BIOORGANIC CHEMISTRY 19, 300–313 (1991)

Evaluation of Squalene Analogs Bearing Photoreactive Groups as Inhibitors of Squalene Epoxidase and Oxidosqualene Cyclase

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Received December 20, 1990

The epoxidation of squalene to (3*S*)-2,3-oxidosqualene by squalene epoxidase (SE) and its subsequent cyclization to lanosterol by oxidosqualene cyclase (OSC) are the committed steps of sterol synthesis (Fig. 1) (*1*). Both enzymes are intriguing from a mechanistic perspective and have been targeted for the development of hypocholesterolemic, antifungal, and herbicidal agents (*3*).

In its epoxidation of squalene, SE selects a single face from among three nearly equivalent trisubstituted olefinic bonds. The epoxidase requires oxygen, FAD, cytochrome P450 reductase, NADPH, and a soluble protein factor for activity (*4*), but apparently contains no heme or bound metals (*5*). The enzyme has been purified from rat liver, but the mechanism of this flavoprotein alkene epoxidation is unknown (*5*).

OSC binds (3*S*)-2,3-oxidosqualene in a chair–boat–chair conformation and mediates the sequential formation of four rings, initiated by protonation of the epoxide and proceeding through a series of carbocation intermediates (*6*). The final cation undergoes a cascade of 1,2-hydride and methyl shifts; proton loss yields lanosterol (*6*). Although the substrate specificity of OSC has been the focus of much attention (*7*), little is known about how the ensemble of active site residues orchestrates this magnificent tetracyclization/rearrangement (*8*). The enzyme has been isolated from a plant source (*9*), but mammalian OSC has only been partially purified (*10*).

In photoaffinity labeling (*11*) a radioactive ligand containing a photolabile group is first allowed to reversibly bind to a protein, irradiation at an appropriate wavelength generates a reactive species which can covalently modify the protein. The radiolabeled, modified protein can be subjected to rigorous purification and characterization. This enables the comparison of the physicochemical properties of the protein from various species or tissues. Degradation can be conducted to isolate labeled oligopeptides, which can be sequenced. Thus, the composition and

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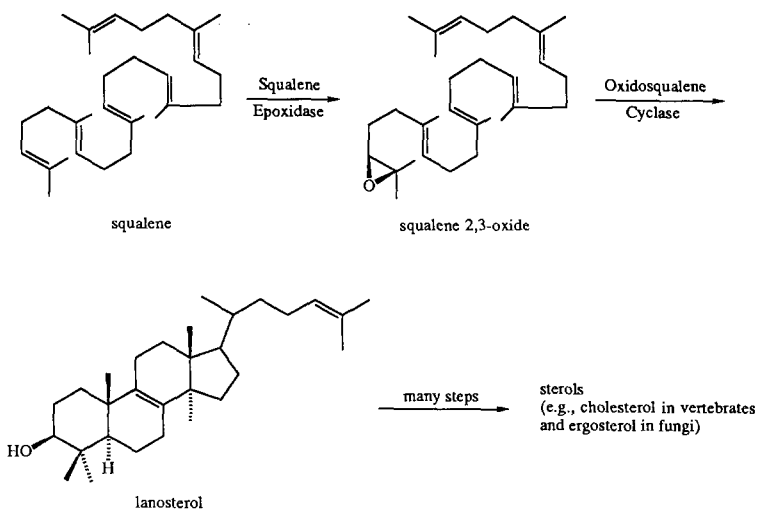


FIG. 1. General scheme of squalene processing in nonphotosynthetic organisms. In plants, squalene (3*S*)-2,3-oxide is converted into cycloartenol and β-amyrin (*2*).

topology of the binding site can be deduced. Herein, the synthesis and evaluation of a series of photolabels for pig liver SE and OSC are described.

DESIGN RATIONALE

Since mammalian SE and OSC do not accommodate aromatic inhibitors with high affinity (*12*), the use of the most commonly employed photolabile group, the aryl azide (*13*), was not attempted. Instead, small photoreactive moieties were incorporated into the squalene system in an effort to minimize structural perturbation. Although these small photoreactive groups do not possess ideal characteristics for photolabeling (intense and long wavelength absorption, no rearrangement to species of less reactivity), each has been used with success in specific biological systems. Because the terminus of squalene is most easily functionalized (*14*), this site was selected for placement of the photoreactive group (*15*).

Our reagents fall into four classes based on the nature of the reactive species formed upon photolysis: carbene (diazirine **1**), *n*, π^* excited state (enone **2**), nitrene (azide **3**), and radical cation (*N*-nitrosamines **4** and **5**) (Fig. 2). Although aliphatic diazirines are known to rearrange to olefins upon irradiation (*16*), this type of reagent has been effectively used to tag the oxysteroid receptor (*17*), bile salt binding proteins (*18*), and IgA X24 (*19*). Enones have been extensively employed in the labeling of steroid receptors (*20*). Furthermore, there is evidence for the presence of a reactive cysteine in the active site of OSC (*21*), so enone **2** may also function as a conventional affinity label (*22*).

The nitrenes resulting from the photolysis of aliphatic azides may rearrange to imines (*23*). However, aliphatic azides have been successfully used to label al-

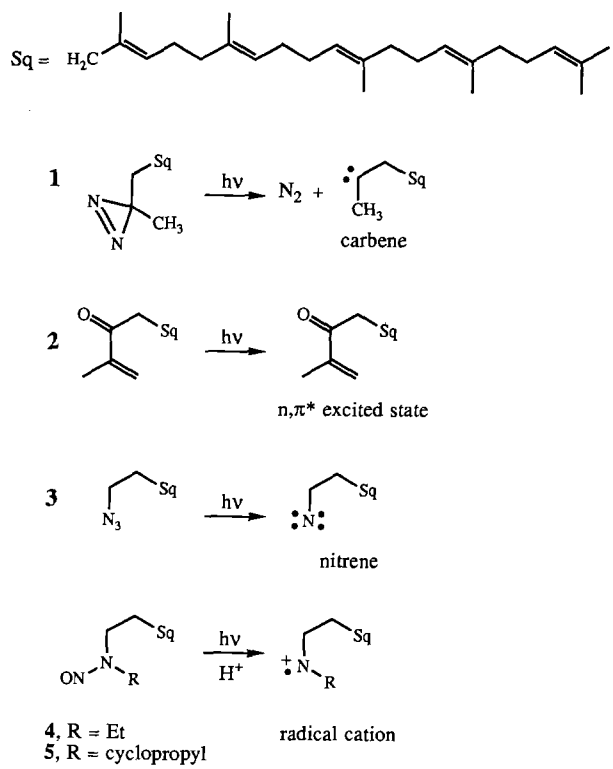


FIG. 2. Proposed photolabels for SE and OSC and the species formed upon photolysis.

bumin (*24*), hydroxycholanoyl transferase (*25*), the calcium channel (*26*), and bile acid binding proteins in whole cells (*27*). Theiler (*28*) has suggested that simultaneous irradiation of azides with uv and visible/near ir light may enhance the efficiency of covalent attachment.

The nitrosamines **4** and **5** were envisioned as photosuicide inhibitors, compounds in which the binding site properties determine the photochemical behavior (*29*). *N*-Nitrosamines are photosensitive only in weakly acidic media of low polarity (*30*). With its putative proton donor site (to initiate the polyene cyclization) and its accommodation of lipophilic squalene, these conditions would appear to be satisfied by the active site of OSC. *N*-Nitrosamines have been used to label acetylcholinesterase (*31*).

Cyclopropyl *N*-nitrosamine **5** embodies a hybridization of the photosuicide inhibitor concept (*29*) and cyclopropylamine mechanism-based inhibitors of monoamine oxidase (*32*) and cytochrome P450 (*33*). These enzyme oxidize cyclopropylamines to radical cations (**6**), which rearrange to iminium homoallyl radicals (**7**), which can react, in turn, with an active site nucleophile (*32, 33*). Likewise, cyclopropyl *N*-nitrosamine **5** was designed to generate, upon photolysis, an identical radical cation intermediate (Fig. 3).

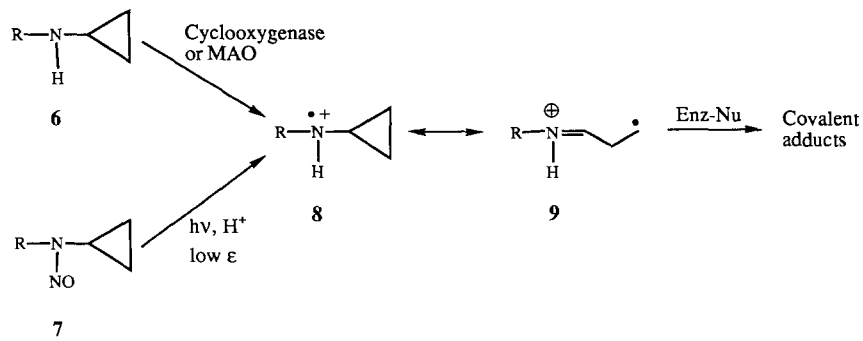


FIG. 3. Enzymatic and photolytic conversion of cyclopropyl amines (**6**) and cyclopropyl *N*-nitrosamines (**7**) into a common radical cation and subsequent covalent labeling. MAO refers to monoamine oxidase; ε is the dielectric constant of the medium; Enz-Nu is an enzymatic nucleophile.

SYNTHESIS

The synthesis of trisnorsqualene azide (**3**) has been described (*34*). The preparation of diazirine **1** and enone **2** is outlined in Scheme I. Squalene was converted to its terminal epoxide (**10**) by the method of van Tamelen and Curphy (*14*). For the enone **2,** epoxide **10** was rearranged under basic conditions (*35*) to the allylic alcohol **11** (*34*). PCC oxidation (*36*) then provided enone **2.** For diazirine **1,** epoxide **10** was first oxidatively cleaved to the aldehyde **8** (*14, 37*). Methyl Grignard addition, followed by PDC oxidation (*38*) gave ketone **14.** This ketone was converted to the diaziridine **15** by the sequential addition of NH_3 and hydroxylamine-*O*-sulfonic acid (*39*). The diaziridine was isolated quickly and immediately converted to the diazirine **1** by silver(I) oxide (*40*).

Nitrosamines **4** and **5** were prepared by treatment of the known amines **16** and **17** (*41*) with nitrous acid (*42*) (Scheme II). This nitrosation method is superior to the use of nitrosonium tetrafluoroborate (*43*) for these substrates because an excess of amine is not required.

Due to hindered rotation about the N–N bond, *N*-nitrosamines **4** and **5** exist as configurational isomers (Fig. 4). For both compounds, proton nuclear magnetic resonance spectroscopy indicates a 3 : 1 ratio of isomers, with the isomer in which the oxygen is *cis* to the trisnorsqualene chain predominating. The geometry assignments are made on the basis of the upfield shifts produced by the shielding effect of oxygen on nearby protons (*44, 45*). For **5,** the two isomers can be distinguished by thin-layer chromatography; the more abundant *E*-isomer has greater TLC mobility (less polar).

ENZYME INHIBITION

The inhibitory potencies of compounds **1–5** for pig liver SE and OSC are presented in Table I. All five photolabels were poor inhibitors of the two enzymes,

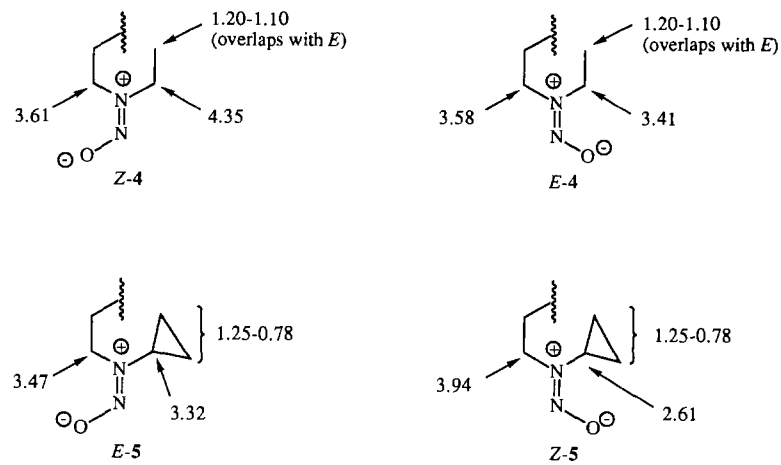


FIG. 4. Field position ($CDCl_3$) of protons on the indicated carbons in the configurational isomers of *N*-nitrosamines **4** and **5**; the chemical shifts are in ppm on the δ-scale.

with only diazirine **1** showing some weak inhibition of OSC. Thus, these compounds are probably unsuitable for labeling studies of the pig liver enzymes.

These results are surprising, considering the small size of the appended photolabile groups, and that, due to the symmetric nature of squalene, these compounds have access to two building modes (Fig. 5) (*47*). The low inhibitory potency of enone **2** may be due to the polarity of this functional group, since the corresponding trisnorsqualene methyl ester **18** (see Structure I) was also a poor inhibitor of both SE (*34*) and OSC (*48*).

The *N*-nitrosamines have considerable dipolar character (*30*). Molecular orbital calculations indicate the dipolar resonance form **19** contributes 48% to the structure of nitrosamines (*30, 49*). Certain zwitterionic compounds, such as amine

TABLE I

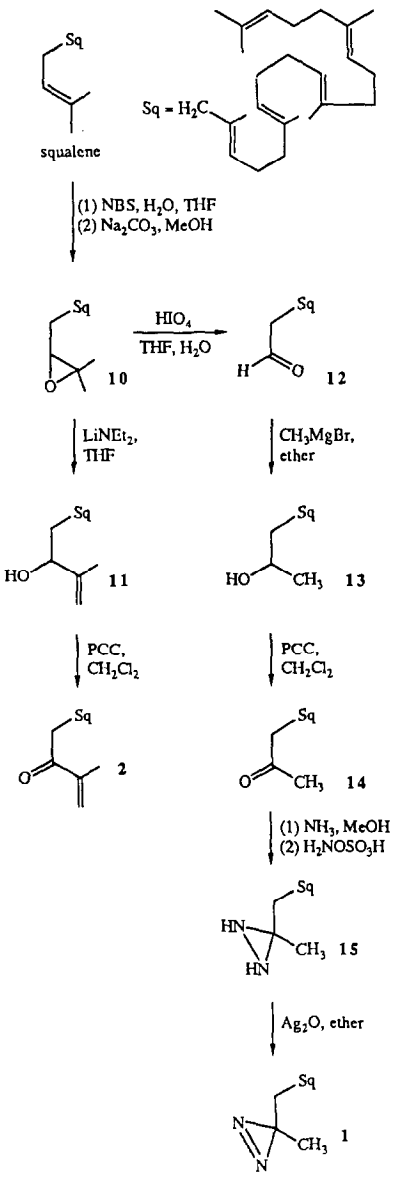
IC_{50} Values[a] for Squalene Analogs **1–5** Using Pig Liver SE[b] and OCS[c]

	IC_{50} (μM)	
Compound	SE	OCS
Diazirine **1**	No inhibition	400
Enone **2**	No inhibition	No inhibition
Azide **3**	>400	>400
Ethyl nitrosamine **4**	No inhibition	No inhibition
Cylcopropyl nitrosamine **5**	>400	>400

[a] Concentration of inhibitor required to reduce enzyme activity by 50%. For a description of the enzyme assays, see Refs. (*34*) and (*46*).

[b] Squalene epoxidase.

[c] Oxidosqualene cyclase.



SCHEME I

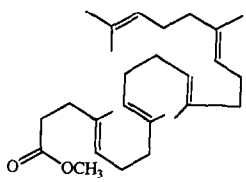
oxide **20,** are potent inhibitors of rat liver OSC and are presumed to mimic the transition state of epoxide opening in the initial step of cyclization (*4*). However, amide **21,** another compound with significant dipolar character, is a poor OSC inhibitor. Alternatively, the nitrosamines may protonate on oxygen (*50*) in the active site of OSC, due to the putative proton donor site. The SE inhibitory potency of squalenoids bearing a terminal hydroxy group is sensitive to the exact

16, R = Et
17, R = cyclopropyl

4, R = Et
5, R = cyclopropyl

SCHEME II

position of the group and to steric demands (*34*, *46*). The potent inhibition of SE by trisnorsqualene cyclopropyl amine (**17**) and OSC by trisnorsqualene cyclopropyl methyl amine *N*-oxide (**22**) (*41*) suggests that the poor inhibition exhibited by **5** is not steric in origin.



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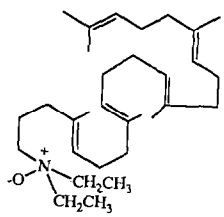

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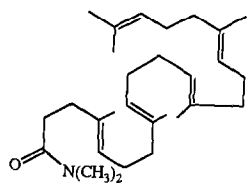

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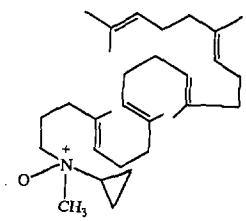

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STRUCTURE I

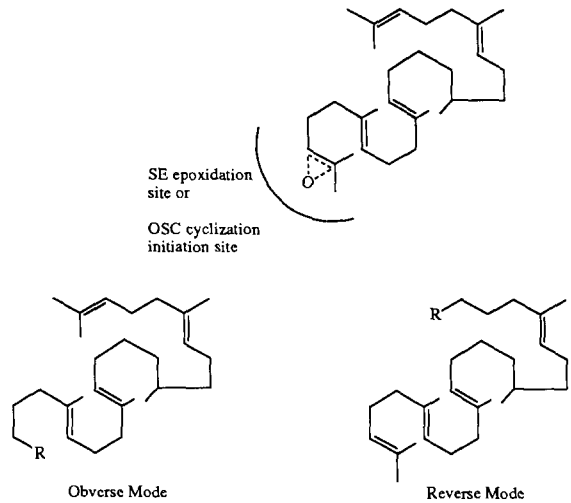

FIG. 5. Two possible binding modes (*47*) of terminally functionalized squalenes relative to squalene or oxidosqualene.

The disappointing inhibition profiles of diazirine **1** and azide **3** are more difficult to rationalize, considering the small size and relatively nonpolar nature of these groups. A possibility for azide **3** is that it may be reduced (*51*) by an active site thiol (*21*) to the corresponding amine. However, trisnorsqualene amine is reported to produce modest inhibition of pig liver SE (IC_{50} = 200 μM), but negligible inhibition of OSC (*41*). These results appear to rule out this possibility.

CONCLUSIONS

Five terminally modified squalenes bearing photolabile functionalities (diazirine, enone, azide, and *N*-nitrosamine) were poor inhibitors ($IC_{50} \geq 400$ μM) for pig liver squalene epoxidase and oxidosqualene cylcase, and therefore were not investigated further as photolabels for these enzymes. However, considering the phyletic variability in SE and OSC (*4*), these compounds may be appropriate for evaluation in other species. These analogs may also be of interest as probes in other systems that process squalene and/or oxidosqualene, such as supernatant protein factor(s) (*52*), membrane components involved in cholesterol biosynthesis (*53*), brain tissue (*54*), and the fowlpox virus (*55*). Furthermore, squalene has been implicated as a possible pheromone or pheromone potentiator in several primates and other organisms (*56*). These compounds may be of utility in identifying high-affinity binding proteins for pheromonal squalene.

With these diverse applications, the further development of photoreactive and other covalently attaching squalenes is certainly mandated. Attempts to prepare mechanism-based inactivators of mammalian SE (*57*) and OSC (*58*) have met with little success, again forcing examination of the photoaffinity labeling option. New

strategies in this regard would be the biological evaluation of squalenoids bearing internal functionality (*15*), which has not received much attention. One candidate is 12,13-didehydrosqualene, which undergoes a triene photocyclization via a zwitterionic mechanism (*59*). Such an intermediate may be trapped by an enzymic electrophile or nucleophile. However, squalene may not be ideal as a backbone for the attachment of photolabeling groups, because the polyene system of squalene may be susceptible to photooxidation during photolysis (*60*). The design and preparation of other photoreactive ligands for OSC and SE is currently in progress.

N-Nitrosamines often display organ-specific carcinogenesis (*61*), so **4** and **5** may add to the armamentarium of experimental tumor inducers. The squalene skeleton may impart the molecular recognition characteristics required for specific biodistribution and metabolism.

EXPERIMENTAL PROCEDURES

Ether and THF were dried with sodium benzophenone ketyl. Analytical thin-layer chromatography (TLC) was performed on MN Polygram Sil G/UV 254 plates; visualization was achieved with vanillin/H_2SO_4. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra (*62, 63*) were obtained on a General Electric QE-300 spectrometer. Infrared spectra were obtained on a Perkin–Elmer 1430 spectrometer as thin films between NaCl plates. Low-resolution mass spectra (MS) were done in the electron impact (EI) mode on a Varian CH-5 or Hewlett–Packard HP 5980A spectrometer. The reported data are from an electron energy of 70 eV and follow the form of *m/z* (intensity relative to base peak = 100). High resolution mass spectra (HRMS) were obtained in the EI mode with a Varian MAT-731 spectrometer. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois. The enzyme (SE and OSC) assays were performed as previously described (*34, 46*).

Warning! *N*-Nitrosamines **4** and **5** are potential carcinogens. Handle and dispose of these materials with respect for their toxic properties (*64*).

Compounds **3** (*34*), **10** (*14*), **11** (*34*), **12** (*37*), **16** (*41*), and **17** (*41*) were prepared as previously described.

(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-one **(2).** Allylic alcohol **11** (1.5 g, 3.5 mmol), dissolved in 10 ml of dichloromethane, was added to a suspension of pyridinium chlorochromate (1.1 g, 5.30 mmol) in dichloromethane (10 ml). After 24 h, the solvent was decanted from the dark residue. The residue was exhaustively rinsed with ether, and the combined organic solvents were evaporated. Purification was achieved by flash chromatography (9:1 hexane–ethyl acetate); 0.35 g (24%) of a clear oil was obtained: R_f = 0.43 (9:1 hexane:ethyl acetate); ir 2922, 1681, 1448, 1381, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.96 (s, 1 H, one H of $=C\underline{H}_2$), 5.76 (s, 1 H, one H of $=C\underline{H}_2$), 5.21–5.05 (m, 5 H, $-C\underline{H}=C(CH_3)$), 2.77 (t, 2 H, J = 8 Hz, $-C(O)C\underline{H}_2-$), 2.28 (t, 2 H, J = 8 Hz, $-C(O)C\underline{H}_2C\underline{H}_2-$), 2.15–1.95 (m, 16 H, $-C\underline{H}_2C\underline{H}_2-$), 1.87 (s, 3 H, $C\underline{H}_3-$

C=CH_2), 1.82–1.45 (m, 12 H, C=C–CH_3), 1.60 (s, 6 H, =$C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 201.5 (CO, 144.5 (CO, 144.5 (C=CH_2): MS, 424.5 (0.1, M^+), 203 (2), 189 (2), 175 (2), 161 (3), 159 (3), 151 (19), 95 (21), 81 (53), 69 (100). *Anal.* Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.62; H, 11.44.

(5E,9E,13E,17E)-5,9,14,18,22-Pentamethyl-5,9,13,17,21-tricosapentaen-2-ol **(13).** Magnesium (155 mg, 6.38 mmol) was suspended in ether (3 ml). Methyl iodide (1.04 g, 7.34 mmol) was added and the mixture was stirred at 0°C for 0.5 h and at 25°C for 1 h. Squalene trisnoraldehyde (**12,** 1.23 g, 3.19 mmol) in ether (10 ml) was added dropwise over 20 min. After 2 h, the reaction was quenched with NH_4Cl solution and filtered through Celite. The Celite was washed with ether and the combined filtrate was partitioned. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography (9 : 1 hexane–ethyl acetate), furnishing a clear oil (886 mg, 69%): R_f = 0.34 (4 : 1 hexane : ethyl acetate); ir 3397, 2924, 1449, 1379, 1082 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.25–5.03 (m, 5 H, –CH=C), 3.79 (m, 1 H, CHOH), 2.20–1.90 (m, 20 H, –CH_2CH_2–), 1.80–1.45 (m, 12 H, C=CCH_3), 1.60 (s, 6 H, C=$C(CH_3)_2$), 1.09 (d, 3 H, J = 6 Hz, CH(OH)CH_3); ^{13}C NMR ($CDCl_3$) δ 68.0 (–CHOH), MS, 400 (0.2 M^+), 203 (1), 191 (2), 177 (3), 175 (2), 163 (3), 149 (11), 136 (10), 121 (15), 107 (77), 81 (59), 69 (100). *Anal.* Calcd for $C_{28}H_{48}O$: C, 83.93; H, 12.07. Found: C, 83.84; H, 12.06.

(5E,9E,13E,17E)-5,9,14,18,22-Pentamethyltricosapentaen-2-one **(14).** Pyridine (1.63 g, 20.6 mmol) was added to dichloromethane (20 ml) and the solution was cooled to 0°C. Chromium trioxide (1.03 g, 10.3 mmol) was added, and the solution was stirred at 0°C for 1 h and at room temperature for 1.5 h. Trisnorsqualene methyl alcohol (**13**) (0.69 g, 1.72 mmol), dissolved in 10 ml of dichloromethane, was added. After 18 h, the solvent was decanted from the dark residue. The residue was taken up in 1 N NaOH and extracted with ether. The combined organic solvents were washed with cupric sulfate solution and brine, dried ($MgSO_4$), and evaporated. Flash chromatography (9 : 1 hexane–ethyl acetate) provided a clear oil (650 mg, 95%): R_f = 0.54 (4 : 1 hexane–ethyl acetate); ir 2918, 1719, 1444, 1382 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.21–5.02 (m, 5 H, =C, 2.53 (t, 2 H, J = 8 Hz, –CH_2CO–), 2.30–1.55 (m, 18 H, –CH_2CH_2–), 2.15 (s, 3 H, –$CH_2C(O)CH_3$), 1.80–1.61 (m, 12 H, C=C–CH_3), 1.57 (s, 6 H, =$C(CH_3)_2$; ^{13}C NMR ($CDCl_3$) δ 208.6 (–CO–); MS, 398.6 (0.3, M^+), 203 (2), 191 (2), 189 (2), 177 (2), 175 (7), 163 (2), 161 (5), 149 (14), 135 (20), 125 (48), 107 (40), 99 (34), 81 (86), 69 (100). *Anal.* Calcd for $C_{28}H_{46}O$: C, 84.36; H, 11.63. Found: C, 84.37; H, 11.62.

(5E,9E,13E,17E)-2,2'-Azo-5,9,14,18,22-pentamethyltricosapentaene **(1).** Ammonia (1.5 ml, ca. 68.2 mmol) was condensed in a graduated tube and then transferred to a flask cooled to −78°C. Ketone **14** (550 mg, 1.38 mmol), dissolved in methanol (10 ml) was added and the solution was stirred at room temperature for 5 h. After cooling to 0°C, hydroxylamine-*O*-sulfonic acid (800 mg, 7.07 mmol) in methanol (10 ml) was added, and the solution was stirred at room temperature for 5 h. Filtration, evaporation, and flash chromatography (1 : 1 hexane–ethyl acetate) afforded the intermediate diaziridine (**15,** 145 mg, ninhydrin-positive, R_f = 0.20,

1:1 hexane–ethyl acetate). The diaziridine was dissolved in ether (15 ml) and freshly prepared silver(I) oxide (1.00 g, 4.32 mmol) (*40a*) was added. After 29 h at room temperature, the solution was filtered and evaporated. Flash chromatography (19:1 hexane:ethyl acetate) gave 109 mg (19% based on ketone **14**) of a clear oil: R_f = 0.77 (1:1 hexane:ethyl acetate); ir 2853, 1449, 1383 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.16–5.02 (m, 5 H, C=C$\underline{H}$), 2.10–1.70 (m, 20 H, -C$\underline{H_2}$C$\underline{H_2}$–), 1.65–1.30 (m, 12 H, -CC$\underline{H_3}$), 1.60 (s, 6 H, =C(C$\underline{H_3}$)$_2$), 0.99 (s, 3 H, -C(N$_2$)C$\underline{H_3}$); ^{13}C NMR ($CDCl_3$) δ 135.1, 134.8, 133.3, 131.2, 125.1, 124.9, 124.2, 39.7, 39.5, 33.7, 33.1, 28.2, 26.8, 26.6, 26.5, 25.7, 25.6, 19.8, 17.6, 16.0, 15.8; MS, 341 (1), 231 (1), 205 (2), 203 (2), 191 (3), 189 (2), 177 (2), 175 (2), 163 (4), 161 (4), 149 (11), 147 (4), 136 (14), 121 (21), 109 (48), 95 (37), 81 (74), 69 (100). *Anal.* Calcd for $C_{28}H_{46}N_2$: C, 81.89; H, 11.29; N, 6.82. Found: C, 81.85, H, 11.38; N, 6.78.

(4E,8E,12E,16E)-N-Ethyl-N-nitroso-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenylamine **(4).** Trisnorsqualene ethyl amine **16** (93 mg, 0.23 mmol) was suspended in THF–water (1:1, 2 ml) and the mixture was cooled to 0°C. Sodium nitrite (47 mg, 0.68 mmol) and methanolic HCl (5 N, 0.14 ml, 0.68 mmol) were added. After 3 h, this same quantity of sodium nitrite and methanolic HCl was again added. Starting material was still apparent after 5 h, so additional sodium nitrite (150 mg) and methanolic HCl (0.4 ml) were added. After a total of 17 h at 0°C, the solution was diluted with ether. The solution was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (97:3 hexane–ethyl acetate) provided 48 mg (48%) of a clear oil: R_f = 0.51 (4:1 hexane:EtOAc; R_f of starting amine = 0.24); ^1H NMR ($CDCl_3$), δ 5.19–5.02 (m, 5 H, C=C$\underline{H}$), 4.35 (q, J = 7 Hz, *Z*-NC$\underline{H_2}$CH$_3$), 3.61 (t, J = 7 Hz, *Z*-C$\underline{H_2}$N(NO)CH$_2$CH$_3$), 3.58 (t, J = 7 Hz, *E*-CH$_2$N(NO)C$\underline{H_2}$CH$_3$), 3.41 (q, J = 7 Hz, *E*-NC$\underline{H_2}$CH$_3$), 2.18–1.90 (m, 18 H, C=$_2$), 1.62 (s, 3 H, *trans*-*C*=C(C$\underline{H_3}$)$_2$, 1.54 (s, 15 H, C=CC$\underline{H_3}$), 1.20–1.10 (overlapping t, 3 H, *E*- and *Z*-NCH$_2$C$\underline{H_3}$) (based on integrations, the *Z*-isomer predominates in a 3:1 ratio); ^{13}C NMR ($CDCl_3$) δ 65.8, 52.7, 45.6, 41.8 (isomers of SqC$\underline{H_2}$N(NO) and SqCH$_2$N(NO)C$\underline{H_2}$CH$_3$).

(4E,8E,12E,16E)-N-Cyclopropyl-N-nitroso-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenylamine **(5).** Trisnorsqualene cyclopropyl amine **17** (200 mg, 0.47 mmol) was suspended in water–acetic acid (1:1, 6 mL) and the mixture was cooled to 0°C. Sodium nitrite (65 mg, 0.94 mmol) was added and the mixture was stirred at 25°C for 18 h. Water was added (20 ml), and extraction with ethyl acetate was performed. The organic layers were washed with 5% $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (96:4 hexane–ethyl acetate), affording 124 mg (58%) of a light yellow oil. This compound exists as two nitroso geometric isomers (see text). On TLC, this compound appears as two closely migrating spots (R_f = 0.40, 0.35 in 9:1 hexane–ethyl acetate). Based on ^1H NMR integrations, the *E*-isomer predominates in a 3:1 ratio and is less polar by TLC. ^1H NMR ($CDCl_3$) δ 5.25–5.00 (m, 5 H, C=C$\underline{H}$), 3.94 (t, J = 7 Hz, *Z*-C$\underline{H_2}$NNO), 3.47 (t, J = 5 Hz, *E*-C$\underline{H_2}$NNO), 3.32 (m, *E*-cyclopropyl C$\underline{H}$), 2.61 (m, *Z*-cyclopropyl C$\underline{H}$), 2.05–1.80 (m, 18 H, C=CC$\underline{H_2}$), 1.61 (s, 6 H, C=CC$\underline{H_3}$ and *trans*-C=C(C$\underline{H_3}$)$_2$), 1.52 (br s, 12 H, C=CC$\underline{H_3}$), 1.25–0.78 (m, 4 H, cyclopropyl C$\underline{H_2}$); MS, 454 (1, M^+), 437 (4), 424 (5), 396 (4), 231 (6), 81 (75), 69 (75), 43 (100); HRMS, calcd/found ($C_{30}H_{50}N_2O$) 454.3923/454.3936.

ACKNOWLEDGMENTS

We acknowledge the Center of Biotechnology (SUNY-Stony Brook), the New York State Science and Technology Program, and the Kirin Brewery Co., Ltd., for financial support. G.M.A. was supported by a National Institutes of Health Research Service Award (Grant HL 0761301).

REFERENCES

1. For reviews see (*a*) SCHROEPFER, G. J., JR. (1983) *Annu. Rev. Biochem.* **51,** 555; (*b*) RILLING, H. C., AND CHAYET, L. T. (1985) *in* Sterols and Bile Acids (Danielsson, H., and Sjövall, J., eds.), pp. 1–39, Elsevier, Amsterdam; (*c*) MULHEIRN, L. J., AND RAMM, P. J. (1972) *Chem. Soc. Rev.*, 259.
2. DEAN, P. D. G. (1971) *Steridologia* **2,** 143.
3. For reviews see (*a*) CATTEL, L., CERUTI, M., VIOLA, F., DELPRINO, L., BALLIANO, G., DURIATTI, A., AND BOUVIER-NAVÉ, P. (1986) *Lipids* **21,** 31; (*b*) RAHLER, A., TATON, M., BOUVIER-NAVÉ, P., SCHMITT, P., BENVENISTE, P., SCHUBER, F., NARULA, A. S., CATTEL, L., ANDING, C., AND PLACE, P. (1986) *Lipids* **21,** 52: (*c*) STÜTZ, A. (1987) *Angew. Chem. Int. Ed. Engl.* **26,** 320; (*d*) CATTEL, L., CERUTI, M., BALLIANO, G., VIOLA, F., GROSA, G., AND SCHUBER, F. (1989) *Steroids* **53,** 363.
4. (*a*) YAMAMOTO, S., AND BLOCH, K. (1970) *J. Biol. Chem.* **245,** 1670; (*b*) NAKAMURA, M., AND SATO, R. (1979) *Biochem. Biophys. Res. Commun.*, **89,** 900.
5. ONO, T., AND IMAI, Y. (1985) *in* Methods in Enzymology (Low, J. H., and Rilling, H. C., eds.), Vol. 110,) p. 375, Academic Press, San Diego.
6. (*a*) VAN TAMELEN, E. E., AND JARES, D. R. (1977) *J. Am. Chem. Soc.* **99,** 950; (*b*) JOHNSON, W. S. (1976) *Bioorg. Chem.* **5,** 51; (*c*) VAN TAMELEN, E. E. (1982). *J. Am. Chem. Soc.* **104,** 6480.
7. (*a*) VAN TAMELEN, E. E., LEOPOLD, E. J., MARSON, S. A., AND WAESPE, H. R. (1982) *J. Am. Chem. Soc.* **104,** 6479; (*b*) VAN TAMELEN, E. E., PEDLAR, A. D., LI, E., AND JARES, D. R. (1977) *J. Am. Chem. Soc.* **99,** 6778; (*c*) VAN TAMELEN, E. E. (1975) *Acc. Chem. Res.* **8,** 152.
8. JOHNSON, W. S., TELFER, S. J., CHENG, S., AND SCHUBERT, V. (1987) *J. Am. Chem. Soc.* **109,** 2517.
9. ABE, I., EBIZUKA, Y., AND SARKAWA, V. (1988) *Chem. Pharm. Bull.* **36,** 5031.
10. (*a*) DEAN, P. D. G. (1969) *in* Methods in Enzymology (Clayton, R. B. ed.), Vol. 15, p. 495, Academic Press, San Diego; (*b*) SCHECHTER, C. I., SWEAT, F. W., and BLOCH, K. (1970) *Biochim. Biophys. Acta* **220,** 463.
11. For reviews see (*a*) Schuster, D. I., Probst, W. C. Ehrlich, G. K., and Singh, H. (1989) *Photochem. Photobiol.* **49,** 785; (*b*) Bayley, H. (1983) Photogenerated Reagents in Biochemistry and Molecular Biology, Elsevier, Amsterdam; (*c*) CAVALLA, D., AND NEFF, N. H. (1985) *Biochem. Pharmacol.* **34,** 2821.
12. DURIATTI, A., BOUVIER-NAVÉ, P., BENVENISTE, P., SCHUBER, F., DELPRINO, L., BALLIARO, G., AND CATTEL, L. (1985) *Biochem. Pharmacol.* **34,** 2765.
13. (*a*) FLEET, G. W. J., PORTER, R. R., AND KNOWLES, J. R. (1969) *Nature* **224,** 511; (*b*) BAYLEY, H., STAROS, J. V. (1984) *in Azides and Nitrenes: Reactivity and Utility* (Scriven, E. F. V., ed.), pp. 433–490, Academic Press, New York.
14. VAN TAMELEN, E. E., AND CURPHEY, T. J. (1962) *Tetrahedron Lett.*, 3769.
15. For approaches to internally functionalized squalenes see (*a*) GRIECO, P., AND MASAKI, Y. (1974) *J. Org. Chem.* **39,** 2135; (*b*) Biellmann, J. F., and Ducep, J. B. (1971) *Tetrahedron* **27,** 5861; (*c*) Pietre, S., and Heathcock, C. H. (1990) *Science* **248,** 1532.
16. BARON, W. J., DE CAMP, M. R., HENDRICK, M. H., JONES, M., LEVIN, R. H., AND SOHN, M. B. (1973) *Carbenes,* Vol. I, Wiley, New York.
17. TAYLOR, F. R., KANDUTSCH, A. A., ANZALONE, L., PHIRWA, S., AND SPENCER, T. A. (1988) *J. Biol. Chem.* **263,** 2264.
18. KRAMER, W., AND SCHNEIDER, S. (1989) *J. Lipid Res.* **30,** 1281.
19. KUHN, C.-S., GLAUDEMANS, C. P. J., AND LEHMANN, J. (1989) *Leibigs Ann. Chem.*, 357.

20. (*a*) Katzenellenbogen, J. A., and Katzenellenbogen, B. S. (1984) *Vitam. Horm.* **41,** 213; (*b*) Groenemeyer, H. (ed.) (1988) *Affinity Labelling and Cloning of Steroid and Thyroid Hormone Research Receptors,* Ellis Horwoord, Chichester, England.
21. Duriatti, A., and Schuber, F. (1988) *Biochem. Biophys. Res. Commun.,* **151,** 1378.
22. (*a*) Means, G. E., and Feeney, R. E. (1990) *Bioconj. Chem.* **1,** 2; (*b*) Shaw, E. (1970) *Physiol. Rev.* **50,** 244.
23. Kyba, E. P. (1984) *in* Azides and Nitrenes: Reactivity and Utility (Scriven, E. F. V., ed.), pp. 1–34, Academic Press, New York.
24. Kramer, W., Buscher, H.-P., Gerok, W., and Kurz, G. (1979) *Eur. J. Biochem.* **102,** 1.
25. Kramer, W., and Kurz, G. (1983) *J. Lipid. Res.* **24,** 910.
26. Naito, K., McKenna, E., Schwartz, A., and Vaghy, P. L. (1989) *J. Biol. Chem.* **264,** 21, 211.
27. Fricker, G., Kramer, W., Buscher, H.-P., Gerok, W., and Kurz, G. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363,** 897.
28. (*a*) Theiler, R. (1988) *Abstr. Pap. Am. Chem. Soc.* **196,** 106-ORGN; (*b*) Theiler, R. (1986) *Biol. Chem. Hoppe-Seyler* **367,** 1197.
29. Goeldner, M. P., Hirth, C. G., Kieffer, B., and Ourisson, G. (1982) *Trends Biochem. Sci.* **7** 310.
30. Chow, Y. L. (1973) *Acc. Chem. Res.* **6,** 354.
31. Eid, P.; Goeldner, M. P.; Hirth, C. G.; and Jost, P. (1981) *Biochemistry* **20,** 2251.
32. Silverman, R. B., and Yamanski, R. B. (1984) *Biochemistry* **23,** 1322.
33. (*a*) Macdonald, T. L., Zirvi, K., Burka, L. T., Peyman, P., and Guengerich, F. P. (1982) *J. Am. Chem. Soc.* **104,** 2050; (*b*) Guengerich, F. P., Willard, R. J., Shea, J. P. Richards, L. E., and Macdonald, T. L. (1984) *J. Am. Chem. Soc.* **106,** 6466; (*c*) Hanzlik, R. P., Kishore, V., and Tullman, R. (1979) *J. Med. Chem.* **22,** 759.
34. Sen, S. E., and Prestwich, G. D. (1989) *J. Am. Chem. Soc.* **111,** 1508.
35. Kissel, C. L., and Rickborn, B. (1972) *J. Org. Chem.* **37,** 2060.
36. Corey, E. J., and Suggs, J. W. (1975) *Tetrahedron Lett.,* 2647.
37. Ceruti, M., Balliano, G., Viola, F., Cattel, L., Gerst, N., and Schuber, F. (1987) *Eur. J. Med. Chem.* **22,** 199.
38. (*a*) Poos, G. I., Arth, G. E., Beyler, R. E., and Sarett, L. H. (1953) *J. Am. Chem. Soc.* **75,** 422; (*b*) Corey, E. J., and Schmidt, G. (1979) *Tetrahedron Lett.,* 399.
39. Church, R. F. R., Kende, A. S., and Weiss, M. J. (1965) *J. Am. Chem. Soc.* **87,** 2665.
40. (*a*) Shih, L. B., and Bayley, H. (1985) *Anal. Biochem.* **144,** 132; (*b*) Vebel, J. J. and Martin, J. C. (1964) *J. Am. Chem. Soc.* **86,** 4618.
41. Sen, S. E., and Prestwich, G. D. (1989) *J. Am. Chem. Soc.* **111,** 8761.
42. (*a*) Singer, G. M., and Andrews, A. W. (1983) *J. Med. Chem.* **26,** 309; (*b*) Challis, B. C., and Challis J. A. (1982) *in The Chemistry of Amino, Nitroso, and Nitro Compounds and Their Derivatives* (Patai, S., Ed.), pp. 1151–1223, Wiley, Chichester, England.
43. Oláh, G., Noszkó, L., Kuhn, S., and Szelke, M. (1956) *Chem. Ber.* **89,** 2374.
44. Karabatos, G. J., and Taller, R. A. (1964) *J. Am. Chem. Soc.* **86,** 4373.
45. For ^{1}H NMR assignments of cyclopropyl nitrosamines see Wiessler, M., and Pool, B. L. (1984) *Carcinogenesis* **5,** 635.
46. Sen, S. E., Wawrzénczyk, C., and Prestwich, G. D. (1990) *J. Med. Chem.* **33,** 1698.
47. For a review of variable orientation modes in protein-ligand binding see (*a*) Biellmann, J. F. (1986) *Acc. Chem. Res.* **19,** 121; (*b*) the symmetry of squalene synthesis and epoxidation is discussed in Cornforth, J., and Ross, F. P. (1977) *Proc. R. Soc. London, Ser. B* **199,** 213.
48. Sen, S. E., and Prestwich, G. D. Unpublished results.
49. Tanaka, J. (1957) *J. Chem. Soc. Japan* **78,** 1647.
50. Keefer, L. K., Hrabie, J. A., Hilton, B. D., and Wilbur, D. (1988) *J. Am. Chem. Soc.* **100,** 7459.
51. (*a*) Cartwright, I. L., Hutchinson, D. W., and Armstrong, V. W. (1976) *Nucleic Acids Res.* **3,** 2311; (*b*) Staros, J. V., Bayley, H., Standring, D. N., and Knowles, J. R. (1978) *Biochem. Biophys. Res. Commun.* **80,** 568; (*c*) Bayley, H., Standring, D. N., and Knowles, J. R. (1978) *Tetrahedron Lett.* 3633.

52. A number of different noncatalytic proteins stimulate SE and OSC activity; see (*a*) CHIN, J. AND BLOCH, K. (1984) *J. Biol. Chem.* 159, 11,735 and references therein; (*b*) NAKAMURA, M., AND SATO, R. (1979) *Biochem. Biophys. Res. Commun.* **89,** 900 and references therein.
53. LANGE, Y., AND MURASAKI, M. F. (1988) *J. Biol. Chem.* **263,** 9366.
54. RAMSEY, R. B., AEXEL, R. T., AND NICHOLAS, H. J. (1971) *J. Neurochem.* **18,** 2245 and references therein.
55. WHITE, JR., H. B., POWELL, S. S., GAFFORD, L. G., AND RANDALL, C. C. (1968) *J. Biol. Chem.* **243,** 4517.
56. (*a*) BELCHER, A. M., SMITH, III, A. B., JURS, P. C., LAVINE, B., AND EPPLE, G. (1986) *J. Chem. Ecol.* **12,** 513; (*b*) MASON, R. J., FALES, H. M., JONES, T. H., PANNELL, L. K., CHIN, J. W., AND CREWS, D. (1989) *Science* **245,** 291; (*c*) WELDON, P. J., SHAFAGATI, A., AND WHEELER, J. W. (1987) *Z. Naturforsch. C Biosci.* **42,** 1345.
57. (*a*) CERUTI, M., VIOLA, F., GROSA, G., BALLIANO, G., DELPRINO, L., AND CATTEL, L. (1988) *J. Chem. Res. Synop.* 18; (*b*) SEN, S. E., AND PRESTWICH, G. D. (1989) *J. Med. Chem.* **32,** 2152.
58. (*a*) CERUTI, M., VIOLA, F., DOSIO, F., CATTEL, L., BOUVIER-NAVÉ, P., AND UGLIENGO, P. (1988) *J. Chem. Soc. Perkin I,* 461; (*b*) XIAO-YI, X., SEN, S. E., AND PRESTWICH, G. D. (1990) *Tetrahedron Lett.* **31,** 2097.
59. MCWHORTER, W. W., AND JAUN, B. (1987) *Helv. Chim. Acta* **70,** 1095.
60. (*a*) DENNIS, K. J., AND SHIBAMOTO, T. (1989) *Photochem. Photobiol.* **49,** 711; (*b*) WITAS, T., AND WEDZINSKA, J. (1979) *Nahrung* **23,** 589.
61. PREUSSMAN, R., AND WIESSLER, M. (1987) *Trends Pharmacol. Sci.* **8,** 185.
62. For ^{1}H NMR assignments of squalene see Clouet, G., and Brossas, J. (1976) *Makromol. Chem.* **177,** 1963.
63. For ^{13}C NMR assignments of squalene see (*a*) VAN DOMMELER, M. E., VAN DER VEN, L. J. M., BUCK, H. M., AND DE HAAR, J. W. (1977) *Recueil* **96,** 295; (*b*) SOZZANI, P., AND DI SILVESTRO, G. (1988) *Gazz. Chim. Italiana* **118,** 385.
64. SANSONE, E. B., AND LUNN, G. (1983) *Am. Lab.* September, p. 82.