2,3-Epoxy-10-aza-10,11-dihydrosqualene, a High-Energy Intermediate Analogue Inhibitor of 2,3-Oxidosqualene Cyclase

Maurizio Ceruti, Gianni Balliano, Franca Viola, Giorgio Grosa, Flavio Rocco, and Luigi Cattel*

Istituto di Chimica Farmaceutica Applicata, Facoltà di Farmacia, Corso Raffaello 31, 10125 Torino, Italy.
Received March 23, 1992

2,3-Epoxy-10-aza-10,11-dihydrosqualene, a high-energy intermediate analogue inhibitor of 2,3-oxidosqualene (SO) cyclase was obtained by total synthesis. This involved the preparation of three main building blocks: (1) C_{17} squalenoid *N*-methylamine, (2) 3-(diphenylphosphinoyl)propanal, and (3) 5,6-epoxy-6-methylheptan-2-one. The final stages of the reconstruction of the 6*E* double bond were obtained by a Wittig-Horner reaction which was modified for poorly reactive systems. This compound was designed to mimic the C-8 carbonium ion formed during SO cyclization. Its inhibitory activity on various SO cyclases was evaluated and compared with the 6 *Z* isomer which has an unfavorable geometry. Only isomer 6 *E*, the carbocation analogue, was active on SO cyclases from rat liver, pig liver, *S. cerevisiae*, and *C. albicans* microsomes, with an I_{50} varying from 3 to 5 μM. Both *E* and *Z* isomers were inactive on squalene epoxidase at the higher concentrations tested.

2,3-Oxidosqualene cyclase (SO cyclase) (EC 5.4.99.7) is a key enzyme in the biosynthesis of animal, plant, and fungal sterols[1-7] through the formation of the acyclic intermediate, (3*S*)-2,3-oxidosqualene.

Our interest in the study of this enzyme is the development of new inhibitors potentially useful as hypocholesterolemic, antifungal, or phytotoxic drugs.[8-10]

Among the inhibitors of cholesterol biosynthesis, clinical application has been found for the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[11] Commercial application has also been found for the inhibitors of lanosterol 14α-demethylation such as the azole antifungal agents miconazole and ketoconazole[12] and for the pesticide agents tridemorph and fenpropimorph which are inhibitors of Δ^8-Δ^7 sterol isomerase and $\Delta^{8,14}$-sterol Δ^{14} reductase.[13]

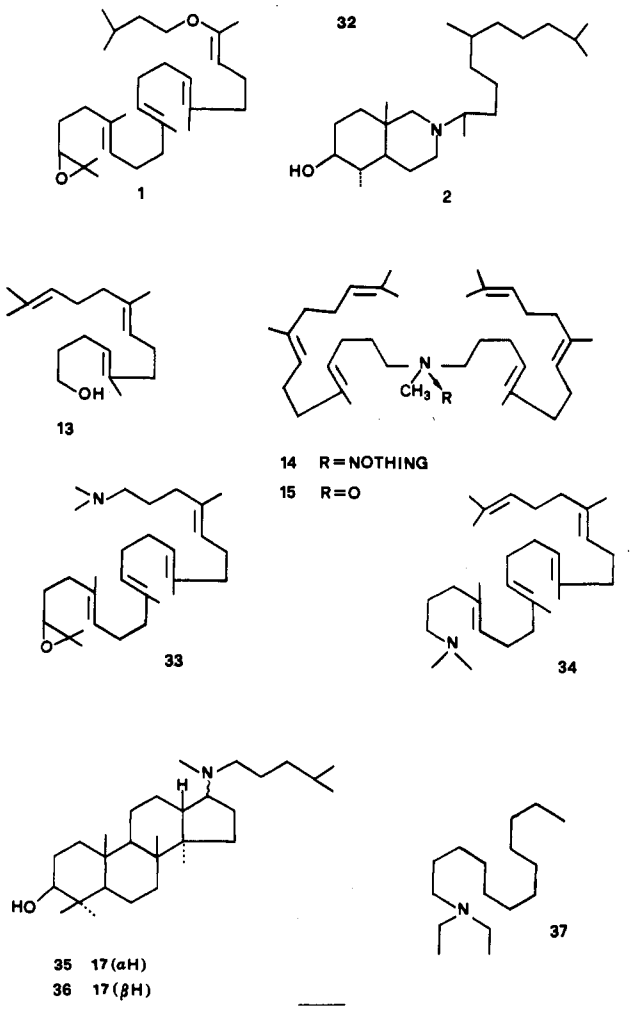
The search for new antimycotics has recently been focused on compounds that interfere at other stages of ergosterol biosynthesis. For example, naftifine and terbinafine, belonging to the class of allylamines, are highly active toward many pathogenic fungi, acting as selective inhibitors of fungal squalene epoxidase.[14]

The design of new high-energy intermediate (HEI) analogue inhibitors of SO cyclase was based on the postulated mechanism, suggesting that the cyclization of SO proceeds through a series of well-defined carbocationic intermediates, leading to a C-20 protosterol intermediate which undergoes further rearrangement to form lanosterol. Recently, the nature of the C-20 ion intermediate was clarified through the synthesis[15] and biocyclization studies[16,17] of an analogue of SO, squalenoid epoxy vinyl ether 1 (Chart I).

A general strategy applied to prepare new potent inhibitors of SO cyclase has been to mimic the above described carbocations by replacing a positively charged carbocation in the structure of a substrate analogue with a protonated nitrogen.[18-20] This strategy has also been applied to inhibit several other enzymes involved in sterol biosynthesis, which catalyse reactions involving carbocationic intermediates.[21]

Among the intermediate carbocations formed during SO cyclization and rearrangement, the C-8 tetracyclic carbonium ion has been mimicked by *N*-(1,5,9-trimethyldecyl)-4α,10-dimethyl-8-aza-*trans*-decal-3β-ol, **2** (Chart I), an azadecalinol-type inhibitor bearing an isoprenoid side chain linked at the C-8, which possessed good activity toward SO lanosterol cyclase, as well as Δ^8-Δ^7 sterol isomerase. Nevertheless, this compound could also mimic the C-8 bicyclic carbonium ion formed during initial SO cyclization.[20-22]

Chart I. Structures of Various SO Cyclase Inhibitors and Intermediates

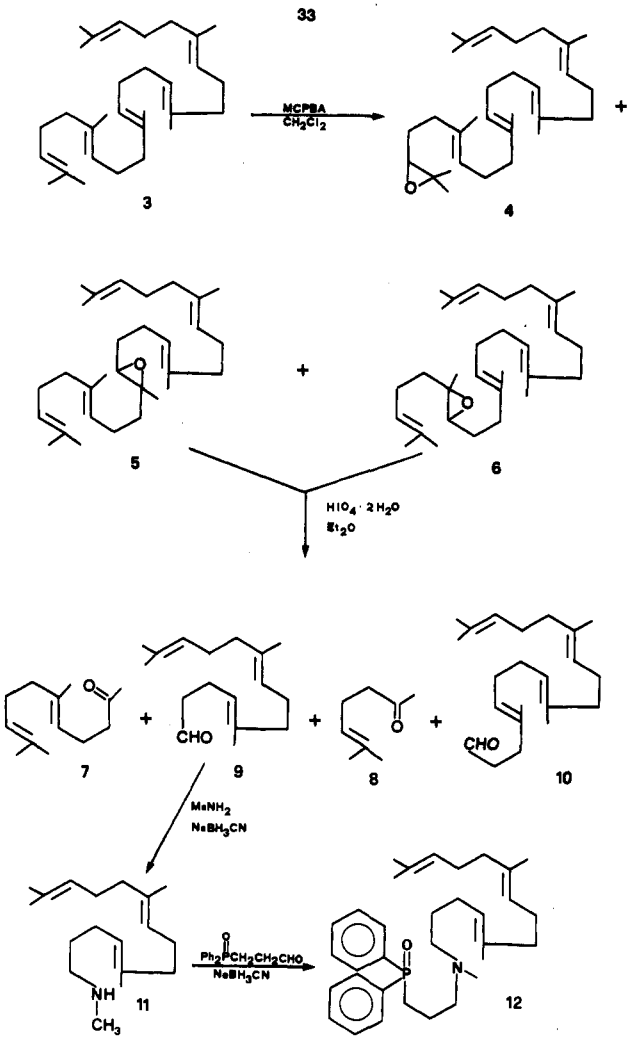


(1) Van Tamelen, E. E. Bioorganic Chemistry: Total Synthesis of Tetra- and Pentacyclic Triterpenoids. *Acc. Chem. Res.* **1975**, *8*, 152-158.
(2) Van Tamelen, E. E.; James, D. R. Overall Mechanism of Terpenoid Terminal Epoxide Polycyclizations. *J. Am. Chem. Soc.* **1977**, *99*, 950-952.
(3) Van Tamelen, E. E. Bioorganic Characterization and Mechanism of the 2,3-Oxidosqualene → Lanosterol Conversion. *J. Am. Chem. Soc.* **1982**, *104*, 6480-6481.

* To whom correspondence should be addressed.

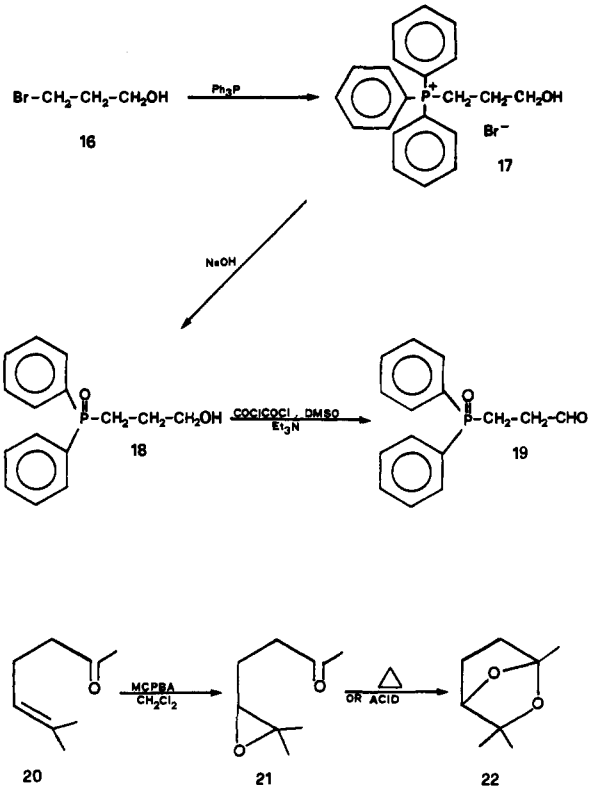
0022-2623/92/1835-3050$03.00/0

Scheme I. Synthesis of Azasqualenoid Phosphinoxide



It was proposed that a more selective inhibitor could be designed by substituting the pro C-8 position of SO with

Scheme II. Synthesis of 3-(Diphenylphosphinyl)propanal and 5,6-Epoxy-6-methylheptan-2-one



a nitrogen atom. Such an acyclic compound would not be expected to interfere with other sterol biosynthesis en-

(4) Yamamoto, S.; Lin, K.; Bloch, K. Some Properties of the Microsomal 2,3-Oxidosqualene Sterol Cyclase. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 110–117.

(5) Shechter, I.; Sweat, F. W.; Bloch, K. Comparative Properties of 2,3-Oxidosqualene-Lanosterol Cyclase from Yeast and Liver. *Biochim. Biophys. Acta* **1970**, *220*, 463–468.

(6) Rilling, H. C.; Chayet, L. T. Biosynthesis of Cholesterol. In *Sterols and Bile Acids*; Danielsson, H., Sjövall, J., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1985; cap. 1, pp 1–39.

(7) Nes, W. D. Control of Sterol Biosynthesis and its Importance to Developmental Regulation and Evolution; in *Recent Adv. Phytochem.* **1990**, *24*, 283–327.

(8) Cattel, L.; Ceruti, M.; Viola, F.; Delprino, L.; Balliano, G.; Duriatti, A.; Bouvier-Navé, P. The Squalene 2,3-Epoxide Cyclase as a Model for the Development of New Drugs. *Lipids* **1986**, *21*, 31–38.

(9) Cattel, L.; Ceruti, M.; Balliano, G.; Viola, F.; Grosa, G.; Schuber, F. Drug Design Based on Biosynthetic Studies: Synthesis, Biological Activity, and Kinetics of New Inhibitors of 2,3-Oxidosqualene Cyclase and Squalene Epoxidase. *Steroids* **1989**, *53*, 363–391.

(10) Cattel, L.; Ceruti, M. 2,3-Oxidosqualene Cyclase and Squalene Epoxidase: Enzymology, Mechanism and Inhibitors. In *Physiology and Biochemistry of Sterols*; Patterson, G. W., Nes, W. D., Eds.; American Oil Chemists' Society: Champaign, IL, 1992; cap. 3, pp 50–82.

(11) Slater, E. E.; MacDonald, J. S. Mechanism of Action and Biological Profile of HMG CoA Reductase Inhibitors. A New Therapeutic Alternative. *Drugs* **1988**, *36*, 72–82.

(12) Heel, R. C.; Brogden, R. N.; Carmine, A.; Morley, P. A.; Speight, T. M.; Avery, G. S. Ketoconazole: A Review of its Therapeutic Efficacy in Superficial and Systemic Fungal Infections. *Drugs* **1982**, *23*, 1–36.

(13) Burden, R. S.; Cooke, D. T.; Carter, G. A. Inhibitors of Sterol Biosynthesis and Growth in Plants and Fungi. *Phytochemistry* **1989**, *28*, 1791–1804.

(14) Stütz, A. Allylamine Derivatives. A New Class of Active Substances in Antifungal Chemotherapy. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 320–328.

(15) Ceruti, M.; Viola, F.; Dosio, F.; Cattel, L.; Bouvier-Navé, P.; Ugliengo, P. Stereospecific Synthesis of Squalenoid Epoxide Vinyl Ethers as Inhibitors of 2,3-Oxidosqualene Cyclase. *J. Chem. Soc., Perkin Trans. 1* **1988**, 461–469.

(16) Corey, E. J.; Virgil, S. C. An Experimental Demonstration of the Stereochemistry of Enzymic Cyclization of 2,3-Oxidosqualene to the Protosterol System, Forerunner of Lanosterol and Cholesterol. *J. Am. Chem. Soc.* **1991**, *113*, 4025–4026.

(17) Corey, E. J.; Virgil, S. C.; Sarshar, S. New Mechanistic and Sterochemical Insights on the Biosynthesis of Sterols from 2,3-Oxidosqualene. *J. Am. Chem. Soc.* **1991**, *113*, 8171–8172.

(18) Ceruti, M.; Balliano, G.; Viola, F.; Cattel, L.; Gerst, N.; Schuber, F. Synthesis and Biological Activity of Azasqualenes, Bis-azasqualenes and Derivatives. *Eur. J. Med. Chem.* **1987**, *22*, 199–208.

(19) Ceruti, M.; Viola, F.; Balliano, G.; Grosa, G.; Caputo, O.; Gerst, N.; Schuber, F.; Cattel, L. Synthesis of a Squalenoid Oxaziridine and Other New Classes of Squalene Derivatives, as Inhibitors of Sterol Biosynthesis. *Eur. J. Med. Chem.* **1988**, *23*, 533–537.

(20) Taton, M.; Benveniste, P.; Rahier, A. N-[(1,5,9)-trimethyldecyl]-4α,10-dimethyl-8-aza-trans-decal-3β-ol. A Novel Potent Inhibitor of 2,3-Oxidosqualene Cycloartenol and Lanosterol Cyclases. *Biochem. Biophys. Res. Commun.* **1986**, *138*, 764–770.

(21) Rahier, A.; Taton, M.; Bouvier-Navé, P.; Schmitt, P.; Benveniste, P.; Schuber, F.; Narula, A. S.; Cattel, L.; Anding, C.; Place, P. Design of High Energy Intermediate Analogues to Study Sterol Biosynthesis in Higher Plants. *Lipids* **1986**, *21*, 52–62.

zymes, usually inhibited by cyclic-type inhibitors. Moreover, it is possible that it might behave as a "pro" inhibitor, since it might be initially recognized and then partially cyclized by SO cyclase to give an ammonium ion, that may represent the best HEI analogue.

For these reasons, we have synthesized two new HEI analogue inhibitors, (6*E*)-**31** and (6*Z*)-2,3-epoxy-10-aza-10,11-dihydrosqualene, **32**, bearing a nitrogen atom in the pro C-8 position (Scheme IV), and we evaluated their inhibitory activity in animal and fungal microsomes.

Chemistry

The overall strategy for the total synthesis of (6*E*)-**31** and (6*Z*)-2,3-epoxy-10-aza-10,11-dihydrosqualene (**32**) involved the preparation of three main building blocks. These were (1) C_{17} squalenoid *N*-methylamine, **11**; (2) 3-(diphenylphosphinoyl)propanal, **19**; and (3) 5,6-epoxy-6-methylheptan-2-one, **21**. Often, conventional methods did not produce the desired products, so various new methods were developed.

Synthesis of C_{17} Squalenoid *N*-Methylamine (20). Direct epoxidation of squalene **3** with *m*-chloroperbenzoic acid (MCPBA) and subsequent separation by flash chromatography gave a mixture of the two trans internal monoepoxides **5** and **6** in 29% yield and then the external monoepoxide **4** (Scheme I). Via a procedure recently developed by us,[15] the mixture of the two trans internal monoepoxides was cleaved by using periodic acid in diethyl ether to give the corresponding C_{17} and C_{22} squalenoid aldehydes **9** and **10**. This method, which avoids treating epoxides with $HClO_4$ and final cleavage of the diols with $NaIO_4$, allows the corresponding aldehydes to be obtained in high yield. After separating the mixture of aldehydes C_{17} and C_{22} (**9** and **10**) from ketones C_{13} and C_8 (**7** and **8**) by flash chromatography, the aldehydes were separated by reversed-phase (octadecyl, C_{18}) flash chromatography, to give pure C_{17} aldehyde **9** and then pure C_{22} aldehyde **10**.

In our first attempt to synthesize C_{17} squalenoid *N*-methylamine **11** from aldehyde **9**, we used standard reductive amination by simply reacting the carbonyl compound with the appropriate amine, in the presence of sodium cyanoborohydride in anhydrous methanol[18] (see Experimental Section, method A). Unfortunately, yields were low (29%) due to the preferential formation of C_{17} alcohol **13** and C_{17} amine dimer **14** (Chart I). To greatly reduce dimer formation, we developed a modified method (method B). In this way, the yield of C_{17} squalenoid *N*-methylamine **11** was increased from 29% to 52%.

Synthesis of 3-(Diphenylphosphinoyl)propanal (19). (3-Hydroxypropyl)triphenylphosphonium bromide (**17**) was obtained from 3-bromo-1-propanol (**16**) and triphenylphosphine by a conventional procedure based on refluxing the mixture in toluene[23] (Scheme II). Subsequent alkaline hydrolysis of **17**, followed by distillation, produced 3-(diphenylphosphinoyl)-1-propanol (**18**) in 65% yield.[24] The low solubility of alcohol **18** at the low temperatures (−60 °C) usually employed for Swern oxidation, together with its low reactivity, probably due to its main cyclic conformation,[25] were overcome by reacting oxalyl chloride activated DMSO with compound **18** at a higher temperature (−40 °C). The yield was good (62%) only if the reaction was performed at a rather high dilution (see the Experimental Section). Increasing the concentration of alcohol **18** gave a dramatic progressive reduction in the yield. This compound could be an important new synthon, as the two functional groups, diphenylphosphinoyl and aldehydic, can react alternatively with various functional groups.

Synthesis of 5,6-Epoxy-6-methylheptan-2-one (21). Compound **21** was obtained by epoxidation of 6-methyl-5-hepten-2-one (**20**) with MCPBA[26] (Scheme II). Conventional oxidation with MCPBA in CH_2Cl_2 was not convenient since it gave rise to partial formation of bicycle **22** under the acidic conditions. So, a two-phase oxidation (MCPBA in CH_2Cl_2/10% aqueous $NaHCO_3$) was employed. The moderate decrease in activity of MCPBA was compensated by a longer reaction time, while formation of bicycle **22** was completely avoided. The same problem was present during distillation, as conventional Claisen distillation produced a mixture of ketone **21** and bicycle **22**. So we developed a procedure for extremely rapid vaporization and distillation with a Kugelrohr apparatus (see the Experimental Section) which produced ketone **21** with a purity of 98% (by ^1H NMR).

Synthesis of (6*E*)- and (6*Z*)-2,3-Epoxy-10-aza-10,11-dihydrosqualene (31 and 32). The first step of the reconstruction of the squalenoid chain was the synthesis of azasqualenoid phosphinoxide **12** (Scheme I). It was obtained by reductive amination[18] of squalenoid *N*-methylamine **11** with 3-(diphenylphosphinoyl)propanal (**19**) and $NaBH_3CN$ in 70% yield.

For the synthesis of epoxyazasqualenes **31** and **32** we turned our attention to the Warren variant of the Horner–Wittig reaction, since the Wittig reaction is usually difficult with complex ketones. In the Warren reaction, the anion of a phosphinoxide, generated by butyllithium, reacts with a carbonyl derivative, followed by an elimination from the alcohol intermediates.[27,28]

Unfortunately in the synthesis of epoxyazasqualenes **31** and **32**, we were faced with many problems due to (1) the very low reactivity of squalenoid derivatives; (2) the Warren reaction with a ketone, which is poorly reactive; (3) the lability of epoxy ketone **21**; (4) the presence of three chiral centers in alcohol diastereoisomers **27–30**; (5) unpredictable stereochemical control in the synthesis of trisubstituted alkenes; and (6) the difficulty in assigning the structure of complex trisubstituted alkenes without having their chrystalline forms.

In our case, the reaction of azasqualenoid phosphinoxide **12** with butyllithium in anhydrous THF by Warren's method did not produce the phosphinoxide anion due to its crowded and poorly reactive structure, common to many

(22) Gerst, N.; Duriatti, A.; Schuber, F.; Taton, M.; Benveniste, P.; Rahier, A. Potent Inhibition of Cholesterol Biosynthesis in 3T3 Fibroblasts by N-[(1,5,9)-trimethyldecyl]-4α,10-dimethyl-8-aza-trans-decal-3β-ol, a New 2,3-Oxidosqualene Cyclase Inhibitor. *Biochem. Pharmacol.* **1988**, *37*, 1955–1964.

(23) Leppard, D. G.; Raynolds, P. W.; Chapleo, C. B.; Dreiding, A. S. Preparation of 4-(4′-methyl-2′-oxo-cyclohex-3′-en-1′-yl)-pentan-1-ol and Derivatives of 3-(4′-methyl-2′-oxo-cyclohex-3′-en-1′-yl)-butan-1-ol. *Helv. Chim. Acta* **1976**, *59*, 695–711.

(24) Aksnes, G. Alkaline Decomposition of Some Quaternary Phosphonium Compounds Containing Oxygen. *Acta Chem. Scand.* **1961**, *15*, 438–440.

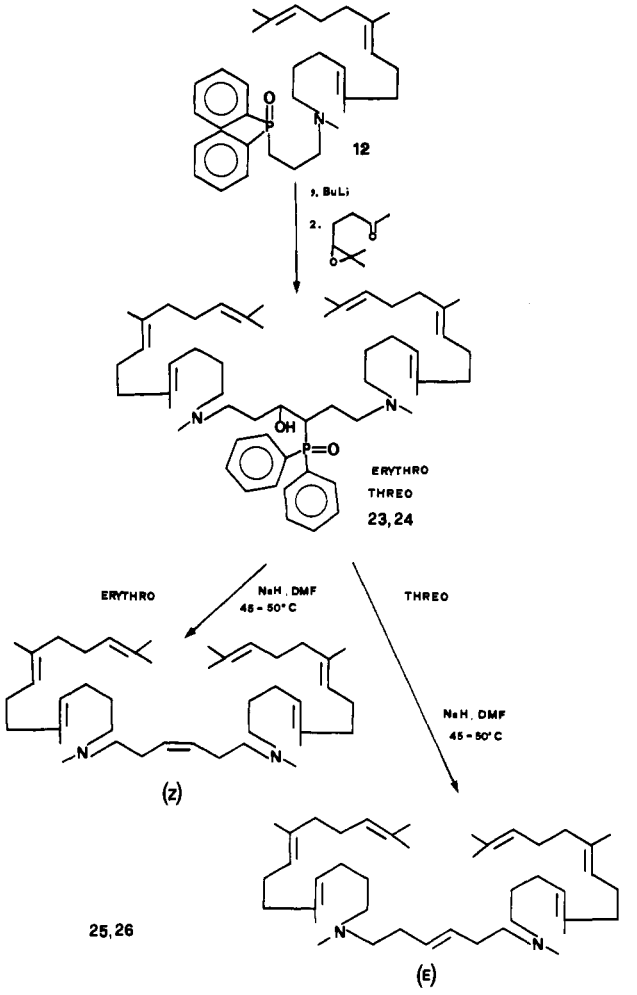
(25) Aksnes, G.; Bergesen, K. Intramolecular Hydrogen Bonding in Hydroxyalkyl Diphenylphosphine Oxides. *Acta Chem. Scand.* **1964**, *18*, 1586–1590.

(26) Anderson, W. K.; Veysoglu, T. A Simple Procedure for the Epoxidation of Acid-Sensitive Olefinic Compounds with m-Chloroperbenzoic Acid in an Alkaline Biphasic Solvent System. *J. Org. Chem.* **1973**, *38*, 2267–2268.

(27) Buss, A. D.; Warren, S. The Stereocontrolled Horner-Wittig Reaction: Synthesis of Disubstituted Alkenes. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2307–2325.

(28) Clough, J. M.; Pattenden, G. The Synthesis of Polyene Isoprenoids by the Horner Variant of the Wittig Reaction. *Tetrahedron Lett.* **1978**, 4159–4162.

Scheme III. Conventional Wittig–Horner Reaction on Azasqualenoid Phosphinoxide

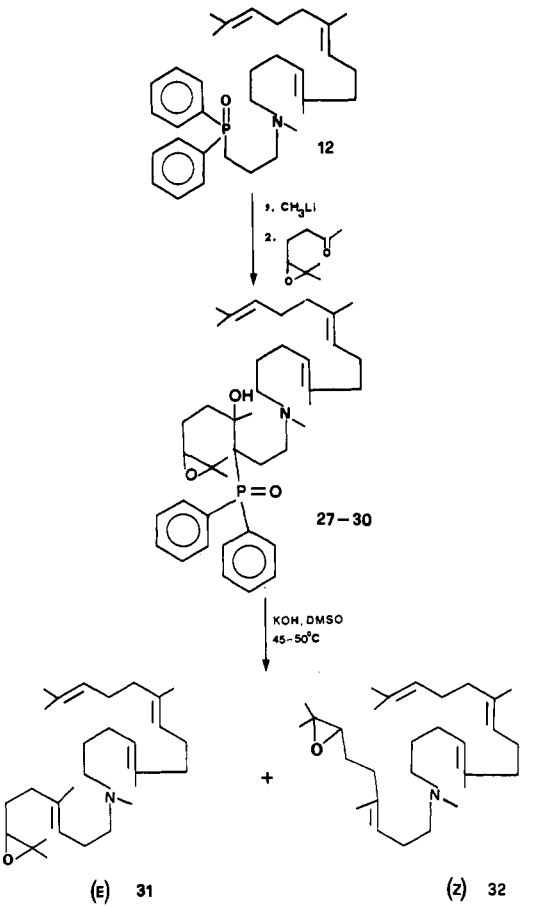


squalenoid molecules,[15,18,19] even in the presence of diphenylphosphinoyl as an anion stabilizing group. Only dimers **25** and **26** were obtained as the main products, together with much of the starting material (Scheme III). A selection of other bases showed that methyllithium (MeLi) allowed the formation of the anion of **12**, as shown by a dark orange solution. Subsequent addition of epoxy ketone **21** resulted in a mixture of the diastereoisomeric alcohols **27–30**, which was purified by flash chromatography. The four isomers could not be separated (Scheme IV).

Further studies are in progress to explore the generality of this variant of the Warren reaction. It allows the synthesis of trisubstituted alkenes from poorly reactive ketones and poorly reactive phosphine oxides.

Treatment of alcohols **27–30** with NaH in DMF[27,28] resulted mainly in the recovery of the starting material, while KOH in DMSO,[29] followed by complex separations (see the Experimental Section), gave the desired products **31** and **32** with *E* selectivity. Usually, the Warren reaction for obtaining disubstituted alkenes is erythro selective and so favors the *Z* olefin,[27] while in the synthesis of trisubstituted alkenes, high stereoselectivity was not usually observed.[29] An exception was the synthesis of *Z*-α-bisabolene, which proceeded with *Z* selectivity.[29]

Scheme IV. Synthesis of (6*E*)- and (6*Z*)-2,3-Epoxy-10-aza-10,11-dihydrosqualene



In the case of the synthesis of alkenes containing an alcoholic group,[30] enamines,[31] allylic tertiary amines,[32] or *N*-allylamides,[33] usually threo (and thus *E*) selectivity was observed for disubstituted alkenes, while a moderate threo selectivity was found for trisubstituted alkenes. The influence of proximal heteroatoms on stereocontrol, chelating the lithium intermediate formed, remains to be completely understood, but other factors, such as a higher reactivity of threo isomers of alcohols **27–30**, together with a possible reverse reaction to erythro isomers to give some of the *E* derivative, may play a role in influencing the threo selectivity.

Assinging the isomerism of the C_6–C_7 double bond was troublesome in the determination of the structure of the two separated geometrical isomers **31** and **32**.

^{1}H NMR spectra at 400 MHz showed little, but significant, differences in the position of epoxidic CH and epoxidic CH_3 signals. These were compared with the

(29) Buss, A. D.; Greeves, N.; Mason, R.; Warren, S. Applications of the Stereochemically-Controlled Horner-Wittig Reaction: Synthesis of Feniculin, (E)-Non-6-en-1-ol, a Pherormone of the Mediterranean Fruit Fly, (E) and (Z)-Dec-5-en-1-ol, Tri-substituted Alkenes, and (Z)-α-Bisabolene. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2569–2577.

(30) Buss, A. D.; Greeves, N.; Levin, D.; Wallace, P.; Warren, S. Synthesis of Single Isomers (E or Z) of Unsaturated Alcohols by the Horner-Wittig Reaction. *Tetrahedron Lett.* **1984**, *25*, 357–360.

(31) Broekhof, N. L. J. M.; Jonkers, F. L.; van der Gen, A. N-Methyl-N-anilinomethyl Diphenylphosphine Oxide: a Versatile Reagent for the Synthesis of Enamines. *Tetrahedron Lett.* **1980**, *21*, 2671–2674.

(32) Cavalla, D.; Warren, S. Regio- and Stereospecific Synthesis of Allylic Tertiary Amines. *Tetrahedron Lett.* **1982**, *23*, 4505–4508.

(33) Cavalla, D.; Warren, S. Regio- and Stereospecific Synthesis of N-Allyl Amides by the Horner-Wittig Reaction. *Tetrahedron Lett.* **1983**, *24*, 295–298.

Table I. I_{50} Values[a] (μM) of Inhibition of Microsomal 2,3-Oxidosqualene Cyclase by (6E)- and (6Z)-2,3-Epoxy-10-aza-10,11-dihydrosqualene 31 and 32

microsomes	isomer E	isomer Z
rat liver[b]	4.8	>20
pig liver[b]	5	ND[e]
S. cerevisiae[c]	5	>100
C. albicans[d]	3	>100

[a] The values are means of two different experiments. [b] Microsomal protein concentration was 1 mg/mL for pig liver and 5 mg/mL for rat liver. [c] Microsomal protein concentration was 2 mg/mL. [d] Microsomal protein concentration was 3 mg/mL. [e] ND = not determined.

corresponding signals of the various other known epoxidic squalenoid derivatives. In the LR_f (low R_f) isomer 31 (E), these signals resonated at a slightly higher field than for the HR_f (high R_f) isomer 32 (Z) and were similar to the corresponding signals of *all-E*-22,23-epoxy-2-aza-2,3-dihydrosqualene 33 (Chart I) and in part of *all-E*-squalene 2,3-epoxide 4 (see the Experimental Section).

The ^{13}C NMR spectra gave important information on the position of the signals of the allylic methylene at C-5 and the allylic methyl at C-6'. The ^{13}C NMR spectra of various (trisubstituted) methyldialkylalkenes with an open chain have different positions in the signal of the CH_3 linked to the double bond and of the allylic vicinal CH_2, depending on isomerism being E or Z.[34,35] The signals of the allylic CH_3 in the E isomers resonated at a higher field than the Z isomers, while the allylic CH_2 of the E isomers resonated at a lower field than for the Z isomers. In our case, the LR_f isomer 31 (E) showed a quartet at δ 15.89 for C-6' and a triplet at δ 36.22 for C-5, while HR_f isomer 32 (Z) showed a quartet at δ 23.21 for C-6' and a triplet at δ 29.59 for C-5, according to the assigned structures.

Finally, the biological results agreed with the assigned structures. Only the E isomer 31 was biologically active.

Biological Results

We have studied the biological activity of (6E)- and (6Z)-2,3-epoxy-10-aza-10,11-dihydrosqualene (31 and 32) as inhibitors of SO cyclase of rat liver, pig liver, *Saccharomyces cerevisiae*, and *Candida albicans* microsomes.

The two isomers 31 and 32 differed greatly in their inhibition of SO cyclase, as expected. Only isomer E, the carbocation analogue with the same configuration of 2,3-oxidosqualene, was active on SO cyclase in all of the biological systems tested, with an I_{50} varying from 3 to 5 μM (Table I). These values are similar to the corresponding ones of the other known inhibitors of SO cyclase, such as the azasqualenes,[8,9,18] but with an important difference: 2-aza-2,3-dihydrosqualene (34) was also active on squalene epoxidase,[36,37] having an I_{50} of 4.5 μM on squalene epoxidase from rat liver. On the other hand, isomers 31 and 32 were completely inactive on squalene epoxidase even at 10 μM, while at this concentration the inhibition of SO cyclase by isomer E was higher than 90%.

Isomer Z (32) was inactive on the various SO cyclases at the higher concentrations tested (Table I). For instance, the residual SO cyclase activity at 10 μM of isomer E was less than 10%, while at 20 μM isomer Z did not inhibit cyclase activity.

The difference in activity shown by the two isomers in the inhibition of SO cyclase from yeasts was even higher, showing that the structure must be similar to 2,3-oxidosqualene.

Discussion

In the past we have synthesized and studied the biological activity of 2-aza-2,3-dihydrosqualene (34) (Chart I) and various series of aza derivatives considered as HEI analogues of the C-2 ion formed by the enzymic opening of the oxirane ring of SO.[8-10,18,19,36-39]

Now we have based this paper on the mimicking of the C-8 carbonium ion transiently formed during SO cyclization to the protosteryl ion by synthesizing the two geometrical isomers E (31) and Z (32), bearing a nitrogen in the pro C-8 position (Scheme IV).

The inhibition activity on SO cyclase of 31 and 32 has been shown to be highly different, since only isomer E, the carbocation analogue corresponding to the natural *all-E* SO, was active. Moreover, both the isomers E and Z, as opposed to 2-aza-2,3-dihydrosqualene (34), were inactive on squalene epoxidase at the higher concentrations tested.

These data have prompted us to make some considerations on the future design of HEI analogue SO cyclase inhibitors.

The starting point is, in order to inhibit the enzymic cyclization process the most, an HEI inhibitor must be superimposable, as much as possible, on one of the discrete cations (i.e. C-8) originating from the cyclization of SO. The fact that compound 32 with an unfavorable 6Z double bond contrary to the isoprenic rule did not inhibit SO cyclase (whereas E isomer 31 did), prompted us to suppose that 31 could be initially recognized and then partially cyclized by the enzyme, generating "in situ" the suitable C-8 ammonium ion analogue inhibitor.

This suggestion is in line with the more general concept that inhibitors having an overall conformation similar to or not very far from that of the substrate may be better accepted by the enzyme.[8,21] For example, (17αH)- (35) and (17βH)-20-azadammaran-3β-ol (36) (Chart I), designed to mimic the C-20 ion originating during the cyclization of SO to β-amyrin, failed to inhibit the cyclase.[40] In this case, the conformation of the enzyme complementary to the C-20 carbocation, which is very different from that occurring in the enzymic ground state binding to the substrate, may be reached only after a catalytic event and not spontaneously.

Contrary to 31, an inhibitor such as 2-aza-2,3-dihydrosqualene 34 did not need a strict relationship between

(34) Breitmaier, E.; Haas, G.; Voelter, W. *Atlas of Carbon-13 NMR Data*, Heyden and Son Ltd., Ed.; Galliard Ltd.: Gt. Yarmouth, Norfolk, Great Britain, 1975.
(35) Brown, J. M.; Martens, D. R. M. An Assessment of the Mobility of Squalene in Part-Aqueous Solutions from Carbon Magnetic Resonance Spin-Lattice Relaxation Times. *Tetrahedron* 1977, *33*, 931–935.
(36) Schmitt, P.; Gonzales, R.; Benveniste, P.; Ceruti, M.; Cattel, L. Inhibition of Sterol Biosynthesis and Accumulation of 2,3-Oxidosqualene in Bramble Cell Suspension Cultures Treated with 2-Aza-2,3-dihydrosqualene and 2-Aza-2,3-dihydrosqualene N-Oxide. *Phytochemistry* 1987, *26*, 2709–2714.
(37) Ryder, N. S.; Dupont, M.-C.; Frank, I. Inhibition of Fungal and Mammalian Sterol Biosynthesis by 2-Aza-2,3-dihydrosqualene. *FEBS Lett.* 1986, *204*, 239–242.
(38) Cattel, L.; Ceruti, M.; Balliano, G.; Viola, F. 2,3-Oxidosqualene Cyclase and Squalene Epoxidase, as Target Enzymes for the Development of New Sterol Biosynthesis Inhibitors. In *Proceeding of the 201st American Chemical Society National Meeting*; Atlanta, 1991; p 40.
(39) Balliano, G.; Viola, F.; Ceruti, M.; Cattel, L. Inhibition of Sterol Biosynthesis in Saccharomyces cerevisiae by N,N-Diethylazasqualene and Derivatives. *Biochim. Biophys. Acta* 1988, *959*, 9–19.
(40) Delprino, L.; Caputo, O.; Balliano, G.; Berta, S.; Bouvier, P.; Cattel, L. Biosynthesis of β-amyrin. Part 3. Synthesis and Biological Evaluation of 17(βH)- and 17(αH)-Azadammaran-3β-ol. *J. Chem. Res.* 1984, (S) 254–255; (M) 2301–2309.

conformation and inhibition activity. Indeed, even a compound such as *N,N*-diethyldodecylamine (**37**) (Chart I), which lacks the complex double bond structure of SO, was at least partially able to inhibit SO cyclase.[8] 2-Aza-2,3-dihydrosqualene (**34**), in contrast to the *all-E*-10-azasqualene derivative **31**, was also able to inhibit squalene epoxidase.[36,37] This latter enzyme, a non-cytochrome P-450 monooxygenase, is inhibited when a suitable group, such as an acetylenic, an allenic, an alcoholic, or a tertiary amine function, is at the end of the squalenoid skeleton.[41-43]

In conclusion, the specific and potent inhibition of SO cyclase by *E* isomer **31** has confirmed our previous approach in designing new inhibitors of sterol biosynthesis by mimicking the HEI intermediates formed during SO cyclization, and it has been a useful tool in studying the mechanism of action of the cyclases, as well as in the development of new antifungal and hypocholesterolemic drugs.

Experimental Section

The ^{1}H NMR spectra were recorded either on a JEOL EX-400, a JEOL GX/270, or a JEOL JNM-PMX 60, with $SiMe_4$ as internal standard. Mass spectra were obtained on a VG Analytical 7070 EQ-HF or a VG ZAB 2F spectrometer by electron impact or by chemical ionization. IR spectra were recorded on a Perkin-Elmer 781. Microanalyses for C, H, and N were within ±0.4% of theoretical values and were performed on an Elemental Analyser 1106 (Carlo Erba Strumentazione), except in the case of P, analyzed according to the method of Schöniger.

The reactions were checked on F_{254} silica gel precoated sheets (Merck); after development, the sheets were exposed to iodine vapor. Purifications were done using column "flash chromatography" on 230–400-mesh silica gel (Merck). Petroleum ether refers to the fraction of bp 40–60 °C. MCPBA refers to *m*-chloroperbenzoic acid.

Squalene Epoxides (as a Mixture of the Two Trans Internal Monoepoxides): (6*E*,10*E*,18*E*)-*trans*-14,15-Epoxy-2,6,10,15,19,23-hexamethyl-2,6,10,18,22-tetracosapentaene (5) and (6*E*,10*E*,14*E*)-*trans*-18,19-Epoxy-2,6,10,15,19,23-hexamethyl-2,6,10,14,22-tetracosapentaene (6). A solution of squalene **3** (10 g, 24.3 mmol) dissolved in CH_2Cl_2 (250 ml) at 0 °C was stirred while MCPBA (85% purity; 1.5 equiv, 6.30 g, 36.5 mmol) was added over a period of 30 min; it was then allowed to react for a further 30 min with continued stirring. The reaction mixture was washed with 20% aqueous $NaHCO_3$ (100 mL × 3) and saturated brine (100 mL × 2), dried over anhydrous sodium sulfate, and evaporated to dryness to give a mixture of products. The resulting oil was purified by flash chromatography (petroleum ether/diethyl ether, 95:5) to give a mixture of the two trans internal monoepoxides **5** and **6** (3.0 g, 29% yield)[15] and then the external monoepoxide **4** (1.5 g, 14% yield) as colorless oils.

5 and **6**: IR (liquid film) 2980, 2910, 2850, 1450, 1385, 1250, 1110, 985 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3 H, oxirane CH_3), 1.58–1.67 (m, 25 H, allylic CH_3 and CH_2-oxirane-CH_2), 1.97–2.05 (m, 16 H, allylic CH_2), 2.70 (m, 1 H, oxirane CH), 5.06–5.15 (m, 5 H, vinylic CH); EIMS *m/z* 426 (4), 400 (2), 383 (2), 357 (10), 339 (4), 289 (4), 276 (4), 247 (30), 203 (20), 191 (15), 177 (17), 161 (20), 149 (43), 135 (75), 109 (100); HRMS *m/z* 426.3867 (calcd for $C_{30}H_{50}O$ 426.3861).

4: ^{1}H NMR ($CDCl_3$) δ 1.242 and 1.283 (2 s, 6 H, oxirane CH_3), 1.58–1.66 (m, 20 H, allylic CH_3 and oxirane-CH_2), 1.98–2.06 (m, 18 H, allylic CH_2), 2.690 (m, 1 H, *J* = 6.2 Hz, oxirane CH), 5.06–5.17 (m, 5 H, vinylic CH).

C_{17} Squalenoid Aldehyde and C_{22} Squalenoid Aldehyde: (4*E*,8*E*)-5,9,13-Trimethyl-4,8,12-tetradecatrienal (9) and (4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenal (10). $HIO_4{\cdot}2H_2O$ (1.5 equiv, 1.60 g, 7.04 mmol) was added to ether (250 mL) with vigorous stirring, and when solution was almost complete, the mixture of squalene epoxides **5** and **6** (2.0 g, 4.69 mmol) in ether (5 mL) was added. Stirring was continued for 15 min after which the reaction mixture was washed with saturated brine (100 mL × 3), dried over anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with various eluants (petroleum ether/CH_2Cl_2, 90:10, 80:20) to give a mixture of C_{17} and C_{22} aldehydes **9** and **10** (1.16 g)[15] and then a mixture of ketones **7** and **8**. The mixture of aldehydes was separated by reversed-phase flash chromatography (octadecylsilane bonded to silica gel; 40-μm average particle diameter) (MeCN/H_2O, 75:25; 80:20; 85:15; 90:10; pure MeCN) to give C_{17} aldehyde **9** (472 mg, 40% yield from **5** + **6**) and C_{22} aldehyde **10** (610 mg, 41% yield from **5** + **6**).

9: IR (liquid film) 2980, 2910, 2850, 1725 (CO), 1445, 1385 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.60–1.71 (m, 12 H, allylic CH_3), 1.97–2.12 (m, 10 H, allylic CH_2), 2.36–2.42 (m, 2 H, CH_2CHO), 5.01–5.20 (m, 3 H, vinylic CH), 9.71 (m, 1 H, CHO); EIMS *m/z* 248 (10), 231 (3), 205 (10), 177 (7), 161 (14), 136 (73), 69 (100).

10: IR (liquid film) 2980, 2910, 2850, 1730 (CO), 1450, 1385 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.58–1.70 (m, 15 H, allylic CH_3), 1.95–2.10 (m, 14 H, allylic CH_2), 2.35–2.43 (m, 2 H, CH_2CHO), 4.98–5.22 (m, 4 H, vinylic CH), 9.70 (m, 1 H, CHO); EIMS *m/z* 316 (8), 299 (2), 273 (5), 247 (14), 205 (12), 192 (15), 149 (24), 137 (50), 69 (100).

C_{17} Squalenoid *N*-Methylamine: (4*E*,8*E*)-*N*-Methyl-5,9,13-trimethyl-4,8,12-tetradecatrienylamine (11). Method A. Methylamine (great excess, about 5 mL) was liquefied at −50 °C, and then anhydrous methanol (10 mL), previously cooled to −50 °C, was added with continuous stirring. A solution of HCl in anhydrous methanol was added dropwise up to pH 4–5. C_{17} squalenoid aldehyde **9** (1.24 g, 5 mmol) dissolved in anhydrous methanol (5 mL) was then added, followed by $NaBH_3CN$ (314 mg, 5 mmol). The reaction mixture was allowed to reach −10 °C, left at this temperature for 30 min, and then brought to room temperature. The mixture was extracted with diethyl ether (100 mL × 3) after addition of water (100 mL), dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo. The resulting oil was purified by flash chromatography using diethyl ether to remove C_{17} squalenoid alcohol **13** (150 mg, 12% yield) and then C_{17} squalenoid amine dimer **14** (670 mg, 54% yield, taking into account that it is a dimer). By elution with diethyl ether/methanol, 50:50, C_{17} squalenoid *N*-methylamine **11** (369 mg, 28% yield) was recovered as a colorless oil.

C_{17} squalenoid alcohol, (4*E*,8*E*)-5,9,13-trimethyl-4,8,12-tetradecatrien-1-ol (13): IR (liquid film) 3400–3200 (broad band), 2980, 2920, 2860, 1450, 1380, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.54–1.66 (m, 14 H, allylic CH_3 and CH_2CH_2OH), 1.95–2.09 (m, 10 H, allylic CH_2), 3.61 (t, 2 H, CH_2OH), 4.98–5.10 (m, 3 H, vinylic CH); EIMS 250 (19), 235 (2), 219 (2), 207 (6), 191 (5), 181 (17), 69 (100)[43].

C_{17} squalenoid amine dimer, *N*-methylbis[(4*E*,8*E*)-5,9,13-trimethyl-4,8,12-tetradecatrienyl]amine (14): IR (liquid film) 2970, 2930, 2860, 1450, 1380 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55–1.70 (m, 28 H, allylic CH_3 and 2 CH_2CH_2N), 1.93–2.11 (m, 20 H, allylic CH_2), 2.24 (s, 3 H, CH_3N), 2.36 (t, 4 H, 2 CH_2N), 5.02–5.12 (m, 6 H, vinylic CH); EIMS *m/z* 495 (15), 427 (45), 358 (100), 276 (43), 140 (12), 126 (11); HRMS *m/z* 495.4809 (calcd for $C_{35}H_{61}N$ 495.4804). Anal. ($C_{35}H_{61}N$) C, H, N.

11: IR (liquid film) 2980, 2910, 2860, 1450, 1385, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55–1.65 (m, 14 H, allylic CH_3 and CH_2CH_2N), 1.97–2.07 (m, 10 H, allylic CH_2), 2.44 (broad s, 3 H, CH_3N), 2.58 (broad t, 2 H, CH_2N), 5.07–5.18 (m, 3 H, vinylic CH); EIMS *m/z* 263 (47), 248 (9), 194 (73), 126 (100); HRMS 263.2615 (calcd for $C_{18}H_{33}N$ 263.2613). Anal. ($C_{18}H_{33}N$) C, H, N.

Method B. Methylamine (great excess, about 5 mL) was liquefied at −50 °C, and then anhydrous methanol (10 mL), previously cooled to −50 °C, was added dropwise up to pH 9. The temperature was then stabilized at −15 °C, and $NaBH_3CN$ (× 1.2, 744 mg, 12 mmol) was added. C_{17} squalenoid aldehyde **9** (2.48 g, 10 mmol) dissolved in 50 mL of anhydrous methanol was added

(41) Cerutí, M.; Viola, F.; Grosa, G.; Balliano, G.; Delprino, L.; Cattel, L. Synthesis of Squalenoid Acetylenes and Allenes, as Inhibitors of Squalene Epoxidase. *J. Chem. Res.* **1988** (S) 18–19; (M) 0239–0260.

(42) Sen, S. E.; Prestwich, G. D. Trisnorsqualene Cyclopropylamine: a Reversible, Tight-Binding Inhibitor of Squalene Epoxidase. *J. Am. Chem. Soc.* **1989**, *111*, 8761–8762.

(43) Sen, S. E.; Wawrzenczyk, C.; Prestwich, G. D. Inhibition of Vertebrate Squalene Epoxidase by Extended and Truncated Analogues of Trisnorsqualene Alcohol. *J. Med. Chem.* **1990**, *33*, 1698–1701.

over a period of 2 h with vigorous stirring. After the mixture was allowed to stand overnight at −15 °C, the pH was brought up to 7 and the reaction mixture was stirred for 1 h at 0 °C. After evaporation of the solution to about half volume, 10% aqueous $NaHCO_3$ (50 mL) was added and the solution was extracted with diethyl ether (50 mL × 3). The organic layers were washed with 10% $NaHCO_3$ (50 mL × 1), dried over anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography using diethyl ether to remove C_{17} squalenoid amine dimer 14 (742 mg, 15% yield) and then diethyl ether/methanol, 50:50, to give 1.37 g of C_{17} squalenoid *N*-methylamine 11 in 52% yield, as a colorless oil.

C_{17} Squalenoid Amine *N*-Oxide Dimer: *N*-Methylbis[(4*E*,8*E*)-5,9,13-trimethyl-4,8,12-tetradecatrienyl]amine *N*-Oxide (15). C_{17} squalenoid amine dimer 14 (250 mg, 0.504 mmol) was dissolved in methanol (1 mL), 30% H_2O_2 (great excess, 5 mL) was added, and the mixture was left at 30 °C with stirring for 24 h. During this time, the reaction mixture progressively cleared. Petroleum ether (50 mL) was then added, the two-phase system was cooled to 0 °C, and MnO_2 was added in small amounts to decompose H_2O_2. When this was complete, the suspension was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography with dichloromethane to remove impurities and then methanol, to give 237 mg of 15 (92% yield) as a colorless oil: IR (liquid film) 2970, 2920, 2860, 1670, 1450, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56–1.68 (m, 28 H, allylic CH_3 and 2 CH_2CH_2N), 1.91–2.08 (m, 20 H, allylic CH_2), 3.16 (s, 3 H, CH_3NO), 3.25 (t, 4 H, 2 CH_2N), 5.00–5.13 (m, 6 H, vinylic CH); EIMS *m/z* 511 (2), 496 (22), 483 (3), 443 (6), 427 (65), 413 (35), 374 (11), 358 (100), 344 (90), 276 (48); HRMS *m/z* 511.4760 (calcd for $C_{35}H_{61}NO$ 511.4753). Anal. ($C_{35}H_{61}NO$) C, H, N, O.

(3-Hydroxypropyl)triphenylphosphonium Bromide (17). Triphenylphosphine (50 g, 0.191 mol) was dissolved in toluene (100 mL), and 3-bromo-1-propanol (16) (26.5 g, 0.191 mol) was added. The reaction mixture was refluxed for 24 h; during this time a white precipitate formed. The solid was filtered, washed with diethyl ether (50 mL × 2), dried, and recrystallized from methanol/ethanol to give, after concentrating and recrystallizing the filtrate, 69.7 g of 17 (91% yield) as white crystals: mp 233–234 °C (lit.[23] mp 232.5–233.5 °C); ^1H NMR (CD_3OD) δ 1.61–2.18 (m, 2 H, $CH_2CH_2CH_2$), 3.42–3.89 (m, 4 H, $CH_2CH_2CH_2$), 7.50–8.07 (m, 15 H, aromatic CH).

3-(Diphenylphosphinoyl)-1-propanol (18). (3-Hydroxypropyl)triphenylphosphonium bromide (17) (50 g, 0.124 mol) was dissolved in ethanol (80 mL) in a one-necked flask, and a 50% (w/v) aqueous NaOH solution (great excess, 80 mL) was added. The mixture was concentrated under vacuum on a rotary evaporator at 50–60 °C to about one-third of the original volume and extracted with CH_2Cl_2 (100 mL × 3) after addition of water (100 mL). The organic layers were washed with water (100 mL × 3), dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude oil was purified by flash chromatography with petroleum ether/ethyl acetate, 90:10, to remove triphenylphosphine, and then ethyl acetate to remove impurities and finally a gradient of ethyl acetate/methanol, 99:1; 98:2; 95:5, to give 20.9 g of 3-(diphenylphosphinoyl)-1-propanol (18) (65% yield) as a white solid. Recrystallized from ethanol/ether it had mp 103–104 °C (lit.[25] mp 103 °C): ^1H NMR ($CDCl_3$) δ 1.82 (m, 2 H, $CH_2CH_2CH_2$), 2.38 (m, 2 H, CH_2PO), 3.63 (t, 2 H, CH_2OH), 7.34–7.92 (m, 10 H, aromatic CH).

3-(Diphenylphosphinoyl)propanal (19). In a three-necked flask, equipped with a fluximeter for N_2 and two dropping funnels, cooled at −40 °C were added oxalyl chloride (× 3, 7.32 g, 57.63 mmol) and 200 mL of anhydrous CH_2Cl_2. Anhydrous DMSO (excess, 12 mL) was then added within 30 min, under nitrogen, with stirring. 3-(Diphenylphosphinoyl)-1-propanol (18) (5 g, 19.2 mmol) dissolved in 40 mL of anhydrous CH_2Cl_2 was added within 15 min, and the mixture was left for one more hour at −40 °C and 1 h at −20 °C. Anhydrous triethylamine (excess, 30 mL) was then added within 15 min. After 30 min, the reaction mixture was allowed to reach room temperature and the flux of nitrogen was stopped. Water (50 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (100 mL × 3). The organic phases were washed with 2 N HCl (50 mL × 2), 10% $NaHCO_3$ (50 mL × 2), and brine (50 mL × 1), dried over anhydrous sodium sulfate, and evaporated in vacuo to give a light brown viscous oil. The crude oil was purified by flash chromatography with dichloromethane/acetone, 95:5, to remove heads, and then acetone to give 3.07 g of 3-(diphenylphosphinoyl)propanal (19) in 62% yield: IR ($CDCl_3$ solution) 3060, 2980, 2930, 2870, 1725 (CO), 1440, 1180, 1125, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.58 and 2.82 (2 m, 4 H, CH_2CH_2), 7.46–7.78 (m, 10 H, aromatic CH), 9.79 (s, 1 H, CHO); EIMS *m/z* 258 (27), 230 (13), 202 (100), 155 (23), 125 (20), 77 (49); HRMS 258.0814 (calcd for $C_{15}H_{15}O_2P$ 258.0809). Anal. ($C_{15}H_{15}O_2P$) C, H, O, P.

5,6-Epoxy-6-methylheptan-2-one (21). To a one-necked flask cooled to 0 °C were added CH_2Cl_2 (300 mL) and 10% aqueous $NaHCO_3$ (80 mL), followed by 6-methyl-5-hepten-2-one (20) (10 g, 79.25 mmol), with stirring. Then MCPBA (× 1.2, 55% purity, containing 10% of *m*-chlorobenzoic acid and 35% water, 29.84 g, 95.1 mmol) was slowly added. The two-phase system was stirred for 2 h at room temperature, and the organic layer washed with aqueous 1 N NaOH (100 mL × 1) and water (100 ml × 2), dried over anhydrous sodium sulfate, and evaporated in vacuo at 30 °C. The crude oil, already of high purity according to ^1H NMR, was rapidly distilled in a Kugelrohr containing three bubbles, at 1.5 mmHg. The first was held in the rotary kiln, the second outside, and the third cooled. The second bubble contained ketone 21 (4.98 g, >98% purity by ^1H NMR), while the third contained ketone 21 (4.71 g), contaminated with about 6–8% of bicycle 22, in total 9.69 g, 86% yield. Using standard Claisen distillation, all the fractions were significantly contaminated with the bicycle (ref 26): IR (liquid film) 2980, 2960, 2940, 1715, 1400, 1365, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 6 H, epoxidic CH_3), 1.68 (m, 2 H, CH_2CH_2CO), 2.15 (s, 3 H, CH_3CO), 2.50–2.80 (m, 3 H, epoxidic CH and CH_2CO).

Azasqualenoid Phosphinoxide: (4*E*,8*E*)-*N*-[3-(Diphenylphosphinoyl)propyl]-*N*-methyl-5,9,13-trimethyl-4,8,12-tetradecatrienylamine (12). Anhydrous methanol (100 mL) was added to C_{17} squalenoid *N*-methylamine 11 (2 g, 7.59 mmol), cooled to 0 °C, and stirred. Then 3-(diphenylphosphinoyl)propanal 19 (× 2, 3.92 g, 15.18 mmol) with a minimum of methanol was added, followed by $NaBH_3CN$ (× 2, 2.954 g, 15.18 mmol). After stirring for 1 h at room temperature, the pH was brought from 9 to 7 by adding HCl in methanol, and the mixture was then kept 1 h more at room temperature; 10% $NaHCO_3$ (100 mL) was then added, and the mixture was extracted with diethyl ether (100 mL × 3), dried over anhydrous sodium sulfate, and evaporated in vacuo. The crude oil was purified by flash chromatography with ethyl acetate/methanol, 95:5, to remove impurities, and then ethyl acetate/methanol, 90:10, to give 2.69 g (70% yield) of product 12 as a colorless oil: IR (liquid film) 3060, 2930, 2860, 2800, 1440, 1380, 1190, 1120, 740, 720, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44–1.75 (m, 16 H, allylic CH_3 and $CH_2CH_2NCH_2CH_2$), 1.95–2.12 (m, 10 H, allylic CH_2), 2.24–2.42 [m, 9 H, $CH_2N(CH_3)CH_2$ and CH_2PO], 5.06–5.16 (m, 3 H, vinylic CH), 7.46–7.78 (m, 10 H, aromatic CH); EIMS *m/z* 505 (3), 490 (2), 436 (31), 368 (100), 286 (24), 243 (83), 215 (48), 205 (72); HRMS 505.3478 (calcd for $C_{33}H_{48}NOP$ 505.3473). Anal. ($C_{33}H_{48}NOP$) C, H, N, O, P.

Aza Phosphinoxide Alcohol Dimers: (6*E*,10*E*,26*E*,30*E*)-19-(Diphenylphosphinoyl)-2,6,10,15,22,27,31,35-octamethyl-15,22-diaza-2,6,10,26,30,34-hexacontahexaen-18-ol (23 and 24). Azasqualenoid phosphinoxide 12 (500 mg, 0.99 mmol) was dissolved in anhydrous THF (20 mL), cooled to −10 °C under nitrogen, and stirred. After 5 min, BuLi (× 2, 15% in hexane, ca. 1.6 M, 1.98 mmol, 1.2 mL) was added; the reaction mixture gradually turned pale orange. After 10 min, it was cooled to −80 °C, and epoxy ketone 21 (× 3, 422 mg, 2.97 mmol) in anhydrous THF (1 mL) was added. The reaction mixture was kept for 1 h at −80 °C and then allowed to reach room temperature. It was poured into diethyl ether/NaCl saturated solution (50 mL) and extracted with ether (50 mL × 3). The organic phases were washed with saturated brine (50 mL × 2), dried over anhydrous sodium sulfate, and evaporated to dryness to give a pale yellow oil. The crude oil was purified by flash chromatography with diethyl ether/methanol, 80:20, to remove impurities and unreacted aza phosphinoxide 12, then diethyl ether/methanol, 65:35, to give HR_f dimer 23 (80 mg, 41%) and then LR_f dimer 24 (116 mg, 59%) with a total yield of 48%.

23: IR (liquid film) 3400–3200, 3060, 2970, 2930, 2860, 1440, 1175, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56–1.67 [m, 32 H, allylic CH_3 and $CH_2CH_2NCH_2CH_2CH(OH)CHPCH_2CH_2NCH_2CH_2$], 1.91–2.14 (m, 20 H, allylic CH_2), 2.21–2.48 [m, 15 H, 2 $CH_2N(CH_3)CH_2$ and CHP], 3.91 (t, 1 H, CHOH), 5.01–5.12 (m, 6 H, vinylic CH), 7.40–7.86 (m, 10 H, aromatic CH); EIMS 826 (8), 688 (5), 620 (2), 548 (2), 535 (4), 466 (3), 416 (5), 398 (6), 360 (11), 276 (100); HRMS m/z 824.6340 (calcd for $C_{54}H_{85}N_2O_2P$ 824.6348). Anal. ($C_{54}H_{85}N_2O_2P$) C, H, N, O, P.

24: IR (liquid film) 3400–3200, 3060, 2970, 2930, 2860, 1440, 1175, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57–1.65 [m, 32 H, allylic CH_3 and $CH_2CH_2NCH_2CH_2CH(OH)CHPCH_2CH_2NCH_2CH_2$], 1.90–2.12 (m, 20 H, allylic CH_2), 2.19–2.50 [m, 15 H, 2 $CH_2N(CH_3)CH_2$ and CHP], 4.08 (t, 1 H, CHOH), 5.01–5.12 (m, 6 H, vinylic CH), 7.42–7.89 (m, 10 H, aromatic CH); EIMS 826 (7), 688 (20), 620 (5), 548 (12), 535 (9), 466 (8), 436 (2), 398 (18), 360 (32), 334 (21), 276 (100); HRMS m/z 824.6344 (calcd for $C_{54}H_{85}N_2O_2P$ 824.6348). Anal. ($C_{54}H_{85}N_2O_2P$) C, H, N, O, P.

Aza Dimers 25 (from HR_f Alcohol 23) and 26 (from LR_f Alcohol 24): (6*E*,10*E*,18*E*,26*E*,30*E*)-2,6,10,15,22,27,31,35-Octamethyl-15,22-diaza-2,6,10,18,26,30,34-hexacontaheptaene and (6*E*,10*E*,18*Z*,26*E*,30*E*)-2,6,10,15,22,27,31,35-Octamethyl-15,22-diaza-2,6,10,18,26,30,34-Hexacontaheptaene. HR_f alcohol dimer **23** (60 mg, 0.0727 mmol) was dissolved in anhydrous DMF and kept under nitrogen flux with stirring. NaH (80% suspension in white oil, washed with pentane, excess, 30 mg) was added, and the reaction mixture was stirred at 45 °C for 3 h. After cooling, diethyl ether was added (30 mL × 3) and the solid eliminated. The organic phases were washed with water (20 mL × 3), dried, and evaporated in vacuo. The crude oil was purified by flash chromatography with diethyl ether/methanol, 95:5, to remove impurities, and then methanol to give 35.7 mg of aza dimer **25** (81% yield) as a colorless oil. From LR_f alcohol **24** (60 mg, 0.0727 mmol) using the same conditions of reaction and purification, 36.5 mg of aza dimer **26** (83% yield) were obtained.

25 (from HR_f alcohol): IR (liquid film) 2980, 2920, 2850, 1450, 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56–1.67 (m, 28 H, allylic CH_3 and 2 $CH_2CH_2CH_2N$), 1.96–2.07 (m, 24 H, allylic CH_2), 2.22 (s, 6 H, 2 CH_3N), 2.35 (m, 8 H, 2 CH_2NCH_2), 5.03–5.14 (m, 6 H, CH of trisubstituted double bonds), 5.424 (m, 2 H, CH=CH); EIMS 607 (6), 592 (0.5), 538 (2), 470 (9), 402 (2), 387 (6), 330 (8), 276 (100); HRMS m/z 606.5850 (calcd for $C_{42}H_{74}N_2$ 606.5852). Anal. ($C_{42}H_{74}N_2$) C, H, N.

26 (from LR_f alcohol): IR (liquid film) 2980, 2920, 2850, 1450, 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56–1.69 (m, 28 H, allylic CH_3 and 2 $CH_2CH_2CH_2N$), 1.98–2.06 (m, 24 H, allylic CH_2), 2.25 (s, 6 H, 2 CH_3N), 2.36 (m, 8 H, 2 CH_2NCH_2), 5.04–5.15 (m, 6 H, CH of trisubstituted double bonds), 5.408 (m, 2 H, CH=CH); EIMS 607 (3), 592 (0.3), 538 (2), 470 (5), 402 (1), 388 (3), 344 (4), 330 (5), 276 (100); HRMS m/z 606.5853 (calcd for $C_{42}H_{74}N_2$ 606.5852). Anal. ($C_{42}H_{74}N_2$) C, H, N.

Epoxy Aza Phosphinoxide Alcohol Diastereoisomers: (14*E*,18*E*)-7-(Diphenylphosphinoyl)-2,3-epoxy-2,6,10,15,19,23-hexamethyl-10-aza-14,18,22-tetracosatrien-6-ol (27–30). Azasqualenoid phosphinoxide **12** (400 mg, 0.791 mmol) dissolved in anhydrous THF (20 mL) was cooled to +10 °C, under nitrogen, with stirring. Methyllithium (5% solution, ca. 1.5 mol, × 2.5, 1.3 mL) was added. The reaction mixture turned deep orange. After 5 min, the temperature was rapidly brought to −70 °C, and then epoxy ketone **21** (× 1.1, 124 mg, 0.870 mmol), in the minimum amount of THF, was added within 5 min. The reaction mixture was left for 15 min at −70 °C and 15 min at −30 °C and then brought to room temperature. It was then poured into a diethyl ether/saturated aqueous NaCl, 1:1, two-phase system (100 mL) and extracted with diethyl ether (50 mL × 3). The organic phases were washed with saturated brine (50 mL × 2), dried over anhydrous sodium sulfate, and evaporated in vacuo. The crude oil was purified by flash chromatography with diethyl ether/methanol, 90:10, to give 261 mg of a mixture of the four diastereoisomers **27–30** in 51% yield: IR (liquid film) 3450–3250, 3060, 2960, 2920, 2860, 1440, 1370, 1180, 1110, 720, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16–1.30 [m, 9 H, epoxidic CH_3 and $CH_3CH(OH)$], 1.59–1.68 (m, 20 H, allylic CH_3, CH_2CH_2CHOH and $CH_2CH_2NCH_2CH_2$), 1.93–2.10 (m, 10 H, allylic CH_2), 2.20–2.72 [m, 9 H, epoxidic CH, CHP, $CH_2N(CH_3)CH_2$], 5.02–5.15 (m, 3 H, vinylic CH), 7.45–7.90 (m, 10 H, aromatic CH); EIMS m/z 647 (18), 632 (10), 588 (28), 578 (31), 510 (95), 446 (20), 428 (9), 414 (4), 385 (41), 358 (20), 299 (90), 290 (45), 276 (70), 205 (61), 69 (100); HRMS 647.4454 (calcd for $C_{41}H_{62}NO_3P$ 647.4467). Anal. ($C_{41}H_{62}NO_3P$) C, H, N, O, P.

(6*E*)-2,3-Epoxy-10-aza-10,11-dihydrosqualene [(6*E*,14*E*,18*E*)-2,3-Epoxy-2,6,10,15,19,23-hexamethyl-10-aza-6,14,18,22-tetracosatetraene (31)] and (6*Z*)-2,3-Epoxy-10-aza-10,11-dihydrosqualene [(6*Z*,14*E*,18*E*)-2,3-epoxy-2,6,10,15,19,23-hexamethyl-10-aza-6,14,18,22-tetracosatetraene (32)]. Epoxy aza phosphinoxide alcohol diastereoisomers **27–30** (200 mg, 0.3087 mmol) dissolved in anhydrous DMSO (5 mL) were stirred under nitrogen. KOH (fine powder, excess, 100 mg) was added, and the reaction mixture was stirred at 50 °C for 4 h. After cooling, the brown solution was poured into a two-phase system consisting of diethyl ether/NaCl saturated solution, 1:1 (50 mL), and extracted with diethyl ether (30 mL × 3). The organic phases were washed with brine (20 mL × 2), dried over anhydrous sodium sulfate, and evaporated in vacuo to give a light brown oil. The crude oil was purified on TLC plates with methanol to give 76 mg of alcohols **27–30** and 80 mg of epoxyazasqualenes **31** and **32** contaminated by aza phosphinoxide **12**. A second purification on TLC with diethyl ether/methanol, 80:20, allowed us to recover 61 mg of pure epoxyazasqualenes **31** and **32** (46% yield).

Separation of (6*E*)- and (6*Z*)-2,3-Epoxy-10-aza-10,11-dihydrosqualene. A mixture of **31** and **32** (20 mg) was separated eight times on several TLC plates with petroleum ether/diethyl ether/methanol, 45:45:10, as eluants to give 3.1 mg of pure HR_f isomer, 3.1 mg of the approximate 1:1 mixture, and 7.2 mg of pure LR_f isomer.

HR_f isomer, *Z* Isomer 32: IR (liquid film) 2960, 2920, 2850, 1450, 1375, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.278 and 1.315 (2 s, 6 H, epoxidic CH_3), 1.44–1.69 (m, 19 H, allylic CH_3, epoxidic CH_2 and $CH_2CH_2CH_2N$), 1.99–2.20 (m, 14 H, allylic CH_2), 2.25–2.48 [m, 7 H, $CH_2N(CH_3)CH_2$], 2.720 (t, 1 H, J = 6.2 Hz, epoxidic CH), 5.08–5.16 (m, 4 H, vinylic CH); ^{13}C NMR ($CDCl_3$) δ 23.21 (q, C-6′), 29.59 (t, C-5); CIMS m/z 430 (100), 429 (3), 428 (10), 412 (8), 360 (3), 346 (3), 292 (3), 276 (85), 262 (5); HRMS 429.3967 (calcd for $C_{29}H_{51}NO$ 429.3970). Anal. ($C_{29}H_{51}NO$) C, H, N.

LR_f isomer, *E* isomer 31: IR (liquid film) 2960, 2920, 2850, 1450, 1375, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.266 and 1.308 (2 s, 6 H, epoxidic CH_3), 1.44–1.69 (m, 19 H, allylic CH_3, epoxidic CH_2 and $CH_2CH_2CH_2N$), 1.98–2.21 (m, 14 H, allylic CH_2), 2.25 (s, 3 H, CH_3N), 2.36 (t, 4 H, CH_2NCH_2), 2.706 (t, 1 H, J = 6.2 Hz, epoxidic CH), 5.08–5.16 (m, 4 H, vinylic CH); ^{13}C NMR ($CDCl_3$) δ 15.89 (q, C-6′), 36.22 (t, C-5); CIMS m/z 430 (100), 429 (3), 428 (10), 412 (7), 360 (2), 346 (3), 292 (2), 276 (80), 262 (5); HRMS 429.3963 (calcd for $C_{29}H_{51}NO$ 429.3970). Anal. ($C_{29}H_{51}NO$) C, H, N.

22,23-Epoxy-2-aza-2,3-dihydrosqualene [(4*E*,8*E*,12*E*,16*E*)-20,21-Epoxy-*N*,*N*-dimethyl-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraenylamine (33)]. This compound was prepared according to the literature[44] starting from squalene diepoxide, cutting off the epoxide with periodic acid in diethyl ether to give epoxysqualene aldehyde, and finally reductive amination with sodium cyanoborohydride and dimethylamine: ^1H NMR ($CDCl_3$) δ 1.262 and 1.304 (2 s, 6 H, epoxidic CH_3), 1.58–1.64 (m, 16 H, allylic CH_3, oxirane-CH_2 and CH_2CH_2N), 1.98–2.15 (m, 16 H, allylic CH_2), 2.29–2.34 [m, 8 H, $(CH_3)_2NCH_2$], 2.708 (t, 1 H, J = 6.0 Hz, epoxidic CH), 5.08–5.15 (m, 4 H, vinylic CH).

Biological Assays. I_{50} on SO-lanosterol cyclase activity were determined in microsomal preparations from rat and pig liver, *S. cerevisiae*, and *C. albicans*.

Microsomes of rat and pig liver and of *S. cerevisiae* were prepared according to the methods previously described.[15,39,44] *C. albicans* microsomes were kindly provided by LEPETIT.

SO-lanosterol cyclase activity and I_{50} were determined by the methods previously described.[39,44]

Squalene epoxidase activity was determined in rat liver microsomes in the presence of supernatant fraction S_{100} and of the inhibitor of the SO cyclase 3β-[β-(dimethylamino)ethoxy]-

(44) Viola, F.; Ceruti, M.; Balliano, G.; Caputo, O.; Cattel, L. 22,23-Epoxy-2-aza-2,3-dihydrosqualene Derivatives: Potent New Inhibitors of Squalene 2,3-Oxide-Lanosterol Cyclase. *Il Farmaco* **1990**, *45*, 965–978.

androst-5-en-17-one (U-14226 A).

The reaction mixture contained in the final volume of 1 mL: [^{3}H]squalene (10000 cpm) diluted with squalene (final concentration = 20 μM), Tween-80 (final concentration = 0.05% w/v), 0.1 M K/K phosphate buffer pH 7.4 containing 1 mM EDTA, microsomes (5 mg of proteins) S_{105} (10 mg of proteins), U-14226A (50 μM), $NADP^+$ (2 mM), glucose-6-P (5 mM), glucose-6-P dehydrogenase (1 UI), $MgCl_2 \cdot 6H_2O$ (5 mM).

Incubations lasted 30 min at 37 °C. The reaction was stopped by the addition of 1 mL of 10% ethanolic KOH and saponification for 30 min at 80 °C. Extraction and chromatographic procedures similar to those of the SO cyclase assay were used.

After developing the TLC in CH_2Cl_2, the areas corresponding to authentic squalene and SO were scraped and counted for radioactivity in a Beckman LS 5000 liquid scintillator. The enzymatic activity was expressed as nanomoles of SO formed/hour.

Acknowledgment. This work was supported by grants from the Ministero dell' Università e della Ricerca Scientifica e Tecnologica and from CNR Progetto di Chimica Fine, n. 91.01677.PF72. We thank the NMR group of the Dipartimento di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Università di Torino; the mass group of the CNR, Area della Ricerca di Padova; and the mass group of the Dipartimento di Chimica Organica e Industriale, Università di Milano.

Novel Indolodioxanes with Antihypertensive Effects: Potent Ligands for the 5-HT_{1A} Receptor

Michael D. Ennis,*,† Mark E. Baze,† Martin W. Smith,‡ Charles F. Lawson,‡ Robert B. McCall,§ Robert A. Lahti,∥ and Montford F. Piercey∥

Departments of Medicinal Chemistry, Chemical and Biological Screening, Cardiovascular Diseases Research, and CNS Research, The Upjohn Company, Upjohn Laboratories, Kalamazoo, Michigan 49001. *Received May 13, 1991*

The synthesis and biological evaluation of a new family of tricyclic indolodioxanes is described. These compounds all contain the 2,3-dihydro-7*H*-1,4-dioxino[2,3-*e*]indole nucleus and bear substituents at the 2 and/or 8 positions. Thirteen members of this class were prepared and shown to be potent ligands for the 5-HT_{1A} receptor, with several compounds displaying subnanomolar inhibition constants. These compounds also bind to the dopamine D-2 receptor, but generally with higher inhibition constants than those for 5-HT_{1A}. Certain members of this novel structural class show in vivo activity in the mouse hypothermia assay. One of these compounds, U-86192A, has been shown to have antihypertensive effects in the cat, completely eliminating sympathetic nerve discharge at 1 mg/kg iv and lowering mean arterial pressure to 50% pretreatment levels. These effects can be reversed by the administration of spiperone, indicating that U-86192A is acting via a central serotonergic mechanism.

The neurotransmitter serotonin (1, 5-hydroxytryptamine, 5-HT) is associated with an ever-growing family of receptor subtypes.[1] One can comfortably reconcile the diversity of pharmacological events with which serotonin has been linked with the existence of these multiple binding sites. For example, there is strong evidence suggesting that activation of one such binding site, the 5-HT_{1A} receptor, inhibits sympathetic nerve discharge and might therefore play a central role in the regulation of blood pressure.[2] Critical to the study of any receptor-mediated event is the availability of chemical agents which bind with high selectivity to the receptor of interest. In this paper we will discuss the design rationale, the chemical synthesis, and the biological evaluation of a novel series of tricyclic indoles which possess remarkable binding affinities for the 5-HT_{1A} receptor. We will provide data which suggest that these compounds hold promise as novel, centrally-acting antihypertensive agents.

When considering structural skeleta as targets for centrally-acting cardiovascular agents, one can justifiably focus upon substituted 1,4-benzodioxanes as prime candidates. These compounds have a long history as antihypertensive agents which act primarily through adrenergic blockade.[3] Recently, however, it has become recognized that certain 1,4-benzodioxanes possess good affinity for the 5-HT_{1A} receptor. For example, the α_1-adrenergic agent WB 4101 binds to 5-HT_{1A} with an IC_{50} = 3.8 nM,[4] and the well-known 5-HT_{1A} antagonist spiroxatrine (2) displays a marked preference for the 5-HT_{1A} receptor over the 5-HT_{1B} or the 5-HT_2 sites.[5] Pharmacological activity believed to be mediated by 5-HT_{1A} receptors has been displayed by the 1,4-benzodioxanes (+)-flesinoxan and MDL 73005EF, the former possessing antihypertensive activity[6] and the latter active as an anxiolytic.[7] We have designed a series of compounds, illustrated generically as 4, which

† Department of Medicinal Chemistry.
‡ Department of Chemical and Biological Screening.
§ Department of Cardiovascular Diseases Research.
∥ Department of CNS Research.

(1) Leff, P.; Martin, G. R. The Classification of 5-Hydroxytryptamine Receptors. *Med. Chem. Rev.* **1988**, *8*, 187-202. Glennon, R. A. Central Serotonin Receptors as Targets for Drug Research. *J. Med. Chem.* **1987**, *30*, 1-12.
(2) Kuhn, D. M.; Wolf, W. A.; Lovenberg, W. Review of the Role of the Central Serotonergic Neuronal System in Blood Pressure Regulation. *Hypertension* **1980**, *2*, 243-255.
(3) Quaglia, W.; Pigini, M.; Giannella, M.; Melchiorre, C. 3-Phenyl Analogues of 2-[[[2-(2,6-Dimethoxy-Phenoxy)ethyl]-amino]-methyl]1,4-benzodioxan (WB 4101) as Highly Selective α_1-Adrenoreceptor Antagonists. *J. Med. Chem.* **1990**, *33*, 2946-2948.
(4) Norman, A. B.; Battaglia, G.; Morrow, A. L.; Creese, I. [^{3}H]-WB4101 labels S_1 serotonin receptors in rat cerebral cortex. *Eur. J. Pharmacol.* **1985**, *106*, 461-462.
(5) Nelson, D. L.; Taylor, E. W. Spiroxatrine: A Selective Serotonin$_{1A}$ Receptor Antagonist. *Eur. J. Pharm.* **1986**, *124*, 207-208.
(6) Wouters, W.; Tulp, M. Th. M.; Bevan, P. Flesinoxan lowers blood pressure and heart rate in cats via 5-HT_{1A} receptors. *Eur. J. Pharmacol.* **1988**, *149*, 213-223.
(7) Moser, P. C.; Tricklebank, M. D.; Middlemiss, D. N.; Mir, A. K.; Hibert, M. F.; Fozard, J. R. Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. *Br. J. Pharmacol.* **1990**, *99*, 343-349.

0022-2623/92/1835-3058$03.00/0