** and **9** show significant size and charge differences. Even analogues **2** and **10**, which have good geometrical and steric overlap with squalene, show poor inhibitory effect in part

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as a result of the charge polarization of the alkene. Only analogues **4**, **7**, **8**, **12**, **15**, and **16** possess the proper geometrical, steric, and charge requirements for activity. For all active isopropylidene mimics, however, the bisfunctionalized molecule shows lower inhibitory effect that its corresponding monofunctionalized analogue.

The apparent K_I values for the mono- and bisfunctionalized alkynols **4** and **12** were obtained by Lineweaver–Burke analysis. Both squalene analogues show mixed function inhibition with K_I = 0.95 and 1.0 mM, respectively.

The alkynol functionality has often been used as a mechanism-based inhibitor of cytochrome P-450 systems. One possible pathway of enzymatic inactivation involves oxidation of the alcohol, followed by Michael addition of the enzyme to the resulting alkynone.[30] To test the importance of the hydroxyl group for inhibitory activity and to elucidate its metabolic outcome, alkynol **4** was chemically converted to the corresponding alkynone **4b**. This "activated" alkynone form showed nearly complete loss of the SE inhibition found for the alkynol. Clearly, oxidative metabolic activation of the alkynol is not required; on the contrary, the alcohol appears to be a requirement for SE inhibitory activity.[41]

During the course of our studies on squalene analogues **1–16** as potential inhibitors of SE and/or OSC derived from pig liver, Ceruti and co-workers[42] independently reported the synthesis of inhibitory effects of several similar squalene analogues toward rat liver SE and OSC. They found that the methylated allene functionality, i.e., compound **5** bearing an additional C-1 methyl group, inhibited rat liver SE with an IC_{50} of 60 μM. They also evaluated both mono- and bisfunctionalized squalene analogues, finding very similar IC_{50} values for each pair. In general, the rat liver system, which has an apparent K_M for squalene of 13 μM showed a higher susceptibility to inhibition by the isopropylidene analogues at >80 μM. Additionally, all bisfunctionalized analogues showed higher inhibitory effects than the corresponding monofunctionalized molecules. Comparison with the results reported herein illustrates that substantial interspecies differences occur for SE in the apparent K_M values for squalene and the apparent K_I values for inhibitory analogues. Pig liver SE (K_M = 40 μM for squalene) appears to have stricter substrate requirements for activity than other systems. It will be important to examine the SE and OSC activities of higher vertebrates and humans and to compare the structures of the purified vertebrate enzymes with those of plants and fungi.

In conclusion, the inhibition of pig liver SE by isopropylidene analogues of squalene suggests certain structural and electronic requirements for acceptance at the enzyme catalytic site. Interpretation of the in vitro assays was facilitated by computer analysis of the steric parameters and charge distributions in the alkene mimics. The results show that squalene analogues require two or more of the following properties: (i) a geometry and size similar to squalene, (ii) a hydrophobic moiety in the region of the isopropylidene methyl groups, (iii) an unpolarized, reactive double bond, and (iv) a hydroxyl group in the isopropylidene region. The fact that none of the bisfunctionalized squalene analogues showed better activity than the corresponding monofunctionalized ones suggests that one terminal isopropylidene group is also necessary for optimal activity.

Experimental Section

Ether and THF were dried from benzophenone sodium ketyl. Methylene chloride was distilled over CaH_2. [^{14}C]Methyl iodide was obtained from Amersham. ^{1}H and ^{13}C NMR were obtained using a General Electric QE 300. IR spectra were obtained on a Perkin-Elmer 1430 infrared spectrometer using NaCl plates. Thin-layer chromatography (TLC) of synthetic reactions was performed on MN Polygram Sil G/UV 254 (4 cm × 8 cm) and all plates were visualized with vanillin/H_2SO_4. For enzyme assays, TLC was performed on duplicate samples, with Whatman 60-Å 250-μm layer, preadsorbent and channelled plates. Radiochemical analysis was performed on an LKB RackBeta Universal liquid-scintillation counter with samples dissolved in Scintiverse II (Fisher) or by using a Bioscan System 500 linear analyzer. All asterisks found in text denote tentative NMR assignments.

[1,30-^{14}C]-(6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene. ^{14}C-labeled squalene was prepared by using a modified procedure of Cane et al.[27] after optimization with unlabeled reagents. To a solution of ethyltriphenylphosphonium bromide (105 mg, 0.29 mmol, recrystallized two times from CH_2Cl_2–hexane) in dry THF (1.6 mL) at 0 °C was added *n*-butyllithium (1.6 M in hexane, 1 equiv). The mixture was stirred for 1 h at 0 °C and cooled to −78 °C, and then a portion of this ylide solution (0.5 mL, 0.08 mmol) was added under an argon atmosphere to a solution of $^{14}CH_3I$ (58 mCi/mmol, 100 μCi) in THF, which had been diluted with 0.04 mmol of unlabeled methyl iodide in 1 mL of dry THF. A second portion of unlabeled methyl iodide solution (0.25 mL, 0.04 mmol) was added 20 min later to give a net 50-fold dilution of radiolabel. The cream-colored suspension was stirred for 1 h at −78 °C, and *n*-BuLi was added (2 equiv) until the solution was cherry red. The isopropyl ylide solution was stirred at −78 °C for 1 h, and then trisnorsqualene aldehyde (50 mg, 1.3 mmol) in THF (2 mL) was added and the solution was stirred overnight at 0 °C. The reaction mixture was diluted with hexane and the entire mixture was concentrated and placed onto a silica column. Flash chromatography with hexane afforded radiochemically homogeneous ^{14}C-labeled squalene (23 mg, 63%, 1.37 mCi/mmol).

(5*E*,9*E*,13*E*,17*E*)-1,1-Dibromo-5,9,14,18,22-pentamethyl-1,5,9,13,17,21-tricosahexaene[42] (1). The dibromoalkene was prepared from trisnorsqualene aldehyde[34] by using a modified procedure of Corey and Fuchs.[35] Flash chromatography using a 5–10% ethyl acetate–hexane gradient (EA/H) afforded pure dibromoalkene 1 (48 mg, 22%, R_f = 0.8, 20% EA/H): IR (neat) 1669, 1625 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.73 (br s, 15 H, C-24, C-25, C-26, C-27, C-28 CH_3), 1.79 (s, 3 H, C-23 CH_3), 2.14 (br m, 20 H, C-3 CH_2, C=CCH_2), 5.27 (br m, 5 H, C=C*H*), 6.47 (t, 1 H, Br_2C=C*H*); ^{13}C NMR ($CDCl_3$) δ 138.32 (C-1), 134.84 (C-2).

(5*E*,9*E*,13*E*,17*E*)-1,1,22,22-Tetrabromo-5,9,14,18-tetramethyl-1,5,9,13,17,21-docosahexaene[42] (9). The bis-dibromoalkene was prepared from hexanorsqualene dialdehyde[42] using the same procedure as above (170 mg, 89%, R_f = 0.9, 20% EA/H): IR (neat) 1668, 1626 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (br s, 12 H, CH_3), 2.01 (br m, 16 H, C=CCH_2), 2.18 (dt, J = 6.6 Hz, J = 6.6 Hz, 4 H, Br_2C=CCH_2), 5.13 (br m, 4 H, C=C*H*), 6.33 (t, J = 6.9 Hz, 2 H, Br_2C=C*H*); ^{13}C NMR ($CDCl_3$) δ 138.38 (C-1), 134.83 (C-2).

(5*E*,9*E*,13*E*,17*E*)-1,1-Dichloro-5,9,14,18,22-pentamethyl-1,5,9,13,17,21-tricosahexaene (2). The dichloroalkene was prepared from trisnorsqualene aldehyde and carbon tetrachloride using the same procedure as above (72 mg, 68%, R_f = 0.75, 20 EA/H): IR (neat) 1666, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (br s, 15 H, C-24, C-25, C-26, C-27, C-28 CH_3), 1.67 (s, 3 H, C-23 CH_3), 2.01 (br m, 18 H, C=CCH_2), 2.26 (dt, J = 7.2 Hz, J = 7.2 Hz, 2 H, Cl_2C=CCH_2), 5.13 (br m, 5 H, C=C*H*), 5.80 (t, J = 7.2 Hz, 1 H, Cl_2C=C*H*); ^{13}C NMR ($CDCl_3$) δ 135.07 (C-1), 129.53 (C-2). Anal. ($C_{28}H_{44}Cl_2$) C, H, Cl.

(5*E*,9*E*,13*E*,17*E*)-1,1,22,22-Tetrachloro-5,9,14,18-tetramethyl-1,5,9,13,17,21-docosahexaene (10). The bis-dichloroalkene was prepared from hexanorsqualene dialdehyde by using the same procedure as above (147 mg, 75%, R_f = 0.73, 20%

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EA/H): IR (neat) 1691.7, 1665.7, 1619.1, 881 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.61 (s, 12 H, CH_3), 2.03 (br m, 16 H, C=CCH_2), 2.29 (dt, J = 14.7 Hz, J = 7.2 Hz, 4 H, Cl_2C=CCH_2), 5.15 (br m, 4 H, C=CH), 5.82 (t, J = 7.2 Hz, 2 H, Cl_2C=CH); ^{13}C NMR ($CDCl_3$) δ 129.53 (C-1, C-22), 119.72 (C-2, C-21), 73.20 (C-3, C-20). Anal. ($C_{26}H_{38}Cl_4$) C, H, Cl.

(5*E*,9*E*,13*E*,17*E*)-5,9,14,18,22-Pentamethyl-5,9,13,17,21-tricosapentaen-1-yne[42] **(3).** The alkyne was prepared from dibromoalkene 1 according to the procedure of Corey and Fuchs.[35] Flash chromatography (heptane–50% heptane–hexane gradient) afforded pure alkyne 3 (82 mg, 77%, R_f = 0.84, 20% EA/H): IR (neat) 3300, 2120, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) d 1.59 (br s, 15 H, C-24, C-25, C-26, C-27, C-28 CH_3), 1.67 (s, 3 H, C-23 CH_3), 1.92 (t, J = 2.5 Hz, 1 H, C-1 CH), 2.00 (br m, 16 H, C-7, C-8, C-11, C-12, C-15, C-16, C-19, C-20 C=CCH_2), 2.23 (m, 4 H, C-3 CH_2, C-4 C=CCH_2), 5.13 (br m, 5 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 84.37 (C-2), 68.30 (C-1), 17.64 (C-3).

(5*E*,9*E*,13*E*,17*E*)-5,9,14,18-Tetramethyl-5,9,13,17-docosatetraene-1,21-diyne[42] **(11).** The bis-alkyne was prepared from bis-dibromoalkene 9 by using the same procedure as above. Flash chromatography (hexane–5% EA/H gradient) gave the bis-acetylene analogue 11 (16 mg, 35%, R_f = 0.63, 20% EA/H): IR (neat) 3317, 2110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57, 1.58, 1.61 (s, 12 H, C-23, C-24, C-25, C-26 CH_3), 1.91 (t, J = 3.0 Hz, 2 H, C-1, C-22 CH), 1.99 (br m, 12 H, C-7, C-8, C-11, C-12, C-15, C-16 C=CH_2), 2.19 (br m, 8 H, C-3, C-20 CH_2, C4, C-19 C=CCH_2), 5.16 (br m, 4 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 84.00 (C-1, C-22), 67.83 (C-2, C-21), 17.28 (C-3, C-20).

(6*E*,10*E*,14*E*,18*E*)-6,10,15,19,23-Pentamethyl-6,10,14,18,22-tetracosapentaen-1-yn-3-ol[42] **(4).** Dry (and acetone-free) acetylene was dissolved into THF (50 mL) at −78 °C for 30 min. *n*-BuLi was added (1.87 M in hexane, 0.04 mL, 1 equiv) and the solution was stirred at −78 °C for 1 h to ensure complete acetylide formation. Trisnorsqualene aldehyde (31 mg, 0.81 mmol) in dry THF (2 mL) was then added, and the solution was warmed to room temperature overnight. Unreacted acetylide was quenched with water and the reaction mixture was extracted several times with ether. The ether extracts were washed (brine), dried ($MgSO_4$), and concentrated to give a colorless oil. Flash chromatography (5–10% EA/H gradient) afforded the propargylic alcohol 4 (24 mg, 72%, R_f = 0.47, 20% EA/H): IR (neat) 3600–3100, 3295, 2100, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52, 1.53, 1.58, 1.60 (s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.66 (s, 3 H, C-24 CH_3), 1.99 (br m, 20 H, CH_2), 2.44 (d, J = 1.8 Hz, 1 H, C-1 CH), 4.32 (br m, 1 H, CHOH), 5.10 (br m, 5 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 85.75 (C-2), 73.88 (C-1), 62.95 (C-3).

(6*E*,10*E*,14*E*,18*E*)-6,10,15,19-Tetramethyl-6,10,14,18-tetracosatetraene-1,23-diyne-3,22-diol[42] **(12).** The bis-alkynol was prepared from hexanorsqualene dialdehyde by using the same procedure as above. Flash chromatography (20% EA/H) afforded the bis-propargylic alcohol 12 (57 mg, 78%, R_f = 0.55, 50% EA/H): IR (neat) 3600–3100, 3290, 2120, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57, 1.59 (s, 12 H, CH_3), 1.79 (td, J = 6.6 Hz, J = 6.6 Hz, 4 H, HC(OH)CH_2), 2.05 (br m, 16 H, C=CCH_2), 2.45 (d, J = 1.8 Hz, 2 H, C-1, C-24 CH), 4.32 (ddd, J = 10.8 Hz, J = 6.0 Hz, J = 1.8 Hz, 2 H, CHOH), 5.15 (m, 4 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 85.89 (C-2, C-23), 72.94 (C-1, C-24), 61.89 (C-3, C-22).

(6*E*,10*E*,14*E*,18*E*)-6,10,15,19,23-Pentamethyl-1,2,6,10,14,18,22-tetracosaheptaene (5). The allene analogue was prepared from alkynol 4 by using the procedure of Fujimoto et al.[36] Flash chromatography of the resulting colorless oil with hexane afforded the allene analogue (49 mg, 59%, R_f = 0.93, 20% EA/H): IR (neat) 1945, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.59 (br s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.67 (s, 3 H, C-24 CH_3), 2.04 (br m, 20 H, CH_2), 4.63 (dt, J = 6.6 Hz, J = 3.0 Hz, 2 H, C=C=CH_2), 5.11 (br m, 6 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 208.41 (C-2), 89.68 (C-3), 74.63 (C-1). Anal. ($C_{29}H_{46}$) C, H.

(6*E*,10*E*,14*E*,18*E*)-6,10,15,19-Tetramethyl-1,2,6,10,14,-18,22,23-tetracosaoctaene (13). The bis-allene analogue was prepared from bis-alkynol 12 by using the same procedure as above (33 mg, 44%, R_f = 0.8, 20% EA/H): IR (neat) 1955, 1665, 1639 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55, 1.60, 1.66 (s, 12 H, CH_3), 2.03 (br m, 20 H, CH_2), 4.64 (br m, 2 H, C=C=CH_2), 4.71 (m, 1 H, HC=C=CH_2), 5.09 (br m, 4 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 208.38 (C-2, C-23), 89.69 (C-3, C-22), 74.64 (C-1, C-24). Anal. ($C_{28}H_{42}$) C, H.

(4*E*,8*E*,12*E*,16*E*)-1-Cyclopropylidene-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaene (6). To a stirred suspension of cyclopropyltriphenylphosphonium bromide (200 mg, 0.52 mmol) in dry THF (10 mL), at −78 °C, was added phenyllithium (2 M in Et_2O–cyclohexane, 0.25 mL, 0.5 mmol). The mixture was stirred for an additional hour to ensure complete ylide formation, and then trisnorsqualene aldehyde (50 mg, 0.13 mmol) in THF (5 mL) was added. The solution was allowed to warm to room temperature overnight. The reaction was quenched by the addition of water, and the resulting mixture was subjected to the usual workup procedures. Flash chromatography with hexane provided pure cyclopropylidene analogue 6 (28 mg, 43%, R_f = 0.8, 20% EA/H): IR (neat) 3397, 1717, 1673, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (br s, 4 H, cyclopropyl CH_2), 1.56 (br s, 15 H, C-23, C-24, C-25, C-26, C-27 CH_3), 1.63 (s, 3 H, C-22 CH_3), 2.02 (br m, 18 H, C-3, C-6, C-7, C-10, C-11, C-14, C-15, C-18, C-19 CH_2), 2.21 (m, 2 H, C-2 CH_2), 5.10 (m, 5 H, C-5, C-9, C-12, C-16, C-20 C=CH), 5.69 (ttt, J = 4.2 Hz, J = 2 Hz, J = 2 Hz, 1 H, C-1 C=CH); ^{13}C NMR ($CDCl_3$) δ 128.72 (C-1), 118.02 (cyclopropyl *C*=CH), 30.46 (C-2), 2.16 (*trans*-cyclopropyl CH_2), 1.80 (*cis*-cyclopropyl CH_2). Anal. ($C_{30}H_{48}$) C, H.

(4*E*,8*E*,12*E*,16*E*)-1,20-Dicyclopropylidene-4,8,13,17-tetramethyl-4,8,12,16-eicosatetraene (14). The bis-cyclopropylidene analogue was prepared from hexanorsqualene dialdehyde by using the same procedure as above (85 mg, 75%, R_f = 0.82, 20% EA/H): IR (neat) 3049, 1666, 1597 cm^{-1}; ^1H NMR δ 1.05 (br s, 8 H, cyclopropyl CH_2), 1.65 (br s, 12 H, CH_3), 2.05 (m, 8 H, C-6, C-10, C-11, C-15 CH_2), 2.14 (t, J = 7.8 Hz, 8 H, C=C(CH_3)CH_2), 2.32 (dt, J = 7.2 Hz, J = 7.2 Hz, 4 H, C-2, C-19 CH_2), 5.18 (br m, 4 H, C-5, C-9, C-12, C-16 C=CH), 5.78 (t, J = 6.6 Hz, 2 H, C-1, C-20 C=CH); ^{13}C NMR ($CDCl_3$) δ 128.69 (C-1, C-20), 118.04 (cyclopropyl *C*=CH), 30.48 (C-2, C-19), 2.18 (*trans*-cyclopropyl CH_2), 1.80 (*cis*-cyclopropyl CH_2). Anal. ($C_{30}H_{48}$) C, H.

(6*E*,10*E*,14*E*,18*E*)-6,10,15,19,23-Pentamethyl-6,10,14,18,22-tetracosapentaen-1-yn-3-one (4b). To a solution of alkynol 4 (75 mg, 0.18 mmol) in dry CH_2Cl_2 (15 mL), at 0 °C, was added pyridinium chlorochromate (138 mg, 3.5 equiv). The mixture was warmed to room temperature and stirred for 7 h, and then ether was added. The reaction mixture was passed through a small pad of Florisil, concentrated, and the resulting residue was chromatographed (7% EA/H) to yield pure alkynone **4b** (55 mg, 77%, R_f = 0.57, 20% EA/H): IR (neat) 3300–3200, 2110, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (br s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.68 (s, 3 H, C-24 CH_3), 2.01 (br m, 16 H, C-8, C-9, C-12, C-13, C-16, C-17, C-20, C-21 CH_2), 2.35 (t, J = 7.6 Hz, 2 H, C-5 CH_2), 2.68 (t, J = 7.7 Hz, 2 H, C(O)CH_2), 3.21 (s, 1 H, C-1 CH), 5.15 (br m, 5 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 186.99 (C-3), 81.41 (C-2), 78.36 (C-1), 44.13 (C-4), 33.36 (C-5). Anal. ($C_{29}H_{44}O$) C, H.

(2*E*,6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol[43] **(17).** The allylic alcohol was prepared from squalene by using a modified procedure of Sum and Weiler.[37] Flash chromatography (5%–10% EA/H gradient) afforded *trans*-squalenol 17 (614 mg, 4%, R_f = 0.43, 20% EA/H): IR (neat) 3620–3140, 1662 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (br s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.66 (s, 3 H, C-30* CH_3), 1.67 (s, 3 H, C-24* CH_3), 2.00 (br m, 20 H, C=CCH_2), 3.97 (br s, 2 H, C=CCH_2OH), 5.12 (br m, 5 H, C=CH), 5.38 (t, J = 5.8 Hz, 1 H, $HOCH_2C$=CH); ^{13}C NMR ($CDCl_3$) δ 134.93 (C-2), 130.97 (C-23), 124.21 (C-3), 68.84 (C-1), 13.48 (C-30).

(2*E*,6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-al (18). To a stirred solution of squalenol (358 mg, 0.84 mmol) in hexane (50 mL) was added activated MnO_2 (1.09 g, 15 equiv). The suspension was stirred at room temperature overnight and then filtered and the filtrate was concentrated. Flash chromatography (5% EA/H) gave pure α,β-unsaturated aldehyde 18 (280 mg, 79%, R_f = 0.33, 20% EA/H): IR (neat) 2708, 1690, 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57, 1.60 (s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.65 (s, 3 H, C-24* CH_3), 1.72 (s, 3 H, C-30* CH_3), 1.99 (m, 18 H, C=CCH_2), 2.41 (dt, J = 7.2 Hz, J = 7.2 Hz, 2 H, OCHC=CCH_2), 5.14 (br m, 5 H, C=CH), 6.43 (dt, J = 7.2 Hz, J = 1.2 Hz, 1 H, OHCC=CH), 9.35 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 194.90 (C-1), 154.13 (C-3), 139.28 (C-2).

(3*E*,7*E*,11*E*,15*E*,19*E*)-3,7,11,16,20,24-Hexamethyl-1,3,7,11,15,19,23-pentacosaheptaene (7). To a suspension of methyltriphenylphosphonium bromide (286 mg, 3.2 equiv) in dry THF, at −60 °C, under a blanket of argon, was added *n*-BuLi (1.6 M in hexanes, 0.5 mL, 3.2 equiv). The solution was warmed to room temperature for 15 min and then cooled to −60 °C, and a solution of α,β-unsaturated aldehyde **18** (104 mg, 0.23 mmol) in THF (15 mL) was added dropwise. The reaction mixture was immediately warmed to room temperature and stirred for 30 min. Water was added and the mixture was extracted several times with hexane (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. Flash chromatography using hexane afforded pure diene **7** (100 mg, 100%, R_f = 0.8, 20% EA/H): IR (neat) 3088, 1666, 1636, 1601 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.61 (s, 15 H, C-26, C-27, C-28, C-29, C-30 CH_3), 1.69 (s, 3 H, C-25* CH_3), 1.74 (s, 3 H, C-31* CH_3), 2.02 (m, 18 H, C-6, C-9, C-10, C-13, C-14, C-17, C-18, C-21, C-22 C=CCH_2), 2.23 (dt, J = 7.5 Hz, J = 7.5 Hz, 2 H, C-5 CH_2), 4.92 (d, J = 10.8 Hz, 1 H, trans C=CC=CH_2), 5.07 (d, J = 17.4 Hz, cis C=CC=CH_2), 5.15 (br m, 5 H, C=C*H*), 5.4m (t, J = 6.7 Hz, 1 H, H_2C=CC=C*H*), 6.37 (dd, J = 17.4 Hz, J = 10.8 Hz, 1 H, C=C*H*C=C); ^{13}C NMR ($CDCl_3$) δ 141.58 (C-3). Anal. ($C_{31}H_{50}$) C, H.

(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*)-Diethyl 2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaenedioate (19). The α,β-unsaturated diester was synthesized from triethyl 2-phosphonopropionate and hexanorsqualene dialdehyde by using the procedure of Wadsworth and Emmons.[39] Flash chromatography (5–10% EA/H gradient) afforded pure bis-α,β-unsaturated ethyl ester **19** (1.33 g, 50g, R_f = 0.77, 20% EA/H): GLC (DB-5 Megabore, 3 m × 0.25 cm) showed a *E,E*:*Z,E*:*Z,Z* ratio of 60:33:7; ^1H NMR ($CDCl_3$) δ 1.28 (t, J = 6.9 Hz, 6 H, C(O)OCH_2CH_3), 1.61 (s, 12 H, C-26, C-27, C-28, C-29 CH_3), 1.83 (s, trans C-25, C-30 CH_3), 1.88 (s, cis C-25, C-30 CH_3), 2.01 (m, 16 H, C=CCH_2), 2.26 (dt, J = 12.6 Hz, J = 6.3 Hz, cis $CH_3CH_2O_2CC$=CCH_2), 2.56 (dt, J = 20.4 Hz, J = 6.0 Hz, trans $CH_3CH_2O_2C$=CCH_2), 4.17 (q, J = 7.2 Hz, 4 H, C(O)OCH_2CH_3), 5.15 (m, 4 H, C=C*H*), 5.92 (t, J = 5.4 Hz, cis $CH_3CH_2O_2CC$=C*H*), 6.74 (t, J = 6.0 Hz, trans $CH_3CH_2O_2CC$=C*H*); ^{13}C NMR ($CDCl_3$) δ 168.00 (C-1, C-24), 142.54 (cis C-3, C-22), 141.76 (trans C-3, C-22), 127.64 (C-2, C-23), 60.26 (trans C(O)OCH_2CH_3), 60.22 (cis C(O)OCH_2CH_3), 14.23 (trans C(O)OCH_2CH_3), 12.28 (cis C(O)OCH_2CH_3).

(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene-1,24-diol[43] (20). To a solution of squalene bis-α,β-unsaturated ethyl ester (1.33 g, 2.5 mmol, isomeric mixture from above) in dry ether (20 mL), at 0 °C, was added $LiAlH_4$ (368 mg, 4 equiv). The solution was stirred at room temperature for 3 h and then quenched by the consecutive addition of H_2O (200 μL), 1 H NaOH (400 μL), and H_2O (3 mL). The mixture was filtered, and the filtrate was washed several times with hot ethyl acetate. The eluent was dried ($MgSO_4$) and concentrated, and the resulting residue was chromatographed (20–60% EA/H gradient) to give pure squalene bis-allylic alcohol **20** (794 mg, 72%, R_f = 0.07, 20% EA/H): IR (neat) 3344, 1686, 1667, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.59 (s, 12 H, C-26, C-27, C-28, C-29 CH_3), 1.64 (s, trans C-25, C-30 CH_3), 1.76 (d, J = 0.9 Hz, cis C-25*, C-30* CH_3), 1.99 (m, 16 H, C=CCH_2), 2.07 (t d, 4 H, $HOCH_2C$=CCH_2), 3.95 (s, trans CH_2OH), 4.08 (s, cis CH_2OH), 5.13 (m, 4 H, C=C*H*), 5.34 (t, J = 7.2 Hz, cis $HOCH_2C$=C*H*), 5.37 (t, J = 6.0 Hz, trans $HOCH_2C$=C*H*); ^{13}C NMR ($CDCl_3$) δ 127.99 (C-3*, C-22*), 68.78 (trans C-1, C-24), 61.36 (cis C-1, C-24).

(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene-1,24-dial (21). To a solution of squalene bis-allylic alcohol **20** (739 mg, 1.67 mmol, isomeric mixture from above) in cyclohexane (75 mL) was added activated MnO_2 (4.3 g, 30 equiv). After stirring at room temperature for 1 day, the suspension was filtered and concentrated. Flash chromatography (5% EA/H) afforded pure bis-enal **21** (530 mg, 72%, R_f = 0.57, 20% EA/H): GLC (DB-5 Megabore, 3 m × 0.25 cm) showed a *E,E*:*Z,E* ratio of 85:15; ^1H NMR ($CDCl_3$) δ 1.48, 1.51 (s, 12 H, C-26, C-27, C-28, C-29 CH_3), 1.62 (s, trans C-25, C-30 CH_3), 1.64 (s, cis C-25*, C-30* CH_3), 1.96 (m, 12 H, C-8, C-9, C-12, C-13, C-16, C-17, C=CCH_2), 2.04 (t, J = 7.5 Hz, 4 H, C-5, C-20 C=CCH_2), 2.33 (dt, J = 7.5 Hz, J = 7.5 Hz, trans OHCC=CCH_2), 2.54 (dt, J = 7.5 Hz, J = 7.5 Hz, cis OHCC=CCH_2), 5.02 (m, C=C*H*, cis OHCC=C*H*), 6.35 (t, J = 6.9 Hz, trans OHCC=C*H*), 9.25 (s, trans C*H*O), 9.98 (s, cis C*H*O); ^{13}C NMR ($CDCl_3$) δ 194.92 (trans C-1, C-24), 190.80 (cis C-1, C-24), 154.22 (trans C-3, C-22), 148.91 (cis C-3, C-22), 139.09 (trans C-2, C-23), 135.83 (cis C-2, C-23), 9.00 (C-25*, C-30*).

(3*E*,7*E*,11*E*,15*E*,19*E*,23*E*)-3,7,11,16,20,24-Hexamethyl-1,3,7,11,15,19,23,25-hexacosaoctaene (15). The bis-diene was synthesized from bis-α,β-unsaturated aldehyde **21** by using the same procedure as for the corresponding monofunctionalized analogue (412 mg, 85%, R_f = 0.73, 20% EA/H): IR (neat) 3088, 1666, 1642, 1606 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.65 (s, 12 H, C-28, C-29, C-30, C-31 CH_3), 1.77 (s, trans C-27, C-32 CH_3), 1.84 (s, cis C-27*, C-32* CH_3), 2.06 (m, 16 H, C=CCH_2), 2.27 (dt, J = 7.5 Hz, J = 7.5 Hz, C=CCC=CCH_2), 4.95 (d, J = 10.8 Hz, cis C=CH_2), 5.10 (d, J = 17.1 Hz, trans C=CH_2), 5.18 (m, 4 H, C-8, C-12, C-15, C-19 C=C*H*), 5.40 (t, J = 7.4 Hz, cis H_2C=CC=C*H*), 5.50 (t, J = 6.6 Hz, trans H_2C=CC=C*H*), 6.39 (dd, J = 17.1 Hz, J = 10.5 Hz, trans H_2C=C*H*), 6.79 (ddd, J = 18.0 Hz, J = 11.1 Hz, J = 0.9 Hz, cis H_2C=C*H*) calcd *E,E*:*Z,E* ratio = 85:15; ^{13}C NMR ($CDCl_3$) δ 141.54 (C-3, C-24), 113.13 (cis C-1, C-26), 110.24 (trans C-1, C-26), 11.60 (C-27, C-32). Anal. ($C_{32}H_{50}$) C, H.

(1*E*,5*E*,9*E*,13*E*,17*E*)-1-(1,5,9,14,18,22-Hexamethyl-1,5,9,13,17,21-tricosahexaenyl)cyclopropane (8). The vinyl cyclopropane analogue was prepared by using the procedure of Suda.[38] To a solution of squalene diene (50 mg, 0.12 mmol) in dry ether (15 mL), at 0 °C, was added diazomethane (0.7 M in Et_2O, from 400 mg of *N*-methyl-*N*-nitrosourea, 1.4 mL, 8 equiv) and $Pd(OAc)_2$ (8 mg, 0.3 equiv). After stirring at 0 °C for 15 min, the reaction mixture was quenched with glacial acetic acid. The solvent was removed under reduced pressure and the crude product was placed directly onto a pipet column and eluted with hexane to afford pure alkenyl cyclopropane **8** (37 mg, 71%, R_f = 0.73, 20% EA/H): IR (neat) 3072, 1666 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.42 (m, 2 H, *trans*-cyclopropyl CH_2), 0.53 (m, 2 H, *cis*-cyclopropyl CH_2), 1.35 (m, 1 H, cyclopropyl C*H*), 1.49 (s, 3 H, C-29* CH_3), 1.60 (s, 15 H, C-24*, C-25, C-26, C-27, C-28 CH_3), 1.68 (s, 3 H, C-23 CH_3), 2.07 (br s, 20 H, C=CCH_2), 5.12 (br m, 6 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 13.8 (cyclopropyl *C*H), 4.16 (cyclopropyl CH_2). Anal. ($C_{32}H_{52}$) C, H.

(1*E*,5*E*,9*E*,13*E*,17*E*,21*E*)-1-(22-Cyclopropyl-1,5,9,14,18,22-hexamethyl-1,5,9,13,17,21-docosahexaenyl)-cyclopropane (16). The bis-alkenyl cyclopropane was prepared from squalene bis-diene **15** (isomeric mixture) by using the same procedure as above (92 mg, 83%, R_f = 0.87, 20% EA/H). The GLC (DB-5 Megabore, 3 m × 0.25 cm) showed a *E,E*:*Z,E* ratio of 84:16: IR (neat) 3072.0, 1666.3 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.41 (m, 2 H, *trans*-cyclopropyl CH_2), 0.52 (m, 2 H, *cis*-cyclopropyl CH_2), 1.35 (m, 1 H, cyclopropyl C*H*), 1.49 s, 6 H, C-23, C-28 CH_3), 1.60 (s, 12 H, C-24, C-25, C-26, C-27 CH_3), 2.01 (br m, 20 H, C=CCH_2), 5.12 (br m, 6 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 13.82 (cyclopropyl *C*H), 4.16 (CH_2). Anal. ($C_{34}H_{54}$) C, H.

Preparative reverse-phase column chromatography (C18/Porasil B, 2-cm pipet column) using a 50% MeOH/H_2O MeOH gradient afforded pure (*E,E*)-bis-alkenyl cyclopropane **16**.

Preparation of SE and OSC Enzyme Solutions. All manipulations for microsome isolation and preparation of enzyme solutions were carried out between 0 and 5 °C.

Microsomal Preparation. With use of a modified procedure of Astruc et al.,[28] 500 g of freshly killed pig liver and 1000 mL of Tris-HCl (0.1 M Tris, 1 mM EDTA in deionized water, pH 7.4) were homogenized for 3 min. The homogenate was centrifuged at 10000*g* for 30 min to remove cellular debris, and the resulting supernatant was filtered through glass wool and centrifuged at 100000*g* for 1 h. The supernatant (which contained supernatant protein factor, SPF) and microsomal pellet (which contained squalene epoxidase, oxidosqualene cyclase, and NADPH-cytochrome P-450 reductase) were stored separately at −80 °C as aliquots (approximately 5 mL of supernatant and 450 mg of microsomes).

Squalene Epoxidase. Crude squalene epoxidase was prepared by using a modification of the procedure of Ono and Imai.[8] A mixture of microsomes (200 mg) and Tween-80 (7–8 mg, 4% detergent) was placed in an ultrasonic bath and sonicated for 10 min. The solubilized microsomes were diluted with 1.8 mL of Tris-HCl buffer (0.1 M, pH 7.4, final detergent concentration = 0.4%). Next, FAD (0.2 mM in Tris-HCl buffer, 40 μL) and NADPH (13 mM in Tris-HCl buffer, 200 μL) were added to give final cofactor concentrations of 40 μM and 1 mM, respectively.

In order to observe epoxidase activity *only*, oxidosqualene cyclase inhibitor, *N,N*-dimethyldodecylamine *N*-oxide,[21] was added (10 μL of a 24 mM solution in 2-propanol, final concentration = 100 μM).

Oxidosqualene Cyclase Plus Squalene Epoxidase. The enzyme preparation was identical with that of squalene epoxidase, except that no *N,N*-dimethyldodecylamine *N*-oxide was added.

Inhibition of SE and OSC: IC_{50} Determinations. Test tubes containing either squalene epoxidase or oxidosqualene cyclase enzyme solutions (240 μL each, as described above) were warmed to 37 °C. After 10 min, squalene analogues were added (1 μL in 2-propanol) to give final inhibitor concentrations of 0, 4, 20, 40, 200, and 400 μM. After an additional 10 min, [^{14}C]-squalene (2 μL in 2-propanol, approximately 20 000 dpm, 33 μM) was added to each enzyme solution. Incubation was continued for another 50 min and then stopped by the addition of 10% KOH–methanol (240 μL). After 1 h at 37 °C, each mixture was extracted with CH_2Cl_2 (1 mL each); the resulting organic extracts were dried ($MgSO_4$) and redissolved in a small amount of CH_2Cl_2 (100 μL), and the triterpene components were separated by TLC. Radiochemical analysis, using either linear analysis or scintillation counting, showed conversion of squalene to either 2,3-oxidosqualene (for the epoxidase assays) or a mixture of lanosterol and 2,3-oxidosqualene (for the cyclase assays). Inhibitor-free assays showed approximately 30% conversion to product, and radiochemical recoveries of 80–90% were routinely achieved.

Inhibition of SE and OSC: Kinetic Analysis. Squalene epoxidase solutions were warmed to 37 °C. After 10 min, inhibitors were added to give three sets of inhibitor concentrations: 0 mM, the IC_{30} value, and the IC_{50} value. After a 10-min preincubation, [^{14}C]squalene was added to give final substrate concentrations of 13, 17, 25, and 40 μM for each set of inhibitor concentrations. The enzyme solutions were incubated for 50 min and then quenched by the addition of 10% KOH–methanol and the products were isolated as described above.

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Supplementary Material Available: Figures representing a Lineweaver–Burk plot of **4** and **12**, and minimization results for the modified isopropylidene region of **1–16**, and superimpositions of each analogue with squalene (12 pages). Ordering information is given on any current masthead page.

In Vitro Metabolic Transformations of Vinblastine: Oxidations Catalyzed by Human Ceruloplasmin

Sayed A. Elmarakby, Michael W. Duffel, and John P. N. Rosazza*

Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242.
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The dimeric *Vinca* alkaloid vinblastine (VLB) undergoes metabolic transformation to three products in a reaction catalyzed by the human serum copper oxidase ceruloplasmin. The enzyme reaction requires chlorpromazine as a shuttle oxidant, and the course of the oxidation reaction appears to be subject to the nature of the shuttle oxidant used. Preparative-scale incubations have resulted in the isolation of three products, which were characterized by chemical and spectral analyses. The metabolites were identified as the ring fission product catharinine, obtained by oxidation of the *Iboga* ring system; an enamine/ether derivative obtained by oxidation of the *Aspidosperma* portion of VLB; and a metabolite embodying the same structural changes in both parts of the vinblastine dimeric structure. Catharinine is identical with the product of VLB oxidation obtained by peroxidase oxidation. The other two products are new metabolites and are derivatives of VLB. All of the metabolites are less active than VLB when tested in vitro vs the human T-cell leukemic cell line (CRFF-CEM).

The *Vinca* alkaloids vinblastine (VLB) (1) and vincristine (VCR) are widely used in cancer chemotherapy.[1] Although several studies have shown that VLB (1) is extensively metabolized in mammals,[2-12] little is known about the metabolites or their possible role in either the mechanism of action or the dose-limiting toxicities. Our investigations have focused on elaborating biochemical and chemical mechanisms by which dimeric and monomeric *Vinca* alkaloids are oxidized by different types of enzymes. The susceptibility of *Vinca* alkaloids to one-electron oxidation reactions is well documented in our laboratories.[13,14] Catalysis by mouse liver microsomes, microorganisms, peroxidases, and copper oxidases of the monomeric *Vinca* alkaloid vindoline yielded several metabolites, all of which were isolated and fully characterized.[13,15-18] Their identification and probes of the mechanism by which they are formed provide a clear basis for understanding metabolic transformations of more complex *Vinca* alkaloid dimers.

Ceruloplasmin is a member of the blue copper oxidases which also include laccases and ascorbate oxidase.[19] Copper oxidases occur widely among mammals, plants, and microorganisms. The enzymes are important electron-transfer agents in biological systems, and they catalyze oxidation reactions by the direct removal of substrate

* To whom requests for reprints should be addressed.

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