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Stereospecific Syntheses of *trans*-Vinyldioxidosqualene and β-Hydroxysulfide Derivatives, as Potent and Time-Dependent 2,3-Oxidosqualene Cyclase Inhibitors

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Abstract—*trans*-Vinyldioxidosqualene and β-hydroxysulfide derivatives were synthesized stereospecifically and evaluated as inhibitors of animal and yeast oxidosqualene cyclases. Only *trans*-vinyldioxidosqualene and 2,3-epoxy-vinyl-β-hydroxysulfides, having the reactive function at crucial positions 14,15 and 18,19, were active as inhibitors of animal and yeast cyclases. (14-*trans*)-28-Methylidene-2,3:14,15-dioxidoundecanorsqualene **27** was the most potent inhibitor of the series of pig liver cyclase, with an IC_{50} of 0.4 μM, and it behaved also as the most active time-dependent inhibitor of the animal enzyme.

Introduction

2,3-Oxidosqualene-lanosterol cyclase (OSC) (EC 5.4.99.7) is a membrane bound enzyme that catalyses the cyclization of (3*S*)-2,3-oxidosqualene (OS) **1** to lanosterol **8** in animals and yeasts, and to cycloartenol **9** in higher plants (Scheme 1).[1–10] OSC binds 2,3-oxidosqualene **1** in a chair–boat–chair conformation and then forwards a sequential formation of distinct carbocationic intermediates **2**–**7**. Recently, Corey[11] proposed a mechanism involved in C-ring formation, showing that a five-membered C-ring cationic structure **5** was an intermediate, followed by a ring expansion, giving six-membered carbocationic intermediate **6**. This makes it possible to avoid the energetically-expensive anti-Markovnikov closure of **4** to **6**, as originally postulated.[12–14] Another important step during OS cyclization is the formation of the protosterol ion **7**, which was initially believed to form a covalent bond with a suitable nucleophile of the enzyme active site, before further rearrangement to lanosterol through a series of hydride 1–2 migrations. It now becomes evident that the H migration from C(17) to C(20) is facilitated by the presence of a β-side chain in **7**,[15,16] and that the protosteryl cation is stabilized by a series of correctly located polarizable π-aromatic groups at the catalytic site of the cyclase.[17,18]

One strategy to gain further insight into the involvement of carbocationic intermediates is to study the inhibition of OSC by mimicking the presumptive carbocationic intermediates.[19–23] In this way, we and others have succeeded in mimicking the transient carbocationic intermediates C-2 **2**, C-8 **4** and C-20 **7** by designing acyclic azasqualene inhibitors[24–28] and partially cyclized derivatives.[29–31] Analogously, various series of sulfur-containing oxidosqualene derivatives, in which sulfur has replaced carbons C5, C6, C8, C9, C10, C11, C13, C14, C15, C16, C18, C19 or C20 (squalene numbering), have been synthesized.[32–36] Some of these compounds were considered to generate sulfonium intermediates during cyclization, thus mimicking the interactions between the intermediate carbocations generated during cyclization of OS and the nucleophilic sites of the enzyme.

Another strategy that has been adopted is to intercept the enzymatic active-site nucleophiles with a stable allylic cation, resulting in an irreversible covalent modification of OSC. Following this strategy, Prestwich[37–40] and Corey[41] groups synthesized various series of 2,3-oxidosqualenoid dienes. We recently succeeded in the stereospecific synthesis of (18*E*)-29-methylidene-2,3-oxidohexanorsqualene, which was found to be a potent

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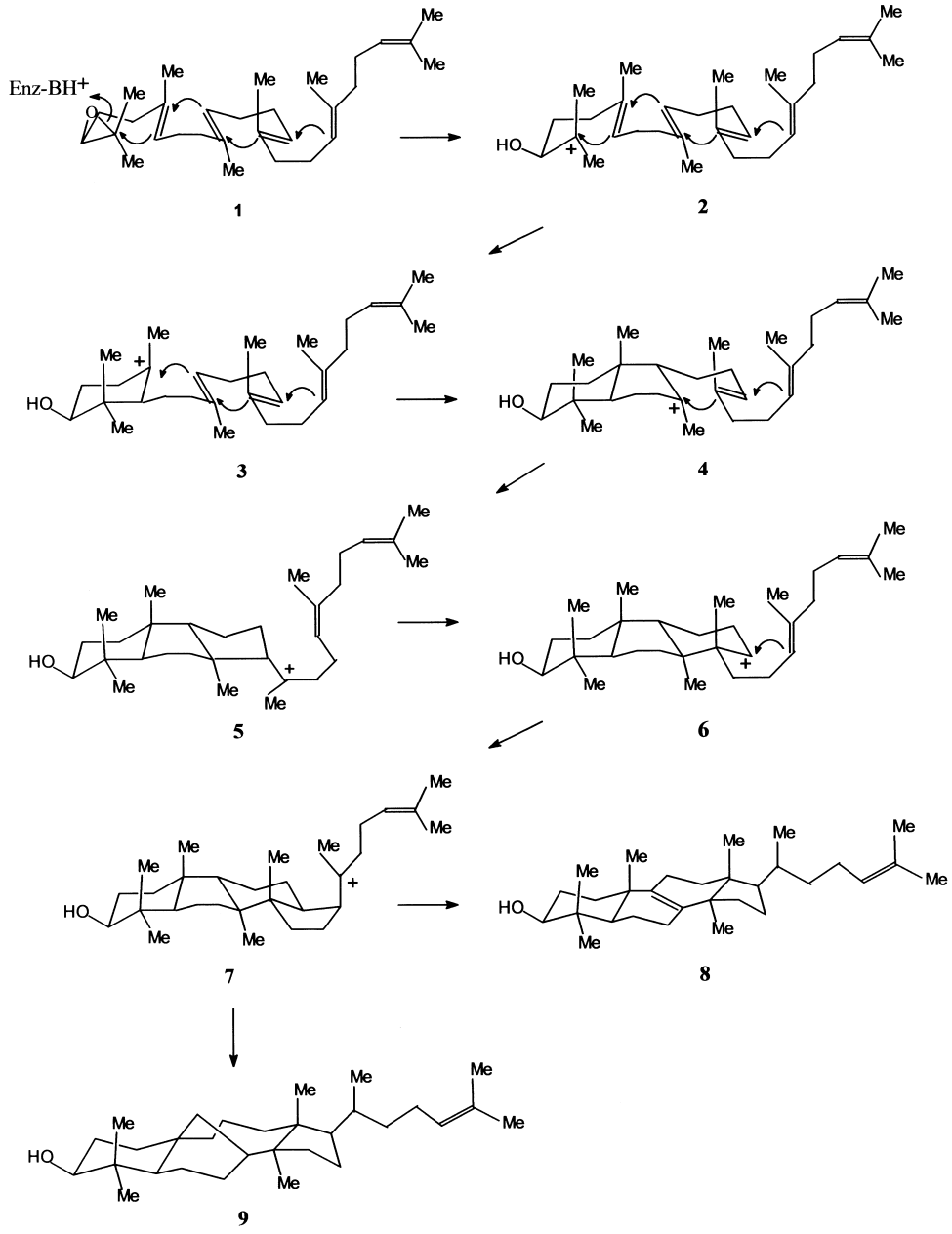


Scheme 1. Mechanism of cyclization of 2,3-oxidosqualene **1** to lanosterol **8** and cycloartenol **9**.

time-dependent inhibitor of yeast OSC, while (18*Z*) isomer was almost inactive.[42] Finally, a strategy followed to achieve new OSC inactivators is the introduction of a second epoxidic ring, replacing a carbon–carbon double bond in the natural substrate OS **1**.[43–46] Among the diepoxysqualene derivatives tested, the most potent is 2,3:18,19-dioxidosqualene, that elicited non-competitive inhibition of OSC, but did not behave as an irreversible inhibitor.

Now we have believed that the stereospecific syntheses of some truncated *trans*-vinyldioxidosqualene derivatives, together with other related compounds, might give better insights into the function and the reactivity of the nucleophiles of the active site of the enzyme, that stabilize the C-13 and C-20 cationic intermediates. These compounds should interfere with C or D ring formation, by two potential mechanisms: (1) by trapping as irreversible inhibitors the enzymatic nucleophiles present near the C14–C15 or the C18–C19 regions of OS and (2) by behaving as competitive reversible inhibitors, strongly interacting with the enzymatic active site.

Chemistry

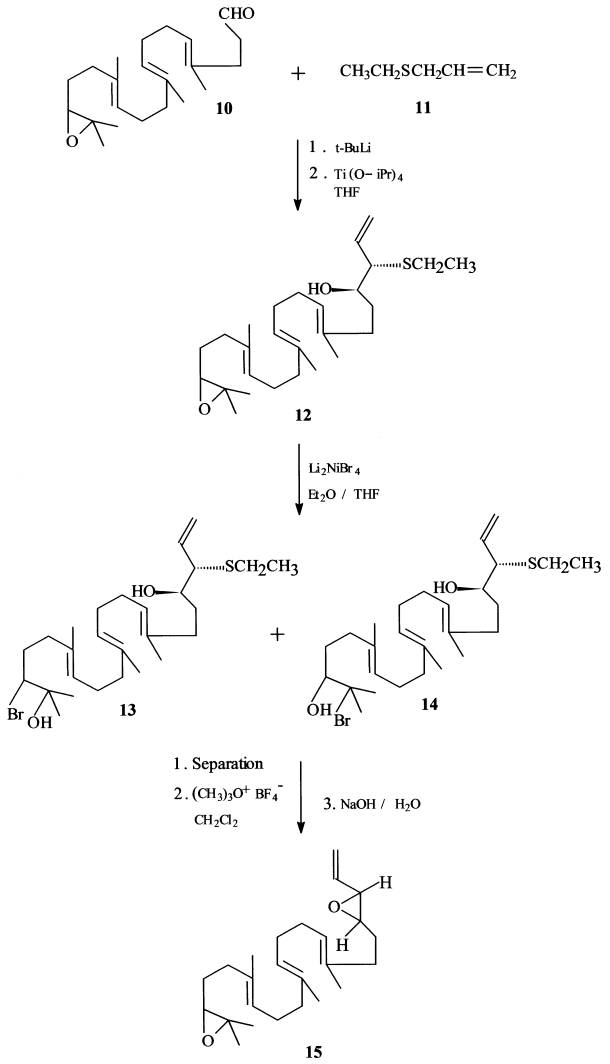
We developed a *trans* stereospecific synthesis of monovinyl diepoxides by adding the vinylepoxide moiety to the monoepoxy squalene derivative. The suitable epoxy aldehyde[24,42] was transformed in three steps into the *trans*-vinyldiepoxide through a new sequence: (1) stereospecific formation of the epoxy-(3*R**,4*S**)-β-hydroxysulfide, (2) protection of the 2,3-epoxidic group as a bromohydrin, and (3) formation of the sulfonium β-hydroxysulfide derivative, followed by base-catalyzed

cyclization of the two vicinal functional groups to epoxides (Scheme 2).

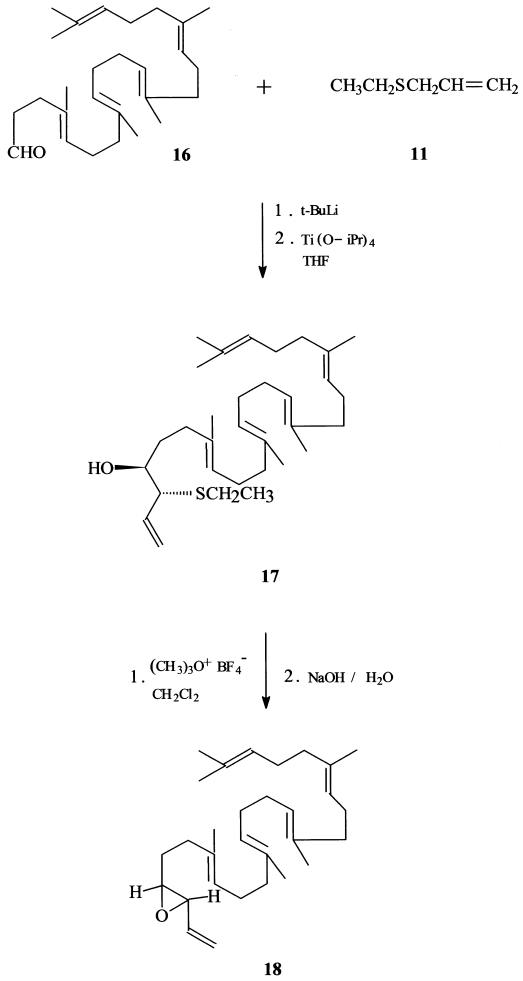
We focused our attention on the reaction of alkylthioallyl anions with aldehydes. The carbanion of allyl ethyl sulfide, generated with a strong base, was reacted with the suitable carbonyl compound.[47] It gave about 30% of the β-hydroxysulfide (the α-adduct) and 70% of the δ-hydroxysulfide (the γ-adduct). Furthermore, the desired β-hydroxysulfide was in (3*R**,4*S**)/(3*R**,4*R**) mixture. It has been shown that the structure and the reactivity of the alkylthioallyl anion is highly dependent on the countercation and on the solvents.[48] The lithium derivatives alone have shown little selectivity, but the addition of other metals modified the α/γ selectivity and the (3*R**,4*S**)/(3*R**,4*R**) ratio of the α-adduct. We thus examined the use of the organotitanium reagents, that have been shown to increase the selectivity of various organic anions. η^3-Trimethylsilylallyltitanium has been shown to react selectively and stereospecifically with aldehydes to give (3*R**,4*S**)-3-trimethylsilyl-4-hydroxy-1-alkenes; on subjecting these intermediates to an acidic or basic elimination, *E* or *Z* dienes were obtained stereospecifically.[49,50]

When we treated the carbanion of allyl ethyl sulfide (or allyl phenyl sulfide) with titanium tetraisopropoxide, followed by the suitable epoxysqualene aldehyde, such as **10**, **16** or **19** (Schemes 2–4), a regiospecific and stereospecific reaction occurred. Only β-hydroxysulfide (the α-adduct) formed exclusively in the (3*R**,4*S**) stereochemistry. This regio and stereospecificity depends on the structure of the organotitanium intermediate. Addition of the allylic organotitanium to the aldehyde takes place through an allylic rearrangement of the organotitanium in a chelate six-membered transition state. The alkylthioallyl anion has a countercation at the γ position, giving the α-adduct. Furthermore, in the chelate transition state, the structure in which both the ethylthio and *R* groups are equatorials is the favoured one, thus forming exclusively the (3*R**,4*S**) isomer.

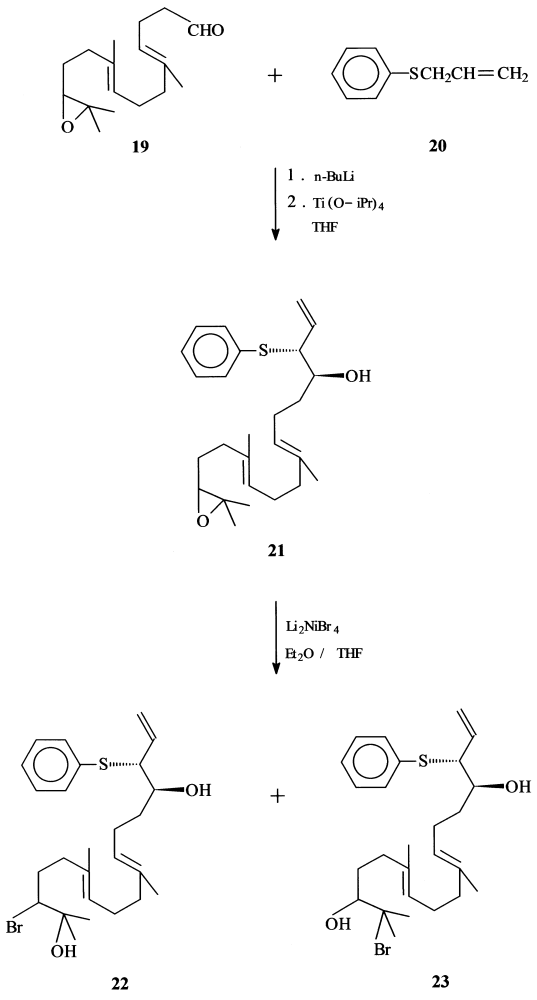
We then subsequently examined the stereospecific closure to epoxide of polyenic (3*R**,4*S**)-β-hydroxysulfide intermediates, such as **12**, **21**, or **24** (Schemes 2 and 4; Fig. 1), which already contain a 2,3-epoxy group. No



Scheme 2. Synthesis of (18-*trans*)-29-methylidene-2,3:18,19-dioxidohexanorsqualene **15**.



Scheme 3. Synthesis of (2-*trans*)-1-methylidene-2,3-oxido-1′-norsqualene **18**.



Scheme 4. Synthesis of C_{17} β-hydroxyphenylsulfide derivatives.

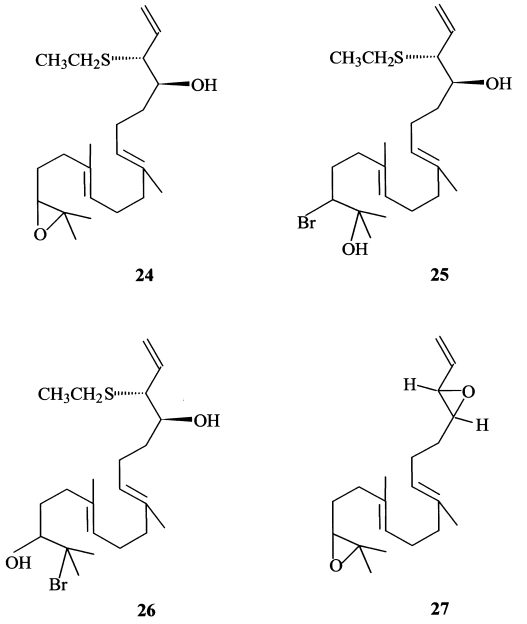


Figure 1. Structures of various C_{17} β-hydroxyethylsulfide derivatives and (14-*trans*)-28-methylidene-2,3:14,15-dioxidoundecanorsqualene **27**.

methods reported in the literature were found to be suitable for this purpose, because the epoxidic group was unstable in the reaction conditions, and a partial cyclization of the epoxy intermediate often occurred. We therefore opened the epoxide to bromohydrin using Li_2NiBr_4. The solution of Li_2NiBr_4 was generated from LiBr and $NiBr_2$ in Et_2O/THF. This two-solvent mixture is a variant we have introduced to the method of Li_2NiBr_4 in THF reported in the literature,[51] giving higher yields than THF alone of the two isomeric bromohydrins (**13**, **14** or **22**, **23**, or **25**, **26**), which were separated by flash chromatography. This non-aqueous reagent was found to be a source of 'soft' nucleofilic bromide that opened the epoxidic group to bromohydrin, without modifying the allylic β-hydroxysulfide or the double bonds. The regioselectivity towards the two isomeric bromohydrins was low, affording, for example, a 55:45 ratio of the two bromohydrins **13** and **14**, from **12** (Scheme 2).

The (3*R**,4*S**)-β-hydroxysulfide, possessing the terminal bromohydrin, was finally converted to the *trans*-vinyldioxidosqualene derivative. The sulfonium β-hydroxysulfide was generated with trimethyloxonium tetrafluoroborate, and transformed to epoxide by NaOH catalyzed cyclization.[52–54] The (3*R**,4*S**)-β-hydroxysulfide was converted to *trans*-epoxide with the contemporary closure of the bromohydrin to epoxide. Bromohydrins with the tertiary hydroxyl, such as **13**, cyclized to the corresponding epoxide **15** in high yields, while bromohydrins with a secondary hydroxyl, such as **14**, afforded the epoxide in lower yields. Other methods for obtaining the vinylepoxides had no stereospecificity, and therefore were unsuitable, because a diastereomeric mixture of *cis* and *trans* alkylic epoxides is practically impossible to separate.

Results and Discussion

The 19-vinyl-2,3:18,19- and 15-vinyl-2,3:14,15-dioxidosqualene derivatives **15** and **27** share some interesting structural features related with other effective OSC inhibitors, such as the time-dependent inhibitors (18*Z*)-29-methylidene-2,3-oxidosqualene [(18*Z*)-29-MOS)] **28**,[37] (18*E*)-29-methylidene-2,3-oxidohexanorsqualene [(18*E*)-hexanor-29-MOS)] **29**,[42] and 2,3:18,19-dioxidosqualene **30**[43–46] (Fig. 2), while some inhibitory behaviors are peculiar of this series. In particular, the IC_{50} values of the two vinyldioxidosqualene derivatives **15** and **27**, obtained using partially purified pig liver or *Saccharomyces cerevisiae* OSC (Table 1), are similar to those found for 29-methylidene derivatives **28** and **29**, previously studied by Xiao and Prestwich,[37] Corey,[41] and our group.[42] Moreover, in pig liver OSC, the IC_{50} of **27** is also similar to that of racemic 2,3:18,19-dioxidosqualene **30**.[43–46]

Another series of compounds, developed as OSC inhibitors both in pig and yeast enzyme, are the vinyl-β-hydroxysulfide derivatives **12**, **21** and **24**. Our aim was to introduce a sulfur atom at (or adjacent to) the critical positions of carbocationic intermediates C-15 and C-19

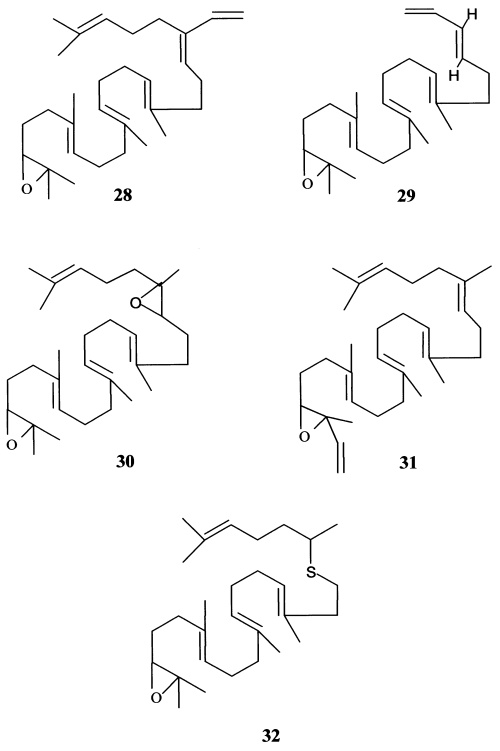


Figure 2. Structures of (18*Z*)-29-methylidene-2,3-oxidosqualene **28**, (18*E*)-29-methylidene-2,3-oxidohexanorsqualene **29**, 2,3:18,19-dioxidosqualene **30**, (2-*trans*)-1-methylidene-2,3-oxidosqualene **31** and 19*H*-18-thia-2,3-oxidosqualene **32**.

(squalene numbering) formed by cyclization of OS. The vinylsulfide system may interact with the nucleophilic group present in the active site of the enzyme near the 14–15 or 18–19 double bonds of the natural substrate. Vinylsulfide **12**, has been shown to have an IC_{50} in pig liver OSC even lower than that found for the corresponding vinyldiepoxide **15**, whereas **21** and **24** were 15- to 30-fold less active than the corresponding vinyldiepoxide **27**, both in pig liver and *S. cerevisiae* OSC.

Vinylepoxy derivative **18** (Scheme 3) did not seem to react with the C-2 cation generated by OSC cyclization, behaving as a modest inhibitor of OSC. In fact, (2-*trans*)-1-methylidene-2,3-oxidosqualene **31**, a C-2 vinyl analogue of 2,3-oxidosqualene (Fig. 2), which has a structure similar to **18**, behaved also as a modest inhibitor of pig liver OSC. Moreover, an increased cyclization rate of **31** to a 4-vinyl analogue of lanosterol was found.[55] Thus, as suggested by Corey,[11] it is possible that the A-ring formation may proceed through a concerted process involving the oxirane ring and the 6,7-double bond (a SN_2 mechanism as long postulated),[19] whereas the formation of an intermediate tertiary cation at C-2 seems unlikely. Results found with compounds **12**, **15**, **21**, **24** and **27** are in agreement with discrete carbocationic intermediates at C15, C14, and C19 (squalene numbering) during the cyclization of OS.

Table 1. IC_{50} and k_{inact}/K_I values of inhibition of solubilized and partially purified OSC by vinyldioxidosqualenes, β-hydroxyvinylsulfides and 29-methylidene-2,3-oxidosqualene derivatives

Compounds	IC_{50} (μM)		k_{inact}/K_I (min^{-1} μM^{-1})
	S. cerevisiae	Pig liver	Pig liver
12	2.5	1.1	5.1×10^{-3}
15	1.5	2.5	1.2×10^{-3}
18	50	100	nd[a]
21	40	7.5	nd
24	25	12	nd
27	1.5	0.4	14.7×10^{-3}
28	1.0	0.4	99.6×10^{-3}
29	1.5	3.5	7.0×10^{-3}

[a]nd, not determined.

To gain further insight into the mechanism of inhibition, we studied the time dependency of pig liver OSC inactivation with the more active compounds **12**, **15** and **27**. Time-dependent inhibition was studied by incubating the enzyme in the presence of the inhibitors. At time intervals, aliquots were withdrawn, diluted at least 40 times and added with substrate, to check residual enzymatic activity with respect to controls, that were pre-incubated in the absence of inhibitors for the same time. Surprisingly, we did not observe time-dependent inactivation at inhibitor concentrations comparable to their IC_{50}. Increasing the concentration of the inhibitors to at least ten times the corresponding IC_{50} value, we observed a time-dependent inhibition. The results obtained with compounds **12**, **15** and **27** at the different concentrations tested are shown in Figure 3. We exclude that the time-dependent decrease of activity could be due to insufficient dilution, as non-preincubated controls have never shown more than a 10% decrease of activity, when tested in the presence of inhibitor at the same final dilution of the tests. The second order inactivation constants for compounds **12**, **15** and **27**, calculated from the $t_{1/2}$ value obtained at 10 μM concentration, were 5.1×10^{-3}, 1.2×10^{-3}, and 14.7×10^{-3} $min^{-1}\,\mu M^{-1}$, respectively (Table 1); again, the most effective compound was **27**. By contrast, the methylidene derivatives (18*Z*)-29-MOS **28** and (18*E*)-hexanor-29-MOS **29** showed time-dependent inhibition at concentrations similar to their IC_{50}.[37,42] Thus, at the lowest concentrations (below 10 μM), the more active compounds **12** and **27** behaved as reversible non-competitive inhibitors, whose effectiveness as OSC inhibitors could be due to a high affinity of the enzyme for a dioxidosqualenoid structure.

It has been suggested that the squalene diepoxides may act as potential modulators of OSC.[43–46] Indeed, 2,3:22,23-dioxidosqualene (DOS) is formed by squalene epoxidase after incubation of OS with pig liver enzyme.[56] It has been shown that DOS is further converted into 24(*S*),25-epoxylanosterol and ultimately into 24(*S*)-epoxycholesterol, which is a repressor of HMG-CoA reductase. Inhibition of OSC produced an accumulation of OS and also of DOS.[57–60] DOS was found to be an inhibitor of mammalian OSC, with an IC_{50} of 16 μM[56] and, in comparison to OS, it is a preferred substrate of OSC (V/Km ($\times10^3$) = 182 for DOS; V/Km ($\times10^3$) = 80 for OS).[59] Moreover, incubation of 6,7- and 10,11-oxidosqualene in rat liver microsomes led to the formation of the corresponding dioxidosqualenes,

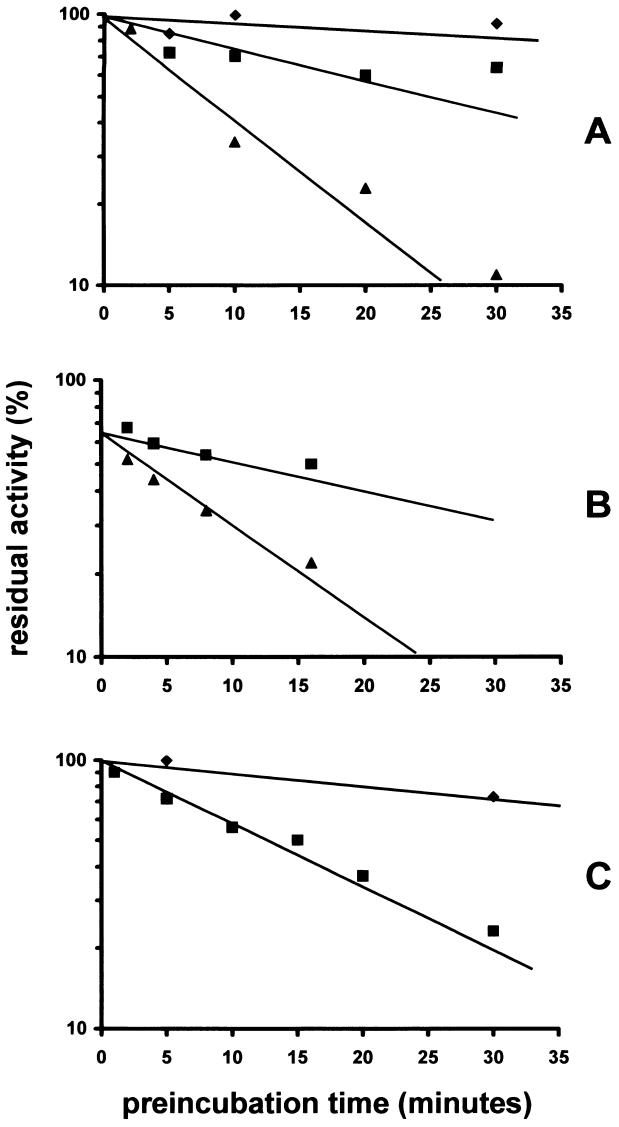


Figure 3. Time dependent inhibition of pig liver OSC by compounds **12**, **15** and **27**. Partially purified OSC was preincubated at 37 °C in the presence of: (A) compound **15**, 5 μM (◆), 10 μM (■), and 100 μM (▲); (B) compound **27**, 10 μM (■), and 20 μM (▲); (C) compound **12**, 3 μM (◆), and 10 μM (■). Residual activity (% of the control preincubated in the absence of inhibitors for the same time) was determined by withdrawing aliquots of 25 μL at time intervals, and diluting to a final volume of 1 mL with substrate.

resulting from the epoxidation of one terminal double bond.[44]

At a concentration considerably above than the IC_{50} (10 μM or more), compounds **12**, **15** and **27** gave time-dependent inhibition. So far we have not been able to prove whether the time-dependent inhibition could be due to irreversible inhibition, as removal of the inhibitor with a DEAE anionic exchanger gave inconsistent results. For example, removal of 50 μM inhibitor **15** with the anionic exchanger, left a residual activity of 60% of the control, after both 5 and 30 min of preincubation. The chromatographic method has not been suitable to give information about irreversibility in our system.

As suggested by Abe[35] for 19*H*-18-thia-2,3-oxidosqualene **32**, a mechanism-based inhibitor of OSC, it is possible that time-dependent inhibition requires partial cyclization of **12**, **15** and **27** to a bicyclic or tricyclic intermediate, with the trapping of a cationic intermediate by an active site nucleophile. In this case, it may be speculated that the vinyldiepoxy or vinylsulfide derivatives, at concentrations near the IC_{50}, in spite of giving covalent linkage with the active site of the enzyme, simply facilitate the interactions of inhibitors with the active site of the enzyme, thus triggering their cyclization and the subsequent π-cationic interactions and stabilizing the bicyclic or the tricyclic intermediates formed.[17,18,39] Indeed, it was found that truncated 2,3-oxidosqualenes were transformed by pig liver OSC into tricyclic compounds as major cyclized components.[61,62] Further work is in progress to assess the nature of these interactions, which will provide important information for the future design of new potent OSC inhibitors useful as hypocholesterolemic agents, and for improving the knowledge of the active site of the enzyme.

Time-dependent inhibition of *S. cerevisae* OSC was tested for compound **15** at concentrations similar to their IC_{50}. Testing the time dependency at higher concentrations was not possible: the dilution needed to remove the inhibitor after preincubation was too high to observe a residual activity, because of the low specific activity of yeast OSC. At the concentrations tested, no time-dependent inhibition was found.

Conclusion

Derivatives bearing a vinylepoxy function, located at crucial positions of the 2,3-oxidosqualenoid moiety, were potent 2,3-oxidosqualene cyclase inhibitors, active at 1 μmolar concentration or even less as reversible inhibitors. Increasing the concentration at least 10-fold, these compounds behaved as time-dependent inhibitors. They are thus one of the few classes of time-dependent inhibitors of animal OSC. (14-*trans*)-28-Methylidene-2,3:14,15-dioxidoundecanorsqualene **27** possessed an IC_{50} of 0.4 μM, as the important irreversible inhibitors of animal cyclase (18*Z*)-29-MOS **28**, although the nature of the interactions with the enzyme may be different. Compound **27** was shown to be the most potent inhibitor of the animal enzyme known so far, possessing a truncated squalenoid structure. Further work is in progress to identify the nature of the interactions with the aminoacids of the active site of OSC.

Experimental

Chemistry

The ^1H NMR spectra were recorded either on a Jeol EX 400 or a Bruker AC 200 instrument in $CDCl_3$ solution at room temperature, with $SiMe_4$ as internal standard. Mass spectra were obtained on a VG Analytical 7070 EQ-HF or a Finnigan MAT TSQ 700 spectrometer, by electron impact or chemical ionization. IR spectra were

recorded on a Perkin–Elmer 781 Spectrophotometer. The reactions were monitored by TLC on F_{254} silica gel precoated sheets; after development, the sheets were exposed to iodine vapour. Flash column chromatography was performed on 230–400 mesh silica gel. Petroleum ether refers to the fraction boiling in the range 40–60 °C. Diethyl ether and THF were dried over sodium benzophenone ketyl. Microanalyses were performed on an elemental analyser 1106 (Carlo Erba Strumentazione). Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values.

C_{22} Squalene aldehyde and C_{17} squalene aldehyde were obtained as previously reported.[24] C_{22} Squalene aldehyde external epoxide **10** was obtained as previously reported.[42] C_{17} Squalene aldehyde external epoxide **19** was obtained starting from C_{17} squalene aldehyde, following the general method previously reported.[42] C_{27} Squalene aldehyde **16** was obtained as previously reported.[63]

(3*R,4*S**,7*E*,11*E*,15*E*)-19,20-Epoxy-3-ethylthio-7,12,16,20-tetramethyl-1,7,11,15-henicosatetraen-4-ol (12).** Allyl ethyl sulfide **11** (1.5 equiv, 276 mg, 2.7 mmol) in anhydrous THF (10 mL) was stirred at −80 °C under dry argon. *tert*-Butyllithium (1.7 M in pentane, 1.5 equiv, 2.7 mmol, 1.6 mL) was added, while the color turned to yellow, and it was left to rest for 15 min at 0–5 °C. It was returned to the bath at −80 °C and titanium tetraisopropoxide (1.5 equiv, 767 mg, 2.7 mmol) was added, forming a dark orange solution. After 10 min at −80 °C, C_{22} squalene aldehyde external epoxide **10** (1 equiv, 600 mg, 1.8 mmol) was added, while the solution partially lightened. It was left to stand for 15 min at −80 °C, then placed in a bath at −10 °C, and gradually allowed to reach room temperature, within 20 min. It was poured into 10% cold NH_4Cl:diethyl ether, 1:1 (80 mL) and extracted with diethyl ether (3×60 mL). The combined extracts were washed with saturated brine (2×50 mL), dried over anhyd sodium sulfate and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether:diethyl ether, 95:5, to remove impurities, then 90:10, to give 641 mg of compound **12** (82% yield from **10**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.25 (2 s and 1 t, 9H, epoxidic CH_3 and CH_3CH_2S), 1.48–1.68 (m, 13H, allylic CH_3, CH_2-epoxide and CH_2CHOH), 2.00–2.15 (m, 12H, allylic CH_2), 2.49 (q, J = 7.4 Hz, 2H, CH_3CH_2S), 2.70 (t, J = 6.2 Hz, 1H, epoxidic CH), 3.30 (m, 1H, CHS), 3.66 (m, 1H, CHOH), 5.00–5.21 (m, 5H, vinylic CH), 5.74 (m, 1H, CH_2=CHCHS); IR (liquid film) 3470, 2960, 2925, 2855, 1665, 1630, 1450, 1380 cm^{-1}; CIMS (isobutane) *m*/*z* 435 (30), 417 (100), 399 (10), 357 (18), 315 (10); HRMS *m*/*z* 434.3215 (calcd for $C_{27}H_{46}O_2S$ 434.3218). Anal. ($C_{27}H_{46}O_2S$) C, H, O, S.

(6*E*,10*E*,14*E*,18*R,19*S**)-3-Bromo-19-ethylthio-2,6,10,15-tetramethyl-6,10,14,20-henicosatetraene-2,18-diol (13) and (6*E*,10*E*,14*E*,18*R**,19*S**)-2-bromo-19-ethylthio-2,6,10,15-tetramethyl-6,10,14,20-henicosatetraene-3,18-diol (14).** Compound **12** (304 mg, 0.70 mmol) was dissolved in anhydrous diethyl ether (15 mL) and stirred at 30 °C under nitrogen. Li_2NiBr_4 freshly prepared,[51] from LiBr and $NiBr_2$ (0.4 M solution in anhyd THF, 3 equiv, 5.3 mL, 2.1 mmol), was added and the blue solution was left to react for 1 day at 30 °C. It was then cooled, poured into a phosphate buffer at pH 7:dichloromethane, 1:1 (80 mL), and extracted with dichloromethane (3×40 mL). The combined extracts were washed with saturated brine (3×40 mL), dried over anhyd sodium sulfate and evaporated in vacuo. Purification was achieved by flash chromatography with petroleum ether:diethyl ether, 90:10, to give unreacted epoxide **12** (8%, 24 mg), then 85:15, to give monobromohydrin **14** (155 mg), and finally 80:20, to give monobromohydrin **13** (127 mg), as light-yellow oils, in 78% total yield of the two monobromohydrins **13** and **14** (55:45).

13. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 1.33 and 1.34 (2 s, 6H, $(CH_3)_2COH$), 1.43–1.73 (m, 13H, allylic CH_3, CH_2CHBr and CH_2CHOH), 2.00–2.25 (m, 12H, allylic CH_2), 2.49 (q, J = 7.3 Hz, 2H, SCH_2CH_3), 3.30 (m, 1H, CHS), 3.67 (m, 1H, CHOH), 3.97 (m, 1H, CHBr), 4.99–5.22 (m, 5H, vinylic CH), 5.75 (m, 1H, CH_2=CHCHS); IR (liquid film) 3450, 2960, 2855, 2360, 1665, 1630, 1450, 1385 cm^{-1}; CIMS (isobutane) *m*/*z* 517 (15), 515 (15), 499 (25), 497 (25), 435 (35), 417 (100), 399 (15), 357 (15); HRMS *m*/*z* 514.2480 (calcd for $C_{27}H_{47}BrO_2S$ 514.2480). Anal. ($C_{27}H_{47}BrO_2S$) C, H, Br, O, S.

14. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 1.45–1.65 (m, 13H, allylic CH_3 and 2 CH_2CHOH), 1.73 and 1.79 (2 s, 6H, $(CH_3)_2CBr$), 2.00–2.26 (m, 12H, allylic CH_2), 2.48 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.30 (m, 1H, CHS), 3.38 (m, 1H, CHOHCBr), 3.67 (m, 1H, CHOHCS), 5.00–5.22 (m, 5H, vinylic CH), 5.75 (m, 1H, CH_2=CHCHS); IR (liquid film) 3450, 2960, 2855, 2360, 1665, 1630, 1450, 1385 cm^{-1}; CIMS (isobutane) *m*/*z* 517 (10), 515 (10), 499 (19), 497 (19), 435 (54), 417 (100), 357 (28), 333 (15); HRMS *m*/*z* 514.2477 (calcd for $C_{27}H_{47}BrO_2S$ 514.2480). Anal. ($C_{27}H_{47}BrO_2S$) C, H, Br, O, S.

(18-*trans*)-29-Methylidene-2,3:18,19-dioxidohexanorsqualene: (3-*trans*-7*E*,11*E*,15*E*)-3,4:19,20-diepoxy-7,12,16,20-tetramethyl-1,7,11,15-henicosatetraene (15). Trimethyloxonium tetrafluoroborate (2 equiv, 58 mg, 0.39 mmol) was added with dichloromethane (2 mL), and vigorously stirred under nitrogen at 0 °C. Bromohydrin **13** (100 mg, 0.194 mmol) was added, the cold bath was removed, and the mixture left to react for 1 h at room temperature, under nitrogen. It was cooled again to 0 °C, aq NaOH (0.5 M, excess, 5 mL) was added in small portions, to avoid excessive warming and foaming. The emulsion was brought to room temperature and allowed to react for 3 h under vigorous stirring. It was extracted with diethyl ether (3×50 mL), after addition of brine (50 mL). The combined extracts were washed with saturated brine (2×30 mL), dried with anhyd sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether:diethyl ether, 97:3 to remove impurities, then 95:5, affording 44 mg (61% yield from bromohydrin

13) of (18-*trans*)-29-methylidene-2,3:18,19-dioxidohexanorsqualene **15**, as a colorless oil (*trans:cis*, >95:5).

The same reaction was also performed under the same conditions starting from bromohydrin **14**, affording compound **15** in 30% yield: ^{1}H NMR ($CDCl_3$) δ 1.258 and 1.300 (2 s, 6H, epoxidic CH_3), 1.43–1.68 (m, 13H, allylic CH_3 and 2 CH_2-epoxide), 1.99–2.24 (m, 12H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1H, CH trisubstituted epoxide), 2.82 (dt, J = 2.1 and 5.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 3.09 (dd, J = 2.1 and 7.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 5.02–5.67 (m, 6H, vinylic CH); IR (CCl_4) 2960, 2930, 2855, 1665, 1645, 1450, 1380 cm^{-1}; CIMS (isobutane) m/z 373 (48), 355 (100), 337 (18), 215 (12); HRMS m/z 372.3031 (calcd for $C_{25}H_{40}O_2$ 372.3028). Anal. ($C_{25}H_{40}O_2$) C, H, O.

(3*R,4*S**,7*E*,11*E*,15*E*,19*E*)-3-Ethylthio-7,11,16,20,24-pentamethyl-1,7,11,15,19,23-pentacosahexaen-4-ol (17).** Compound **17** was obtained according to the method reported for the synthesis of **12**, starting from C_{27} squalene aldehyde **16**, in 85% yield: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.3 Hz, 3H, CH_3CH$_2$S), 1.51–1.68 (m, 20H, allylic CH_3, and CH_2CHOH), 2.00–2.25 (m, 18H, allylic CH_2), 2.49 (q, J = 7.3 Hz, 2H, CH$_3$CH_2S), 3.29 (m, 1H, CHS), 3.67 (m, 1H, CHOH), 5.00–5.22 (m, 7H, vinylic CH), 5.76 (m, 1H, CH$_2$=CHCHS); IR (liquid film) 3460, 2980, 2920, 2860, 1450, 1385 cm^{-1}; CIMS (isobutane) m/z 487 (100), 471 (12), 469 (35). EIMS m/z 486 (3), 457 (1.5), 407 (2), 385 (5), 349 (3), 231 (10), 191 (12), 161 (15), 149 (25), 69 (100); HRMS m/z 486.3894 (calcd for $C_{32}H_{54}OS$ 486.3895). Anal. ($C_{32}H_{54}OS$) C, H, O, S.

(2-*trans*)-1-Methylidene-2,3-oxido-1′-norsqualene: (3-*trans*-7*E*,11*E*,15*E*,19*E*)-3,4-epoxy-7,11,16,20,24-pentamethyl-1,7,11,15,19,23-pentacosahexaene (18). Compound **18** was obtained according to the method reported for the synthesis of **15**, starting from **17**, in 73% yield (*trans:cis*, >95:5): ^{1}H NMR ($CDCl_3$) δ 1.43–1.72 (m, 20H, allylic CH_3 and CH_2-epoxide), 2.00–2.26 (m, 18H, allylic CH_2), 2.82 (dt, J = 2.1 and 5.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 3.09 (dd, J = 2.1 and 7.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 5.00–5.66 (m, 8H, vinylic CH); IR (liquid film) 2975, 2930, 2860, 1665, 1645, 1450, 1410, 1380 cm^{-1}; EIMS m/z 424 (0.7), 381 (0.4), 355 (0.6), 245 (2), 231 (5), 203 (80), 69 (100); CIMS (isobutane) m/z 425 (100), 407 (70); HRMS m/z 424.3709 (calcd for $C_{30}H_{48}O$ 424.3705). Anal. ($C_{30}H_{48}O$) C, H, O.

(3*R,4*S**,7*E*,11*E*)-15,16-Epoxy-8,12,16-trimethyl-3-phenylthio-1,7,11-heptadecatrien-4-ol (21).** Allyl phenyl sulfide **20** (1.5 equiv, 545 mg, 3.63 mmol) in anhyd THF (10 mL) was stirred at −80 °C under dry argon. *n*-Butyllithium (1.6 M in hexane, 1.5 equiv, 3.63 mmol, 2.3 mL) was slowly added at −80 °C, at which the color turned to orange, and it was left to stand for 30 min at 0 °C. During this time the color turned to orange–red. It was returned to the bath at −80 °C, and titanium tetraisopropoxide (1.5 equiv, 1.03 g, 3.63 mmol) was added, forming an orange solution. After 10 min stirring at −80 °C, C_{17} squalene aldehyde external epoxide **19** (1 equiv, 600 mg, 2.42 mmol) was added, at which the solution partially lightened. It was left to stand for 15 min at −80 °C, then transferred to a bath at −10 °C and gradually allowed to reach room temperature, within 20 min. It was poured into 10% cold NH_4Cl:diethyl ether, 1:1 (80 mL), and extracted with diethyl ether (3×60 mL). The combined extracts were washed with saturated brine (2×50 mL), dried over anhyd sodium sulfate and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether:diethyl ether, 95:5, to remove impurities, then 90:10, to give 512 mg of compound **21** (51% yield from **19**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.26 and 1.29 (2 s, 6H, epoxidic CH_3), 1.50–1.69 (m, 10H, allylic CH_3, CH_2-epoxide and CH_2CHOH), 2.00–2.16 (m, 8H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1H, epoxidic CH), 3.68 (m, 2H, CHOH and CHS), 5.02–5.18 (m, 4H, vinylic CH), 5.84 (m, 1H, CH$_2$=CHCHS), 7.20–7.44 (m, 5H, aromatic CH); IR (liquid film) 3450, 3060, 2970, 2930, 2860, 1585, 1440, 1380 cm^{-1}; CIMS (isobutane) m/z 415 (22), 397 (100), 379 (18); HRMS m/z 414.2595 (calcd for $C_{26}H_{38}O_2S$ 414.2592). Anal. ($C_{26}H_{38}O_2S$) C, H, O, S.

(6*E*,10*E*,14*R,15*S**)-3-Bromo-2,6,10-trimethyl-15-phenylthio-6,10,16-heptadecatrien-2,14-diol (22) and (6*E*,10*E*,14*R**,15*S**)-2-bromo-2,6,10-trimethyl-15-phenylthio-6,10,16-heptadecatrien-3,14-diol (23).** Compounds **22** and **23** were obtained according to the method described for **13** and **14**, in 81% yield (**22:23** = 60:40).

22. ^{1}H NMR ($CDCl_3$) δ 1.33 and 1.34 (2 s, 6H, (CH_3)$_2$COH), 1.43–1.68 (m, 10H, allylic CH_3, CH_2CHBr and CH_2CHOH), 2.00–2.37 (m, 8H, allylic CH_2), 3.68 (m, 2H, CHOH and CHS), 3.97 (m, 1H, CHBr), 5.00–5.20 (m, 4H, vinylic CH), 5.82 (m, 1H, CH$_2$=CHCHS), 7.20–7.45 (m, 5H, aromatic CH); IR (liquid film) 3450, 3080, 2970, 2920, 2860, 1635, 1585, 1480, 1440, 1385 cm^{-1}; CIMS (isobutane) m/z 497 (4), 495 (5), 479 (75), 477 (76), 471 (100). EIMS m/z 496 (0.01), 494 (0.01), 347 (0.7), 345 (0.8), 327 (2), 309 (3), 265 (15), 229 (20), 150 (100), 135 (40); HRMS m/z 494.1852 (calcd for $C_{26}H_{39}BrO_2S$ 494.1854). Anal. ($C_{26}H_{39}BrO_2S$) C, H, Br, O, S.

23. ^{1}H NMR ($CDCl_3$) δ 1.40–1.68 (m, 10H, allylic CH_3, and 2 CH_2CHOH), 1.73 and 1.79 (2 s, 6H, (CH_3)$_2$CBr), 2.00–2.28 (m, 8H, allylic CH_2), 3.40 (m, 1H, CHOHCBr), 3.70 (m, 2H, CHOHCHS), 5.00–5.20 (m, 4H, vinylic CH), 5.82 (m, 1H, CH$_2$=CHCHS), 7.20–7.45 (m, 5H, aromatic CH); IR (liquid film) 3450, 3070, 2970, 2920, 2860, 1635, 1580, 1480, 1440, 1385 cm^{-1}; CIMS (isobutane) m/z 497 (4), 495 (5), 479 (100), 477 (100), 471 (60); EIMS m/z 496 (0.01), 494 (0.01), 422 (0.05), 397 (0.05), 355 (1), 327 (5), 265 (40), 229 (20), 150 (100); HRMS m/z 494.1857 (calcd for $C_{26}H_{39}BrO_2S$ 494.1854). Anal. ($C_{26}H_{39}BrO_2S$) C, H, Br, O, S.

(3*R,4*S**,7*E*,11*E*)-15,16-Epoxy-3-ethylthio-8,12,16-trimethyl-1,7,11-heptadecatrien-4-ol (24).** Compound **24** was obtained according to the method reported for the synthesis of **12**, starting from C_{17} squalene aldehyde external epoxide **19**, in 80% yield. ^{1}H NMR ($CDCl_3$) δ 1.24 (2 s and 1 t, 9H, epoxidic CH_3 and CH_3CH$_2$S), 1.43–1.69 (m, 10H, allylic CH_3, CH_2-epoxide and

CH_2CHOH), 2.00–2.16 (m, 8H, allylic CH_2), 2.51 (q, J = 7.4 Hz, 2H, CH_3CH_2S), 2.71 (t, J = 6.2 Hz, 1H, epoxidic CH), 3.31 (m, 1H, CHS), 3.67 (m, 1H, CHOH), 5.05–5.22 (m, 4H, vinylic CH), 5.74 (m, 1H, CH_2=C*H*CHS); IR (liquid film) 3450, 2960, 2930, 2860, 1715, 1630, 1450, 1380 cm^{-1}; CIMS (isobutane) m/z 367 (28), 349 (100); HRMS m/z 366.2590 (calcd for $C_{22}H_{38}O_2S$ 366.2592). Anal. ($C_{22}H_{38}O_2S$) C, H, O, S.

(6*E*,10*E*,14*R,15*S**)-3-Bromo-15-ethylthio-2,6,10-trimethyl-6,10,16-heptadecatrien-2,14-diol (25) and (6*E*,10*E*,14*R**,15*R**)-2-bromo-15-ethylthio-2,6,10-trimethyl-6,10,16-heptadecatrien-3,14-diol (26).** Compounds **25** and **26** were obtained and separated according to the method described for **13** and **14**, in 83% yield (**25:26** = 55:45).

25. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 1.33 and 1.34 (2 s, 6H, $(CH_3)_2$COH), 1.45–1.64 (m, 10H, allylic CH_3, CH_2CHBr and CH_2CHOH), 2.00–2.22 (m, 8H, allylic CH_2), 2.48 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.30 (m, 1H, CHS), 3.67 (m, 1H, CHOH), 3.98 (m, 1H, CHBr), 5.00–5.22 (m, 4H, vinylic CH), 5.75 (m, 1H, CH_2=C*H*CHS); IR (liquid film) 3450, 2960, 2855, 2360, 1665, 1630, 1450, 1385 cm^{-1}; CIMS (isobutane) m/z 449 (18), 447 (18), 431 (28), 349 (100); HRMS m/z 446.1860 (calcd for $C_{22}H_{39}BrO_2S$ 446.1854). Anal. ($C_{22}H_{39}BrO_2S$) C, H, Br, O, S.

26. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 1.45–1.64 (m, 10H, allylic CH_3 and 2 CH_2CHOH), 1.74 and 1.80 (2 s, 6H, $(CH_3)_2$CBr), 2.00–2.25 (m, 8H, allylic CH_2), 2.48 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.30 (m, 1H, CHS), 3.38 (m, 1H, CHOHCBr), 3.67 (m, 1H, CHOHCS), 5.00–5.22 (m, 4H, vinylic CH), 5.75 (m, 1H, CH_2=C*H*CHS); IR (liquid film) 3450, 2960, 2855, 2360, 1665, 1630, 1450, 1385 cm^{-1}; CIMS (isobutane) m/z 449 (14), 447 (14), 431 (22), 349 (100); HRMS m/z 446.1855 (calcd for $C_{22}H_{39}BrO_2S$ 446.1854). Anal. ($C_{22}H_{39}BrO_2S$) C, H, Br, O, S.

(14-*trans*)-28-Methylidene-2,3:14,15-dioxidoundecanorsqualene: (3-*trans*-7*E*,11*E*)-3,4:15,16-diepoxy-8,12,16-trimethyl-1,7,11-heptadecatriene (27). Diepoxide **27** was obtained according to the method described for **15**, in 58% yield (*trans:cis*, >95:5), starting from bromohydrin **25**. ^{1}H NMR ($CDCl_3$) δ 1.258 and 1.300 (2 s, 6H, epoxidic CH_3), 1.52–1.68 (m, 10H, allylic CH_3 and 2 CH_2-epoxide), 1.95–2.22 (m, 8H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1H, CH trisubstituted epoxide), 2.83 (dt, J = 2.1 and 5.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 3.10 (dd, J = 2.1 and 7.6 Hz, 1H, epoxidic CH−CH−CH=CH_2), 5.08–5.62 (m, 5H, vinylic CH); IR (CCl_4) 2960, 2925, 2855, 1665, 1645, 1450, 1380 cm^{-1}; CIMS (isobutane) m/z 305 (60), 287 (100); HRMS m/z 304.2406 (calcd for $C_{20}H_{32}O_2$ 304.2402). Anal. ($C_{20}H_{32}O_2$) C, H, O.

Solubilisation and purification of OSCs

Partially purified OSCs from pig liver microsomes and yeast microsomes were obtained as previously described.[62,63]

Assay of OSC activity and kinetic determination

Enzyme activity of OSC was determined by incubating the partially purified pig enzyme for 30 min at 45 °C, and the solubilized yeast enzyme for 30 min at 35 °C, with [3-^{3}H]-3-(*R*,*S*)-2,3-oxidosqualene (50,000 cpm), as previously described.[64,65] IC_{50} values (the concentration of inhibitor that reduces the enzymatic conversion of 2,3-oxidosqualene to lanosterol by 50%) were determined at 25 μM substrate concentration, in the presence of different concentrations of inhibitors.

Time-dependent inactivation of the OSC

Time-dependent inactivation was determined at 35 °C by adding the inhibitors to the enzyme solution in the absence of the substrate. Aliquots were withdrawn at time intervals from 30 s to 45 min, and diluted 40-fold for pig enzyme or 10-fold for yeast enzyme, by transfer to test tubes containing cold and labeled substrate 2,3-oxidosqualene (25 μM) and Tween-80 (0.5 mg/mL) in Na/K phosphate buffer. Residual activity was determined by incubating pig or yeast enzyme under the same conditions. Second-order inactivation constants were determined from $t/2$ values obtained in the time-dependent inactivation experiments.

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