BIOORGANIC CHEMISTRY 17, 108–120 (1989)

Azadecalin Analogs of 4,4,10β-Trimethyl-*trans*-decal-3β-ol: Synthesis and Assay as Inhibitors of Oxidosqualene Cyclase

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Received June 9, 1988; accepted August 11, 1988

Azadecalins **4, 7, 8, 9** and **10** have been synthesized and tested as inhibitors of oxidosqualene cyclase in a rat liver microsomal assay. These compounds, which structurally resemble the known cyclase inhibitor 4,4,10β-trimethyl-*trans*-decal-3β-ol (TMD) **(1),** were selected because, under physiological conditions, they all have at least partial positive character at or near the initial putatively carbocationic site formed during the cyclization of squalene oxide and thus were potential transition state analog inhibitors. All of these azadecalins are, in fact, cyclase inhibitors, but, disappointingly, none is as effective as TMD itself.

In 1978 it was discovered in our laboratory that 4,4,10β-trimethyl-*trans*-decal-3β-ol **(1),** known as TMD,[2] is an effective specific inhibitor of the enzymatic cyclization of squalene 2,3(*S*)-oxide to lanosterol in rat liver homogenates and in Chinese hamster ovary cells (*1, 2*). Since that time TMD has proved to be a useful research tool in a number of laboratories (*3–10*),[3] and it is clear that development of even more efficient oxidosqualene cyclase inhibitors is desirable. The mechanism of inhibition by TMD has not been elucidated, but its obvious resemblance to the A and B rings of lanosterol naturally leads to speculation that it could be functioning as a product-like competitive inhibitor of the cyclase.[4] If TMD's inhibitory property is indeed related to its structural similarity to that portion of lanosterol, then one potential source of more effective inhibitors would be transition state analogs (*11–13*) for the first stage of the cyclization of squalene oxide. Although it is not certain whether the cyclization process is stepwise or essentially concerted, it is reasonable to assume that at least partial carbocationic character

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[2] Abbreviations used: TMD, 4,4,10β-trimethyl-*trans*-decal-3β-ol.

[3] In addition to the workers whose research is described in Refs. (*3–10*), TMD has been supplied by us to over 25 other investigators, and some researchers (e.g., Ref. (*17*)) have obtained TMD from other sources.

[4] Two points should be noted here. First, it has been shown that both enantiomers of TMD inhibit oxidosqualene cyclase (Ref. (*1*)) and it is difficult to see how both enantiomers could bind through their resemblance to part of lanosterol. Second, Benveniste (Ref. (*17*)) has presented kinetic evidence that TMD is a noncompetitive inhibitor, although whether these results are meaningful is not certain, as he points out.

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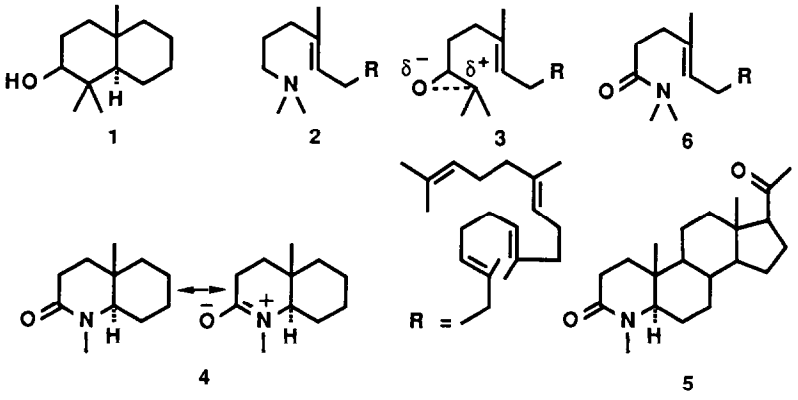
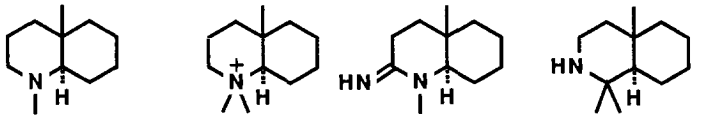
will develop at the carbon atom (C2) from which the bond to the epoxide oxygen is being broken.

Benveniste (*14*) and Poulter (*15*) and their co-workers first demonstrated that ammonium ion analogs of carbocationic transition states or intermediates can act as effective enzyme inhibitors. Benveniste (*16*) then applied this idea to oxidosqualene cyclase by showing that 2-aza-2,3-dihydrosqualene **(2),** which, in its protonated form was conjectured to resemble putative cyclization intermediate species **3,** inhibits a plant cyclase. These findings prompted us to initiate the study of azadecalin analogs of TMD described herein. During the course of this investigation, the Benveniste group published the results of an extensive study of approximately 30 prospective plant and mammalian cyclase inhibitors, most of which were also based on the idea of ammonium ions as mimics of carbocationic intermediates or transition states (*17*), and a few of which were azadecalins different from those described herein.

The first prospective inhibitor to be considered by us was lactam **4,** whose dipolar canonical form bears an obvious electron relationship to **3.** An analogous steroidal lactam, **5,** is a potent inhibitor of progesterone 5α-reductase, an effect rationalized by the resemblance of the dipolar resonance form of **5** to an enolate anion-like transition state for the reductase-catalyzed reaction (*18*). As it later transpired, on the basis of reasoning similar to ours, Benveniste prepared and assayed lactam **6** in his squalene analog series (*17*). In addition to **4,** we have prepared and tested the known amine **7** (*19*), ammonium salt **8,** and amidine **9.** Benveniste has studied the analogs of each of these compounds in his 2-aza-2,3-dihydrosqualene series (*17*).

Benveniste and his co-workers have also shown (*20*) that efficient inhibition (in the case of cycloeucalenol-obtusifoliol isomerase) can be obtained when the positively charged nitrogen atom is at a position adjacent to the carbocationic site which develops in the enzyme-catalyzed reaction. This finding led us to consider one further prospective cyclase inhibitor, amine **10,** which, when protonated, would have such an adjacent positive charge as well as a geometry very similar to that of TMD. Description of the synthesis of azadecalins **4, 7, 8, 9,** and **10** and their testing as inhibitors of oxidosqualene cyclase in a rat liver microsomal assay follows.

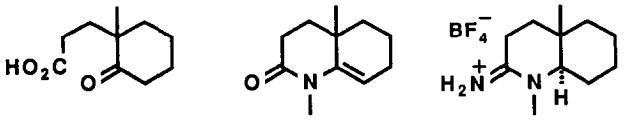




7 8 9 10

Preparation of lactam **4** was readily accomplished by use of the approach employed by Rasmusson *et al.* (*21*) in the synthesis of **5.** Keto acid **11,** prepared by a known procedure (*22*), was heated with methylamine in ethanol at 180°C in a sealed tube to afford 73% of unsaturated lactam **12.** This procedure worked better than one employing phosphorus pentachloride and methylamine in chloroform (*23*), which was erratic and gave at best 62% of **12.** Hydrogenation of **12** over platinum oxide then provided 69% yield of a single saturated lactam which was assigned the desired *trans* structure **4** on the basis of precedent and ^{1}H NMR data. Both Winternitz (*23*) and Rasmusson (*21*) observed exclusive formation of *trans* stereochemistry in analogous hydrogenations with platinum oxide as catalyst. Furthermore, the angular hydrogen in **4** appears as a well-defined doublet of doublets (J = 11.9 and 4.0 Hz) at δ = 3.02, in excellent agreement with the signal for the angular hydrogen of the *N*-desmethyl *trans* isomer, which appears as a doublet of doublets (J = 11 and 4 Hz) at δ = 3.05, whereas the angular hydrogen of the *N*-desmethyl *cis* isomer appears as a broad singlet at δ = 3.10 (*23*).

Further confirmation of this stereochemical assignment was obtained by means of the preparation of the next target compound, known tertiary amine **7** (*19*), which was readily accomplished in 80% yield by reduction of **4** with lithium aluminum hydride. The product had a ^{13}C NMR spectrum essentially identical to that reported for **7** (*19*), but distinctly different from that reported for the *cis*-fused isomer of **7** (*24*). Tertiary amine **7** was then readily converted to quaternary salt **8** in 78% yield by treatment with methyl iodide at reflux. Amidine **9** was prepared by treatment of lactam **4** with trimethyloxonium tetrafluoroborate followed by ammonia (*25*) to afford 62% of tetrafluoroborate salt **13,** which was converted to the free amidine in 87% yield by exposure to aqueous base.

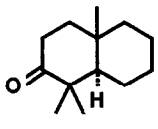


11 12 13

The synthesis of the final target compound, 3-azadecalin **10,** required a substantially greater number of steps, starting from TMD **(1)** itself. After oxidation of **1** to known (*26*) ketone **14** in 90% yield, Baeyer-Villiger oxidation (*27*) afforded 92% of lactone **15.** Initial plans to hydrolyze **15** to a hydroxy acid were abandoned when it was discovered that pyrolysis of **15** at 420°C led very cleanly (95% yield) to unsaturated acid **16.** It was anticipated that by subjecting the thus readily available **16** to Curtius rearrangement, followed by aminomercuration of the resulting **17,** the synthesis of **10** could be completed.

Traditional Curtius rearrangement via acyl chloride, acyl azid, and isocyanate did not give very good yields of **17.** In particular, the hydrolysis of the isocyanate intermediate to **17** was inefficient under either acidic or basic conditions. Furthermore, aminomercuration of **17** was ineffective in our hands, affording only very low yields of **10.** Far better success was achieved by use of the Yamada diphenylphosphoryl azide procedure (*28*) under conditions described by Poulter (*29*), which, after addition of benzyl alcohol to the intermediate isocyanate, afforded carbamate **18** in 78% yield from **16.**

Closure of the A ring was then accomplished by amidomercuration (*30*) using mercuric trifluoroacetate in nitromethane (*31*) which afforded carbamate **19** in 41% yield (52% based on unrecovered **18**). A variety of procedures was tried, but the yield of **19** could not be improved. Since complete disappearance of **18** was observed during the mercuration step, but **18** was recovered after demercuration with sodium borohydride, apparently "retroamidomercuration" (*32*) was occurring. Removal of the carbobenzyloxy group from **19** was then effected with hydrogen chloride in acetic acid (*30*) to afford the target amine **10** as its hydrochloride **20** in 80% yield. The overall yield of **20** from **14** was 22%. With pure samples of **4, 7, 8, 9,** and **20** in hand, the stage was set for testing these compounds as oxidosqualene cyclase inhibitors.



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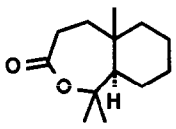

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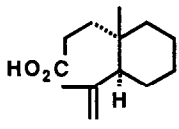

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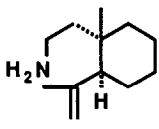

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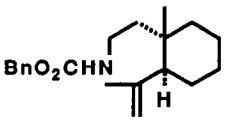

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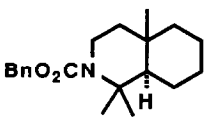

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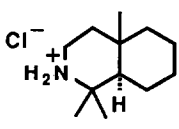

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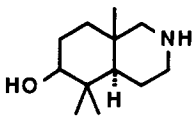

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Since the primary purpose of this study was to determine if any of these azadecalin analogs would be a more effective inhibitor of oxidosqualene cyclase than TMD **(1)** itself, a dose–response curve for each of these inhibitors was established under identical incubation conditions with a rat liver microsomal preparation and compared to that obtained for TMD. The assay used was similar to that employed by Benvensite (*17*), based on previous protocol by Bloch (*33*). It was decided to utilize commercially available radiolabeled squalene as the substrate in initial assays to screen the compounds for effectiveness as inhibitors. If any of the compounds appeared promising, assays employing the natural substrate, squalene 2,3(*S*)-oxide, would follow. The assay was conducted and the results were analyzed as described under Experimental.

All of the azadecalins **4, 7, 8, 9,** and **20** did indeed inhibit the conversion of squalene to lanosterol, but none of them, unfortunately, was as effective as TMD **(1).** The results are given in Table 1, expressed as inhibitor concentrations re-

TABLE 1

Concentration of Inhibitor Required to Reduce by One-Half the Amount of Lanosterol Formed from Squalene (I_{50} Value) in Rat Liver Microsomes under Standard Assay Conditions

Compound	**1**	**4**	**7**	**8**	**9**	**10**
I_{50} (μM)	65	165	250	420	215	180

quired under the standard assay conditions to reduce by one-half the amount of lanosterol produced (I_{50} values). Since squalene was the substrate in these incubations, it was necessary to confirm that the azadecalins were, like TMD, acting to inhibit oxidosqualene cyclase rather than squalene epoxidase. Accordingly, the products from incubations of each of compounds **1, 4, 7, 8, 9,** and **20** in the presence of approximately I_{50} values of inhibitor concentration were analyzed by HPLC for squalene 2,3-oxide. In every case the amount of squalene oxide was increased relative to control by an amount comparable to the increase caused by TMD, thus establishing that the azadecalins are indeed cyclase inhibitors. For some reason, however, the I_{50} value found for TMD in our assay (65 μM) is much larger than that reported by Benzeniste (9 μM) (*17*) in an apparently similar assay, despite the fact that we were using squalene rather than squalene oxide as substrate. Since our primary purpose was to determine whether any of **4, 7, 8, 9,** and **10** would be distinctly superior to TMD as an inhibitor, no attempt was made to discover the source of this discrepancy.

The results shown in Table 1 clearly indicate that none of our azadecalins is a particularly effective inhibitor and suggest that the idea of mimicking putative intermediate structure **3** by an ammonium ion analog is not likely to lead to more effective oxidosqualene cyclase inhibitors. Benveniste (*17*) made a similar finding with 8-azadecalin **21,** designed to bear a positive charge, when protonated, at the carbocationic site which is formed during the second ring closure in the cyclization of squalene oxide. Compound **21** was very much less effective than TMD **(1)** in his assay, showing only a marginal inhibitory effect (*17*).[5] In view of the current findings, it was decided not to explore further the properties of azadecalins **4, 7, 8, 9,** and **10.**

EXPERIMENTAL

Synthesis

General synthetic procedures. Melting points were determined by using a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra

[5] It should be noted, however, that the *N*-1,5,9-trimethyldecyl derivative of an 8-azadecalin closely related to **21** is a significantly more potent inhibitor than TMD in rat liver microsomes [M. Taton, P. Benveniste, and A. Rahier (1986) *Biochem. Biophys. Res. Commun.* **138,** 764–770] and particularly in 3T3 fibroblasts [N. Gerst, A. Duriatti, F. Schuber, M. Taton, P. Benveniste, and A. Rahier (1988) *Biochem. Pharmacol.* **37,** 1955–1964].

were recorded on a Perkin–Elmer 599 spectrometer and referenced to the 1601 cm^{-1} band of polystyrene. Liquid samples were analyzed as thin films on NaCl plates and solid samples were analyzed as transparent KBr disks. The ^1H nuclear magnetic resonance spectra were recorded on Perkin–Elmer R-24 or Varian EM 360A (60 MHz) and Varian XL-300 (300 MHz) instruments using $CDCl_3$ as solvent. ^{13}C NMR spectra were determined on a Varian XL-300 spectrometer using $CDCl_3$ as solvent. Both ^1H and ^{13}C NMR spectral data are presented in parts per million (δ) downfield relative to tetramethylsilane as internal standard. The ^1H NMR data are tabulated in order: multiplicity, coupling constants, and number of protons. Low resolution mass spectra (MS) were performed on a Finnigan 4023 gas chromatograph/mass spectrometer at 70 eV (direct insertion). High resolution MS were performed at the Mass Spectrometric Biotechnology Resource at Rockefeller University. Elemental analyses were carried out by Atlantic Microlab, Inc. (Atlanta, GA). Analytical thin-layer chromatography was performed on EM silica gel 60 G_{254} plates (0.2 mm) and visualized under ultraviolet light or with *p*-anisaldehyde–H_2SO_4–EtOH spray. Flash chromatography (*34*) was carried out with EM reagent silica gel 60 (230–400 mesh). Spectrograde hexane, ethyl acetate, and methanol were used as received. Other solvents were purified according to Perrin *et al.* (*35*). Brine refers to a saturated aqueous NaCl solution.

4,10-Dimethyl-Δ5-4-azaoctal-3-one (***12***). 1-Methyl-2-oxocyclohexanepropionic acid (**11**) was synthesized by the method of House (*22*). A solution of 0.160 g (0.868 mmol) of **11** in 2.5 ml of absolute ethanol which had been saturated with methylamine (Matheson) was heated in a sealed tube at 180°C for 8 h. The mixture was then cooled and evaporated to afford 0.155 g of yellow oil. Flash chromatography with 1 : 1 hexane : ethyl acetate afforded 0.113 g (73%) of **12** as an oil which was homogeneous by TLC. Distillation in a microdistillation apparatus at 0.35 Torr at an oil bath temperature of 135–140°C afforded an analytical sample of **12:** ir 1720, 168 cm^{-1}; ^1H NMR δ 5.04 (d of d, J = 2.4 and 7.4 Hz, 1), 3.11 (s, 3), 2.7–1.1 (m, 13, with a sharp peak at 1.11); ^{13}C NMR δ 168.49, 143.35, 104.68, 37.84, 34.20, 32.12, 30.66, 28.98, 24.42, 22.78, 17.63; MS *m/e* 179. Anal. Calcd for $C_{11}H_{17}NO$: C, 73.69, H, 9.56; N, 7.80. Found: C, 73.63; H, 9.58; N, 7.75.

4,10-Dimethyl-trans-4-azadecal-3-one (***4***). A solution of 0.500 g (2.78 mmol) of **12** in 9 ml of freshly distilled glacial acetic acid was added to a 250-ml Parr hydrogenation bottle containing 80 mg of amorphous PtO_2 (Aldrich) and the mixture was shaken for 15 h under an initial hydrogen pressure of 50 psi. The catalyst was removed by filtration through a Celite bed, which was then rinsed with 2 × 5 ml of acetic acid. The filtrates were evaporated and the residue was dissolved in 20 ml of $CHCl_3$ which was washed with 10 ml of 5% aqueous $NaHCO_3$ solution and 10 ml of brine, dried over $MgSO_4$, and evaporated to give 0.430 g of yellow liquid which was flash chromatographed with 1 : 6 hexane : ethyl acetate to afford 0.350 g (69%) of **4** which was homogeneous by TLC. Distillation in a microdistillation apparatus at 0.35 Torr at an oil bath temperature of 135°C afforded an analytical sample of **4:** ir 3500, 1700 cm^{-1}; ^1H NMR δ 3.02 (d of d, J = 4.0 and 11.9 Hz, 1), 2.91 (s, 3), 2.45 (m, 2), 1.86 (m, 2), 1.51 (m, 4), 1.32 (m, 4), 0.99 (s, 3); ^{13}C NMR δ 170.08, 64.24, 38.51, 33.46, 29.19, 28.84, 25.46, 25.05, 20.23, 15.06; MS *m/e* 181. Anal. Calcd for $C_{11}H_{19}NO$: C, 72.88; H, 10.56; N, 7.72. Found: C, 72.61; H, 10.67; N, 7.63.

4,10-Dimethyl-trans-4-azadecalin (**7**). As in the procedure of Cope and Ciganek (*36*), 0.090 g (2.3 mmol) of lithium aluminum hydride was suspended in 25 ml of dry ether. To this was added 0.470 g (2.60 mmol) of **4** and the resulting mixture was stirred under N_2 at reflux overnight. The solution was cooled and quenched with 10% NaOH solution and extracted with 3 × 100 ml of ether. The ethereal extracts were dried over $MgSO_4$ and evaporated to afford 0.330 g (77%) of **7,** which was purified by microdistillation: ir 2925, 1375, 1190 cm^{-1}; ^1H NMR δ 3.1 (dd, 1), 2.5–1.5 (m, 14), 1.0 (s, 3); ^{13}C NMR δ 71.99, 59.19, 43.23, 40.50, 40.33, 34.10, 26.10, 25.10, 22.08, 21.12, 17.29 (lit. (*19*) ^{13}C NMR δ 71.92, 59.19, 43.11, 40.67, 40.32, 34.10, 26.14, 25.08, 22.15, 21.19, 17.35).

4,10-Dimethyl-trans-4-azadecalin methiodide (**8**). To a solution of 0.025 g (0.155 mmol) of **7** in 10 ml of ethanol was added 0.4 ml (2.8 mmol) of methyl iodide (Aldrich) and the resulting mixture was heated at reflux for 6 h. Evaporation of the solvent left 0.043 g of red–orange solid residue, which was recrystallized several times from isopropyl alcohol to afford 0.036 g (78%) of **8** as an off-white solid: mp > 230°C; ir 2940, 1475, 1450 cm^{-1}; ^1H NMR δ 3.8 (dd, 1), 3.45 (s, 3), 3.05 (s, 3), 2.1–1.4 (m, 14), 1.1 (s, 3); ^{13}C NMR δ 77.4, 65.7, 55.9, 45.3, 43.5, 36.0, 35.4, 25.7, 21.2, 20.3, 17.7; HRMS *m/e* Calcd for $C_{12}H_{24}N$: 182.1903. Found: 182.1909.

3-Imino-4,10-dimethyl-trans-4-azadecalin hydrotetrafluoroborate (**13**). According to the procedure of Rasmusson (*25*), 0.100 g (0.55 mmol) of **4** was added rapidly to a solution of 0.700 g (3.98 mmol) of trimethyloxonium tetrafluoroborate (Alfa) in 2 ml of dry methylene chloride and the resulting mixture was heated at reflux for 10 min and then stirred for 2 h at room temperature. Anhydrous ammonia was bubbled through the solution rapidly until reflux was obtained and the addition was continued for 5–10 min. A white precipitate formed which was assumed to be NH_4BF_4. The mixture was allowed to sit overnight, after which it was filtered and the solvent was evaporated, yielding a yellowish liquid, to which were added 2 ml of isopropyl alcohol and 2 ml of ether, and the resulting solution was placed in the freezer overnight. The white precipitate which had formed was collected and recrystallized from isopropyl alcohol to afford 0.120 g (62%) of **13:** mp 133–134°C; ir 3265, 2935, 1660, 1620, 1060 cm^{-1}; ^1H NMR 7.3 (s, 1) 7.0 (s, 1), 3.2 (dd, 1), 3.0 (s, 3), 2.0–1.0 (m, 14), 0.9 (s, 3); ^{13}C NMR δ 165.6, 65.3, 38.2, 33.5, 32.9, 32.3, 24.9, 24.3, 19.8, 15.4; HRMS *m/e* Calcd for $C_{11}H_{21}N_2$: 181.1700. Found: 181.1705.

3-Imino-4,10-dimethyl-4-azadecalin (**9**). To a mixture of 2 ml of ether and 3 drops of water was added 0.020 g of **13** and then 0.5 ml of 50% NaOH solution. The resulting mixture was vigorously shaken and the ether layer was separated. The aqueous layer was washed with 2 × 3 ml of ether and the ether layers were combined and evaporated to afford 0.013 g (87%) of **9** as an oil: ir 3300, 1610 cm^{-1}; ^1H NMR δ 6.2 (s, 1), 5.3 (s, 1); HRMS *m/e* Calcd for $C_{11}H_{20}N_2$: 180.1621. Found: 180.1626.

4,4,10-Trimethyl-trans-decal-3-one (**4**). Oxidation of 2.35 g (11.9 mmol) of TMD **(1),** which had been prepared as previously described (*1, 26*), with Jones reagent (*37*) at 0°C for 20 min afforded, after a normal workup, 2.10 g (90%) of **14,** bp 90–93°C (1 Torr), which had spectroscopic properties consistent with those published for **14** (*26*).

2,2,7-Trimethyl-trans-3-oxa-4-oxobicyclo[5.4.0]undecane (***15***). According to a procedure of Meinwald and co-workers (*27*), a solution of 6.20 g (31.9 mmol) of **14** in 20 ml of CH_2Cl_2, which had been freshly distilled from P_2O_5, was added dropwise to a solution of 9.60 g (44.5 mmol) of *m*-chloroperoxybenzoic acid (Aldrich, 80–85%) in 75 ml of CH_2Cl_2. The mixture was protected from light with Al foil and stirred at room temperature for 36 h, at which time TLC showed no **14** remaining. The precipitate was removed by filtration, and the filtrate was washed successively with 30 ml of 10% aqueous Na_2SO_3 solution, 2 × 30 ml of saturated aqueous $NaHCO_3$ solution, 10 ml of H_2O, and 20 ml of brine, dried over $MgSO_4$, and evaporated to afford 7.41 g of material which partially solidified upon standing in the refrigerator. Trituration with 10 ml of hexane furnished 4.30 g of **15,** mp 55–58°C. The residue was flash chromatographed with 5 : 1 hexane : ethyl acetate to afford an additional 1.84 g of **15,** mp 54–57°C, for a total yield of 6.14 g (92%) of **15,** which was homogeneous by TLC. Two recrystallizations from hexane afforded an analytical sample of **15:** mp 58.5–59.5°C; ir 1730 cm^{-1}; ^1H NMR δ 2.70 (m, 2), 2.0–1.0 (m, 20, with singlets at 1.40 and 1.10); ^{13}C NMR δ 175.60, 86.80, 54.56, 45.15, 38.55, 36.90, 33.35, 32.18, 26.91, 25.25, 23.84, 21.49, 18.73; MS *m/e* 210. Anal. Calcd for $C_{13}H_{22}O_2$: C, 74.23; H, 10.54. Found: C, 74.51; H, 10.65.

1α-[2-Carboxyethyl]-1β-methyl-2β-isopropenylcyclohexane (***16***). A 5-ml addition funnel situated on the top of a pyrolysis tube which had been heated to 420°C was charged with 3.90 g (18.5 mmol) of **15.** After the **15** had melted, it was slowly dropped through the hot column accompanied by a stream of N_2. Material emerging from the column was collected in a trap at dry ice temperature. After the addition of **15** was completed, the column was flushed with 10 ml of hexane. Evaporation yielded 3.70 g (95%) of **16,** mp 59–63°C, which was homogeneous by TLC. Two recrystallizations from ether : hexane afforded an analytical sample of **16:** mp 68–70°C; ir 3250–2550, 1715, 1645, 900 cm^{-1}; ^1H NMR δ 10.10 (bs, 1), 4.82 (m, 2), 2.35 (m, 2), 2.0–1.0 (bm 17, with sharp singlets at 1.75 and 0.89); ^{13}C NMR δ 181.48, 147.48, 112.90, 53.17, 38.28, 37.74, 35.85, 28.72, 28.15, 26.74, 23.43, 21.93, 18.79; MS *m/e* 210. Anal. Calcd for $C_{13}H_{22}O_2$: C, 74.23; H, 10.54. Found: C, 74.46; H, 10.64.

1α-[2-Aminoethyl]-1β-methyl-2β-isopropenylcyclohexane (***17***). To 0.610 g (2.90 mmol) of **16** under N_2 at 0°C was added 0.60 ml (8.2 mmol) of freshly distilled thionyl chloride (Fisher) and the **16** slowly dissolved. The resulting yellow solution was stirred at room temperature for 2 h and then was concentrated *in vacuo*. Microdistillation of the residue at 0.8 Torr at an oil bath temperature of 130–140°C afforded 0.586 g of acid chloride: ir 3030, 1800, 1650, 900 cm^{-1}. To a warm solution of this 0.586 g (2.56 mmol) of acid chloride in 6 ml of toluene, which had been freshly distilled from sodium, under N_2 was added 0.51 ml (3.8 mmol) of trimethylsilyl azide (Aldrich) and the resulting mixture was heated at reflux for 4 h, during which time the reaction progress was monitored by ir. The mixture was evaporated *in vacuo* to afford 0.510 g of crude isocyanate: ir 3030, 2270, 1650, 900 cm^{-1}. To a solution of 0.470 g (2.26 mmol) of this crude isocyanate in 1.5 ml of ether was added 3 ml of 50% aqueous KOH solution with vigorous stirring and the resulting mixture was heated at reflux for 15 min. The ether layer was separated and the aqueous layer was extracted with 3 × 5 ml of ether, saturated with NaCl,

and extracted with two additional 5-ml portions of ether. The combined ethereal extracts were dried over Na_2CO_3 and evaporated to give 0.290 g of yellow oil. Microdistillation at 0.7 Torr at an oil bath temperature of 125–150°C afforded 0.248 g (51% from **16**) of **17** which was homogeneous by TLC (99:1 CH_3OH:NH_4OH as eluent): ir 3380, 3300, 3080, 1650, 1600, 900 cm^{-1}; ^1H NMR δ 4.74 (s, 1), 4.58 (s, 1), 2.62 (m, 2), 1.82–0.85 (m, 16, with sharp singlets at 1.65 and 0.87); ^{13}C NMR δ 147.59, 112.20, 53.29, 47.70, 38.72, 36.81, 35.67, 27.76, 26.58, 23.26, 21.74, 18.92; HRMS *m/e* Calcd for $C_{12}H_{23}N$: 181.1830. Found: 181.1821.

N-Carbobenzyloxy-1α-[2-aminoethyl]-1β-methyl-2-isopropenylcyclohexane (***18***). According to methodology of Yamada (*28*) and Poulter (*29*), to a solution of 0.720 g (3.42 mmol) of **16** in 3 ml of toluene, which had been freshly distilled from sodium, was added 0.47 ml of freshly distilled triethylamine and 0.940 g (3.42 mmol) of diphenylphosphorylazide (Aldrich). The resulting mixture was heated at 85–90°C for 1 h, and then 0.940 g (8.70 mmol) of benzyl alcohol was added and the yellowish mixture was heated at 85–90°C for an additional 4 h. The mixture was evaporated and the residue was dissolved in 30 ml of CH_2Cl_2, which was then washed with 10 ml of 5% aqueous NaOH solution, 10 ml of H_2O, and 10 ml of brine, dried over $MgSO_4$, and evaporated to afford 1.52 g of viscous yellow oil. Flash chromatography with 500 ml of 10:1 hexane:ethyl acetate and then 1000 ml of 4:1 hexane:ethyl acetate yielded 0.852 g (78%) of **18** as a clear liquid which solidified on standing and which was homogeneous by TLC. Recrystallization from hexane gave an analytical sample of **18:** mp 68–69.5°C; ir 3340, 1710, 1650 cm^{-1}; ^1H NMR δ 7.36 (s, 5), 5.13 (s, 2), 4.76 (m, 3), 3.20 (m, 2), 1.76 (s, 3), 1.50 (bm, 11), 0.89 (s, 3); ^{13}C NMR δ 147.60, 136.61, 128.40, 128.10, 127.99, 127.95, 112.81, 66.44, 53.51, 43.23, 38.47, 36.63, 35.76, 27.90, 26.70, 23.29, 21.87, 18.87; MS *m/e* 315. Anal. Calcd for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.26; N, 4.43. Found: C, 76.19; H, 9.30; N, 4.36.

4,4,10-Trimethyl-3-carbobenzyloxy-trans-3-azadecalin (***19***). According to a procedure of Harding (*31*), a mixture of 1.02 g (3.17 mmol) of **18** and 1.60 g (3.80 mmol) of mercuric trifluoroacetate (Aldrich) in 45 ml of nitromethane, which had been freshly distilled from calcium hydride, was stirred at room temperature under nitrogen and covered with Al foil. TLC after 1 h showed the presence of some **18,** so 0.800 g (1.80 mmol) of additional mercuric trifluoroacetate was added and the mixture was stirred for another 0.5 h. The reaction mixture was evaporated, and the residue was taken up in 10 ml of CH_2Cl_2, which was then filtered. The filtrate was added slowly to a vigorously stirred solution of 3.50 g of benzyltriethylammonium chloride (Aldrich) in 20 ml of 10% aqueous NaOH solution. To this mixture was then slowly added a solution of 0.115 g (3.02 mmol) of $NaBH_4$ in 6 ml of 10% aqueous NaOH solution, during which time the mixture turned black. After the black metallic particles settled on the bottom of the flask (ca. 15 min), the supernatant was carefully decanted and the residue was rinsed with ca. 10 ml of CH_2Cl_2. The layers were separated and the aqueous layer was extracted with 3 × 15 ml of CH_2Cl_2. The combined organic layers were washed with 2 × 15 ml of brine, dried over $MgSO_4$, and evaporated to give 1.74 g of yellow oil, which was flash chromatographed with 10:1 hexane:ethyl acetate to afford 0.420 g (41%) of **19,** which solidified on standing and which was homogeneous by TLC.

Recrystallization from hexane gave an analytical sample of **19:** mp 47.5–49°C; ir 1710 cm^{-1}; ^1H NMR δ 7.38 (m, 5), 5.13 (s, 2), 3.76 (m, 1), 3.43 (m, 1), 2.0–1.0 (m, 17, with sharp singlets at 1.55, 1.30, and 1.06); ^{13}C NMR δ 157.30, 137.14, 128.36, 127.74, 127.68, 66.44, 58.63, 53.03, 44.45, 40.04, 39.05, 32.23, 28.76, 27.59, 23.66, 21.81, 20.03, 19.12; MS *m/e* 315. Anal. Calcd for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.26; N, 4.43. Found: C, 75.99; H, 9.24; N, 4.30. Also obtained from the flash chromatography were 0.040 g of an unidentified substance and 0.220 g (21%) of unreacted **18.**

4,4,10-Trimethyl-trans-3-azadecalin Hydrochloride (***20***). A solution of 0.400 g (1.26 mmol) of **19** in 9 ml of glacial acetic acid which had been saturated with HCl gas was stirred at room temperature for 1 h at 55–60°C for 6 h, with a slow stream of HCl gas bubbling through the mixture. The mixture was concentrated *in vacuo* to one-third its original volume and diluted with 80 ml of ether, which had been freshly distilled from $LiAlH_4$, causing formation of a white precipitate. After the mixture had been stored in the freezer overnight, filtration yielded 0.220 g (80%) of **20,** mp 218–220°C (dec.) Three recrystallizations from absolute ethanol : ether gave an analytical sample of **20:** mp 226–227°C (dec.); ir 2480–2440 cm^{-1}; ^1H NMR δ 9.40 (bs, 2), 3.11 (m, 2), 1.8–0.95 (bm, 20, with sharp singlets at 1.47, 1.26, and 0.97); ^{13}C NMR δ 58.10, 50.60, 43.63, 36.73, 36.40, 32.15, 28.63, 26.43, 21.52, 20.98, 18.77, 18.60; Anal. Calcd for $C_{12}H_{24}ClN$: C, 66.18; H, 11.10; Cl, 16.32; N, 6.43. Found: C, 66.06; H, 11.12; Cl, 16.26; N, 6.42.

Squalene oxide. Squalene 2,3(*R,S*)-oxide was prepared by the procedure of Nadeau and Hanzlik (*38*) and was purified to homogeneity by HPLC under the conditions described below. [^3H]Squalene 2,3(*S*)-oxide was prepared by incubation of (*R,S*)-[2-^3H]mevalonic acid lactone with S_{10} rat liver homogenate which had been preincubated with TMD, exactly as previously described (*39*). The [^3H]squalene 2,3(*S*)-oxide was also purified to homogeneity by HPLC.

Microsomal Assay Procedures

Materials. Squalene, Na_2EDTA, NADP, D-glucose 6-phosphate, reduced glutathione, L-å-phosphatidyl-DL-glycerol, NADPH, and FAD were purchased from Sigma Chemical. Enzyme grade Tris was obtained from Bethesda Research Laboratories. Bovine serum albumin was a gift from Dr. D. L. Schneider, Dartmouth Medical School. [4,8,12,13,17,21-^3H]Squalene (sp act 19.4 Ci/mmol) and Biofluor high efficiency emulsifier cocktail were purchased from New England Nuclear. (*R,S*)-[2-^3H]Mevalonic acid lactone (sp act 1.26 Ci/mmol) was obtained from Amersham. Organic solvents were obtained from Fisher.

Preparation of rat liver microsomes. This preparation was performed in a cold room. Three fed male rats (200–220 g, Sprague–Dawley strain from Charles River Breeding Laboratory) were sacrificed by stunning and subsequent decapitation. The rat body cavity was rinsed with Buffer A [0.1 M phosphate, pH 7.4, at 21°C containing potassium phosphate dibasic (19 mM), potassium phosphate monobasic (81 mM), $MgCl_2 \cdot 6H_2O$ (5.0 mM), Na_2EDTA (1.0 mM), and sucrose (250 mM) in glass-distilled water]. Prior to use, the buffer was filtered through a 0.2-μm nylon 66 membrane (Alltech Associates, Inc.). The livers were excised, suspended in a volume of Buffer A equal to three times the liver weight and homogenized with a

loose-fitting serrated Teflon pestle. The homogenate was centrifuged at 10,000 rpm for 20 min (2–4°C) in a Sorvall Superspeed RC2-B centrifuge. A 5.0-ml portion of the S_{10} supernatant was reserved for an activity test and the remainder was spun in a T-865 rotor at 40,000 rpm for 1 h (2–4°C) using a Sorvall OTD-65 ultracentrifuge with oil turbine drive. The S_{105} supernatant, which contained 9–10 mg protein/ml, was removed and stored in small aliquots at −70°C. The pellet was resuspended in Buffer B [100 mM Tris, pH 7.3, at 37°C containing Na_2EDTA (1.0 mM) in glass-distilled water] and recentrifuged under the same conditions. The second supernatant was discarded and the pellet (P_{105}) was resuspended in 10.0 ml of Buffer B per liver. The resulting suspension contained 5–7 mg protein/ml.

Activity test for S_{10} rat liver homogenate. A 5.0-ml portion of S_{10} and 0.5 ml of cofactor solution [5.0 ml of glass-distilled water containing NADP (10.0 mg), D-glucose 6-phosphate (66.0 mg), and reduced glutathione (92.0 mg)] were incubated for 10 min with constant shaking in a water bath at 37°C. (*R,S*)-[2-^{3}H]Mevalonic acid (1.0 μCi) was added and incubation allowed to proceed for 1 h. The reaction was terminated by addition of 5.0 ml of 4 N KOH in 1:1 ethanol:H_2O and the mixture was heated for 1 h at 75°C. The mixture was cooled and extracted three times with an equivalent volume of petroleum ether (bp 35–60°C). The combined organic layers were washed with brine and petroleum ether was added until a final volume of 100 ml was reached. The recovered radioactivity was then counted to determine what percentage of the (*R*)-[2-^{3}H]mevalonic acid had been converted to sterol.

Oxidosqualene cyclase assay. The assay protocol was based on that of Saat and Bloch (*33*) and was optimized with respect to the amounts of P_{105}, S_{105}, and cofactors used. Squalene (10 nmol in hexane), [4,8,12,13,17,21-^{3}H]squalene (70,000 cpm in hexane), L-*å*-phosphatidyl-DL-glycerol (0.2 mg in 98:2 chloroform:methanol), Tween-80 (40 μl of a 0.1% solution in acetone), and varying amounts of the inhibitor to be tested were added to the incubation tubes (125 × 20-mm Pyrex culture tubes with screw caps). The organic solvents in the incubation tubes were evaporated under a stream of nitrogen. The resulting residue was emulsified with 470 μl of Buffer B. Miocrosomes (200 μl) were added to the buffer solution and the mixture was incubated with shaking at 37°C for 15 min. Following this preincubation, 300 μl of S_{105} and 30 μl of cofactors (1.0 mM NADPH and 0.01 mM FAD in Buffer B) were added sequentially and the incubation was allowed to proceed for an additional 60 min. The reactions were quenched with 2.0 ml of 4 N KOH in 1:1 ethanol:H_2O, heated for 1 h at 75°C, and cooled overnight at 20°C. Each tube was then extracted three times with 4.0-ml portions of petroleum ether (bp 35–60°C). The organic extract from each tube was evaporated under N_2 to a volume of 0.1 ml. This was spotted onto 20 × 20-cm channeled Whatman LK-5D silica plates and allowed to dry for 20 min. Each plate also had a reference channel on which were spotted pure lanosterol and cholesterol. Plates were developed in 4:1 hexane:ethyl acetate and dried. Spots were rendered visible by spraying with a solution of phosphomolybdic acid (25 g of phosphomolybdic acid in 500 ml of isopropyl alcohol) and heating briefly at 110°C. The channels were divided into bands of approximately equal width corresponding to the reference bands and each band was scraped into a scintillation vial containing 1.0 ml of methanol and

10.0 ml of Biofluor scintillation cocktail. After brief vortexing, the vials were counted in a Beckman LS 7500 liquid scintillation system. Percentage conversion to sterol, which was essentially all lanosterol, was then determined from the ratio of cpm in the sterol bands to total cpm recovered.

Protein determination. Portions of P_{105} or S_{105} were diluted 1 : 20 with water and various amounts were aliquoted in duplicate into 100 × 12-mm Bausch and Lomb cuvettes. A 100-μl portion of 1.0% deoxycholate in water and up to 900 μl of water were added to each tube to bring the total volume to 1.0 ml. Trichloroacetic acid (100 μl of a 40% solution in H_2O) was added and the solutions were thoroughly mixed. The tubes were allowed to sit at room temperature for 15 min and then at 0–4°C overnight. The tubes were centrifuged at 2000 rpm for 10 min, the supernatant was discarded, and the pellet was dissolved in 100 μl of water and 100 μl sodium dodecyl sulfate (1.0% in 0.1 M NaOH). The resulting solutions were then analyzed for protein content by the method of Lowry, using bovine serum albumin as standard (*40*).

HPLC analysis. The HPLC system used was a Waters Model 510 solvent delivery system with Model 680 automated gradient controller, a Model 481 Lambda-Max variable wavelength detector, a Model U6K universal injector, a Beckman Ultrasphere ODS column (5 μm, 250 × 4.5 mm), and a Buchner LC 100 automatic fraction collector. The HPLC solvents were prepared by filtering the appropriate solutions through a 0.2-μm nylon 66 membrane, followed by degassing with He. The mobile phase for these analyses was 98 : 2 CH_3OH (HPLC grade) : nanopure H_2O. Prior to analysis the solvents were allowed to pass through the system overnight at a rate of 0.1 ml/min. Nonsaponifiable extracts to be analyzed were dried under a stream of nitrogen and then redissolved in 100 μl of methanol and 100 μl of dichloromethane prior to injection. The conditions for the analysis were ambient temperature, flow rate of 1.0 ml/min, chart speed of 1.0 cm/min, attenuation of 0.10 AUFS, and detection wavelength of 210 nm. The flow rate was modified to 2.0 ml/min after 32 min in order to allow squalene to pass more quickly through the column. Fractions were collected at 1-min intervals and analyzed for radioactivity. Those fractions corresponding to the observed retention time for authentic squalene oxide (19.4 min) were used to determine how much squalene oxide had formed in a given incubation.

ACKNOWLEDGMENTS

This research was supported by Grant HL23083 from the National Institutes of Health. The authors are grateful to Dr. Jean Chin for invaluable advice, assistance, and encouragement.

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