Stereospecific Synthesis of Squalenoid Epoxide Vinyl Ethers as Inhibitors of 2,3-Oxidosqualene Cyclase

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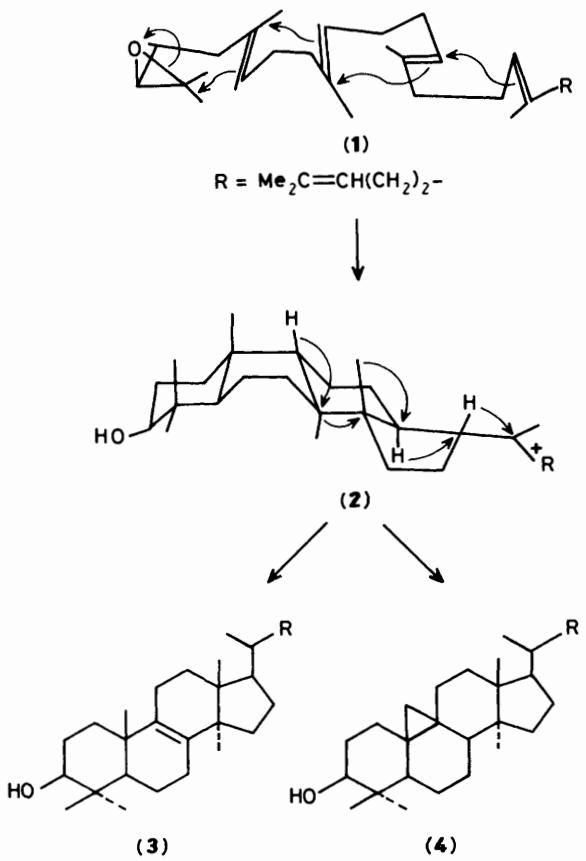
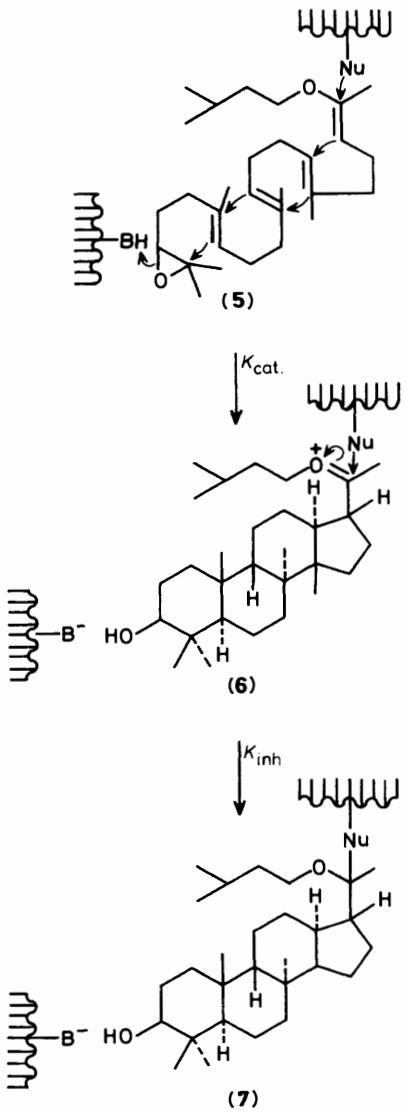
The stereospecific synthesis of squalenoid epoxide vinyl ethers with an isopentyloxy group is described. The synthesis involves the preparation of the C_{22} squalenoid aldehyde bromohydrin (**15**) by a new method *via* a one-step cleavage of lipophilic epoxides using periodic acid in diethyl ether, and the preparation of (1-isopentyloxyethyl)diphenylphosphine oxide (**24**). The structure of this compound has been confirmed by *X*-ray analysis. The configuration of vinyl ethers, synthesized using a Wittig-Horner reaction, has been determined by ^{13}C n.m.r. Biological results show that vinyl ethers (**5**) and (**27**) are competitive inhibitors of 2,3-oxidosqualene cyclase from rat liver.

It is assumed that the biosynthesis of sterols involves cyclization of 2,3-oxidosqualene (SO) (**1**) to the cation (**2**) (or its functional equivalent), and subsequent rearrangement by a sequence of 1,2-shifts either to lanosterol (**3**) (in animals and fungi) or cycloartenol (**4**) in higher plants[1–9] (Scheme 1).

2,3-Oxidosqualene cyclase (SO cyclase) (EC 5.4.99.7) can cyclize to ring systems other than sterols, as in the production of α- or β-amyrin in higher plants.[10–15] In order to discuss the



Scheme 1.



Scheme 2.

biosynthetic reactions from SO to tetra- or penta-cyclic triterpenoids, Cornforth[6] suggested the possible neutralization of some intermediate ions by a suitable nucleophilic prosthetic group, followed by a 120° rotation around the bond, to achieve the postulated axial antiparallel orientations.[8]

Following our previous work dealing with the synthesis of new inhibitors of SO cyclase,[16–20] we here report a study of whether substitution of the terminal isoprenic unit of SO with an isopentyl vinyl ether produces an irreversible 'suicide' inhibitor of SO cyclase of rat liver. If such an inhibitor is produced, the squalenoid epoxide vinyl ether (**5**) would give, after enzymatic cyclization, a C_{20} ion (**6**), which could be stabilized through formation of an oxenium ion. This latter could interact with the postulated nucleophilic group of the enzyme,[6] yielding a stable covalent bond (**7**) (Scheme 2).

For the synthesis of *E* and *Z* vinyl ethers we developed a new procedure for the epoxidation and cleavage of functionalized squalenoid substrates. In addition, we applied for the first time the Horner-Warren variant of the Wittig reaction for the synthesis of vinyl ethers with an alkoxy group containing more carbon atoms than the methoxy group.

The preliminary biological results showed that (**5**) and (**27**) are competitive inhibitors of SO cyclase and not irreversible 'suicide' inhibitors.

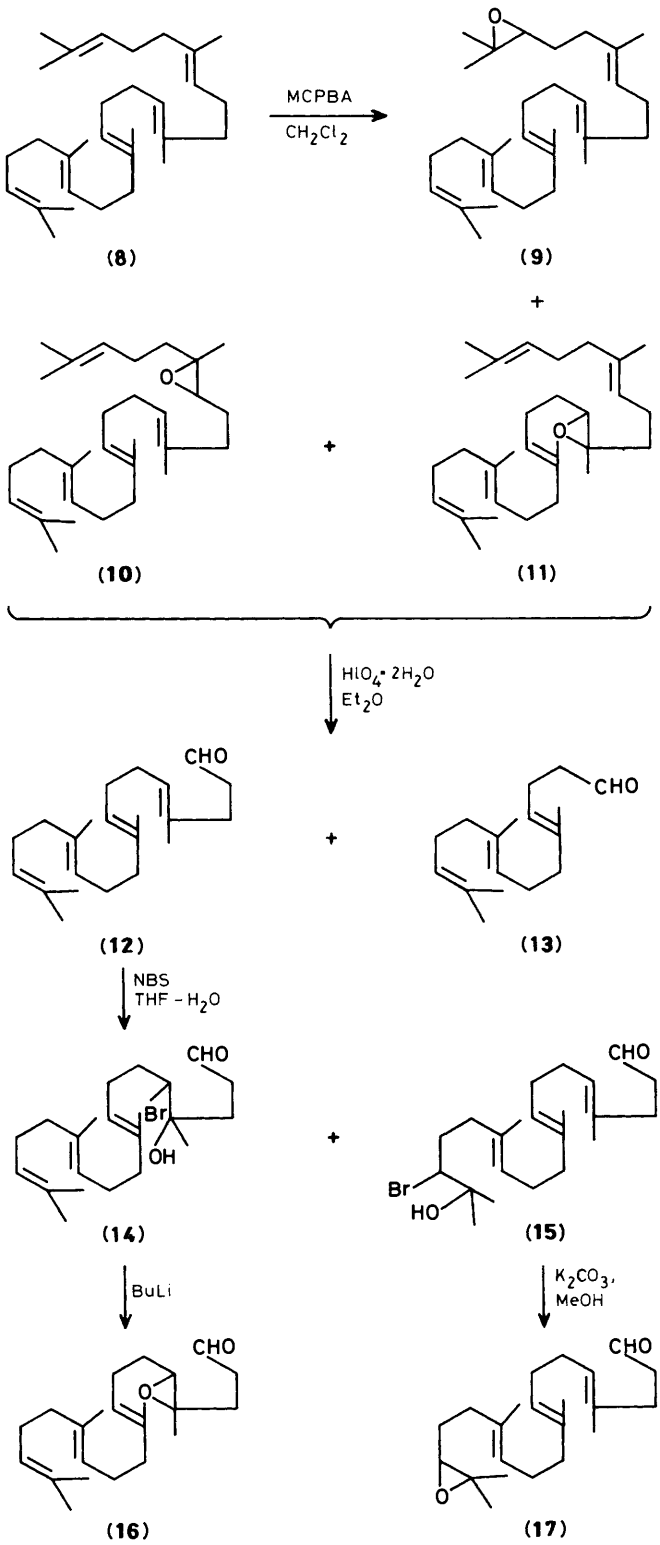
Results and Discussion

The overall strategy for the stereospecific synthesis of vinyl ethers (**5**) and (**27**) involved (*a*), the preparation of C_{22} squalenoid aldehyde bromohydrin (**15**) from squalene (**8**) and (*b*), the reconstruction of the terminal chain bearing an oxygen atom at C_{23}, associated with the closure of the oxirane ring through a Wittig-Horner reaction between (**15**) and (1-isopentyloxyethyl)diphenylphosphine oxide (**24**).

Synthesis of the C_{22} *Squalenoid Aldehyde Bromohydrin* (**15**).—The C_{22} squalenoid aldehyde (**12**) was initially prepared following a known procedure[21–23] by controlled ozonization of squalene (**8**). In our hands, this method led to unsatisfactory results (overall yields = 0.1%), due to the complex experimental procedure needed for the separation of the carbonylic fragments. An alternative approach was based on epoxidation of squalene (**8**)[24] with *m*-chloroperbenzoic acid (MCPBA), separation of the two internal *trans* epoxides from the external epoxide by flash chromatography, treatment with aqueous $HClO_4$, and finally cleavage of the corresponding diols with $NaIO_4$.[25] Since the method involved low-yield multistep reactions, we developed a new procedure for a one-step cleavage of epoxides to aldehydes, by using HIO_4 in diethyl ether (Scheme 3).

Since the yields are very high, this procedure provides a new way for the direct oxidation of lipophilic polyene epoxides to aldehydes.[26]

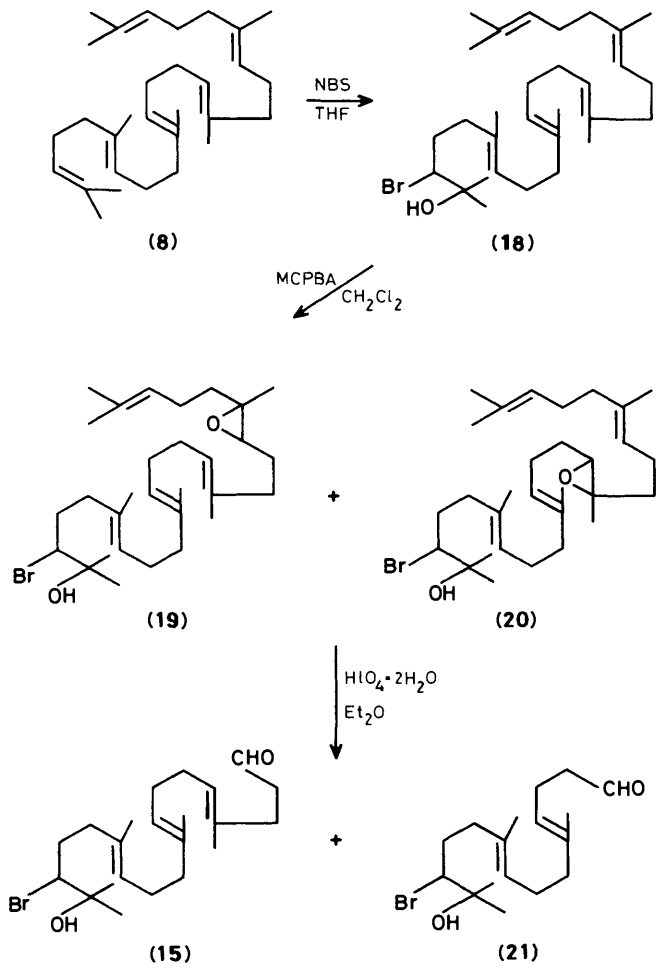
The C_{22} squalenoid aldehyde (**12**) was treated with *N*-bromosuccinimide (NBS) in aqueous tetrahydrofuran, as described by van Tamelen,[27–29] in order to allow the regiospecific formation of the terminal bromohydrin (**15**) (Method A). The reaction gave a mixture of products. The aldehydic signal was absent in the ^1H n.m.r. spectrum of the main product, as a result of hemiacetal formation. Since the bromohydrin group failed to close to epoxide using K_2CO_3, we employed butyl-lithium (BuLi). The epoxide proved, on the basis of ^1H n.m.r. evidence, to be internal and, in consequence, the bromohydrin was near the aldehydic group (**14**). The desired C_{22} aldehyde external bromohydrin (**15**) was a secondary product; the structure was confirmed by derivatization of the bromohydrinic group to epoxide, in the presence of K_2CO_3. Because of the above results, we decided to improve our



Scheme 3.

synthesis of the C_{22} squalenoid aldehyde bromohydrin (**15**) by epoxidation of squalene monobromohydrin (**18**) followed by cleavage of the internal monoepoxides (**19**) and (**20**) with $HIO_4 \cdot 2H_2O$ (Method B) (Scheme 4).

Thus, treatment of squalene monobromohydrin (**18**) with MCPBA in CH_2Cl_2 led, after purification, to a mixture of



Scheme 4.

epoxides (see Experimental section), which were treated with $HIO_4 \cdot 2H_2O$ in diethyl ether to give the expected C_{22} and C_{17} aldehyde bromohydrins (**15**) and (**21**).

Stereospecific Synthesis of Squalenoid Epoxide Vinyl Ethers.—The Wittig reaction has been used to prepare vinyl ethers by using the corresponding alkyl ylides.[30–33] Nevertheless, the ylide involved appeared very unstable, since the oxygen atom may further destabilize the anion of the adjacent atom.[31,34,35] In addition, poor yields of vinyl ethers are often obtained, particularly with enolizable aldehydes and ketones, and it is difficult to separate the vinyl ethers from triphenylphosphine oxide.[31,36] In our studies we required the stereospecific synthesis of both *E* and *Z* squalenoid epoxide vinyl ethers (**5**) and (**27**), which is very difficult to achieve through use of triphenylphosphorane reagents.

The Horner-Warren variant of the Wittig reaction, using diphenylphosphinoyl as an anion-stabilizing group and lithium di-isopropylamide (LDA) as base, largely solved these problems, since it permits the isolation and separation of the intermediates and the subsequent elimination gives the pure geometrical isomers of alkenes.[37–46]

This reaction has been applied to the synthesis of trisubstituted vinyl ethers.[42,44] We have now observed that this method could be extended to the synthesis of complex vinyl ethers, such as the isopentyloxy derivatives *E* or *Z* (**5**) and (**27**) (Scheme 5).

Thus, by treating 1-(1-chloroethoxy)-3-methylbutane (**22**), prepared from isopentyl alcohol and acetaldehyde by Henry's method,[47,48] with triphenylphosphine, the corresponding triphenylphosphonium salt[49] (**23**) was obtained. Careful alkaline hydrolysis[50] gave the desired (1-isopentyloxyethyl)-diphenylphosphine oxide (**24**) in acceptable yield. By treating the C_{22} squalenoid aldehyde bromohydrin (**15**) with phosphine oxide (**24**) at −78 °C in THF, in the presence of LDA,[51] the diastereoisomeric epoxy alcohols (**25**) and (**26**) were obtained. In addition to the expected Horner condensation, the concomitant closure of the bromohydrin to epoxide was also obtained. After separation, (**25**) and (**26**) were treated with NaH in THF giving the pure geometrical isomers of the vinyl ethers *Z*-(**27**) and *E*-(**5**) respectively.

X-Ray analysis of (1-isopentyloxyethyl)diphenylphosphine oxide (**24**) was performed and the structure is shown in Figure 1. Bond distances and angles do not show significant deviations from standard values.

The isopentyloxy terminal is folded allowing one of the two terminal methyl groups to point towards one of the phenyl rings. The only relevant intermolecular contact is that between the phosphine oxygen of one molecule and the more acidic hydrogen of the CH group between the two heteroatoms of another molecule in the unit cell [H · · · O = 2.37(2) Å]. A full report on this and other related structures, now being investigated, will be published elsewhere.[52]

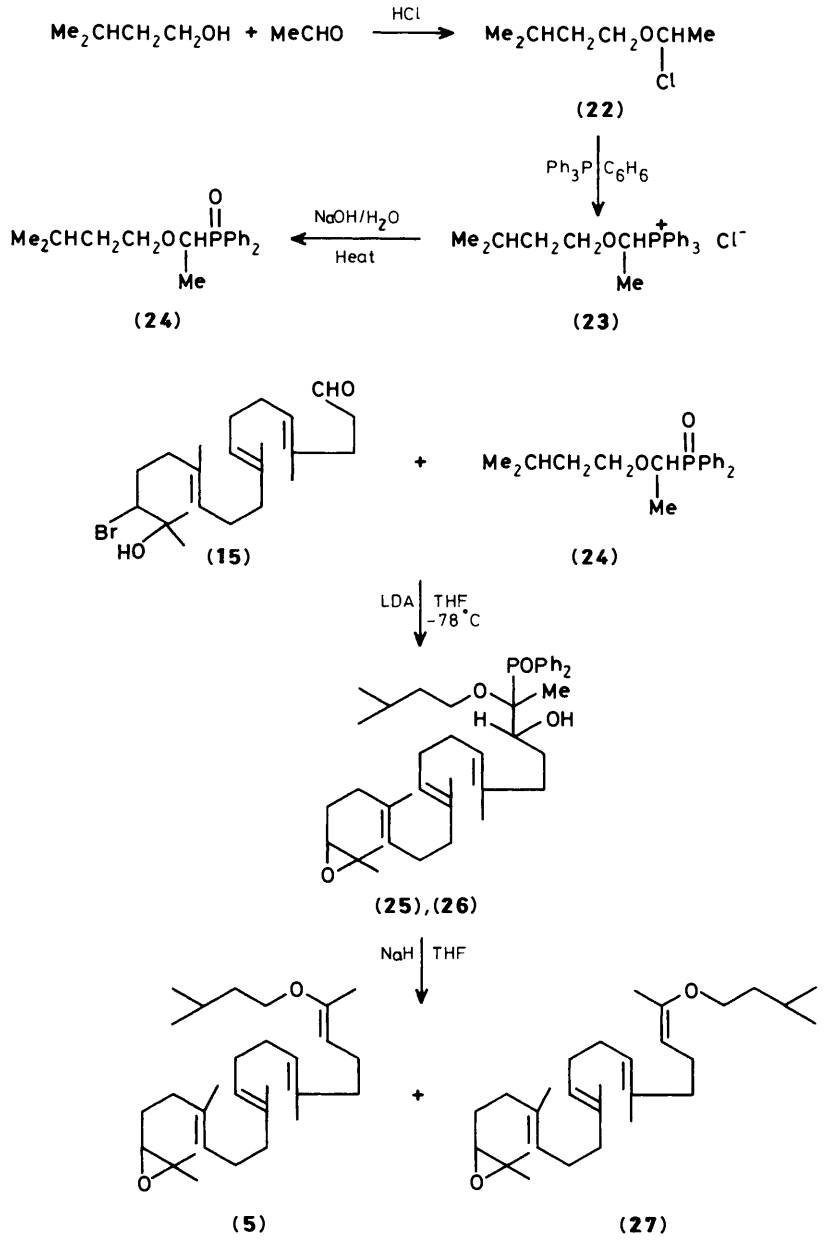
The structures of the vinyl ethers were assigned on the basis of their ^1H and ^{13}C n.m.r. spectra. The ^1H n.m.r. spectrum of the two isomers (**5**) and (**27**), showing approximatively the same features (see Experimental section), is not of diagnostic value in determining the *E* and *Z* configuration. The method of choice for determination of the configuration of α,β-disubstituted alkyl vinyl ethers is provided by ^{13}C n.m.r. spectroscopy,[53] since the configurational assignment by ^1H n.m.r. was reported as complex and contradictory.[44,54–57] According to the literature,[53] the signal of β-C of the *Z* isomer is always found in a significant lower field than that of the *E* isomer, the difference being 11—15 p.p.m.

This increase in shielding has been attributed to the reduced conjugation in the vinyloxy system of the *Z* isomer, which makes the resonance effect less favourable.[53,58,59] In our case, we assigned the *Z* configuration to the isomer derived from the alcohol (**25**) and the *E* configuration to the derivative of the alcohol (**26**), according to the difference in the ^{13}C n.m.r. signals of C-β: $\delta(C\text{-}\beta)^Z - \delta(C\text{-}\beta)^E = 12.4$ p.p.m. The signals of the methyleneoxy group were also in agreement with the assigned configuration, the difference $\delta(CH_2O)^Z - \delta(CH_2O)^E$ being 1.5 p.p.m.[53]

The ^{13}C n.m.r. spectra of (**5**) and (**27**) also supported the squalenoid carrier assignment,[60] it being taken into account that the Wittig-Horner reaction did not modify the geometry of the double bond system.[61] It is well known that *Z* and *E* isomers are derived by stereospecific elimination from *erythro* and *threo* alcohols.[44,62–64] Consequently, we were able to assign the *erythro* configuration (*RS,RS*) to the alcohol (**25**), which ran faster on t.l.c. (HR_F isomer) and produced the *Z* vinyl ether (**27**) and the *threo* configuration (*RS,SR*) to the alcohol (**26**), which ran slower on t.l.c. (LR_F isomer) and gave rise to the *E* vinyl ether (**5**).

Biological and Kinetic Results.—The I_{50} values (inhibitor concentration required to reduce reaction rate by half) of the two vinyl ethers (**5**) and (**27**) were determined on the SO cyclase in microsomes of rat liver. Both (**5**) and (**27**) were inhibitors of SO cyclase, the I_{50} values being 80 and 120 μM respectively. Furthermore, inhibition proved competitive with respect to SO (Figures 2 and 3).

All the correlation coefficients for the lines fitted in both Dixon and Cornish-Bowden plots were significant ($p < 0.005$).



Scheme 5.

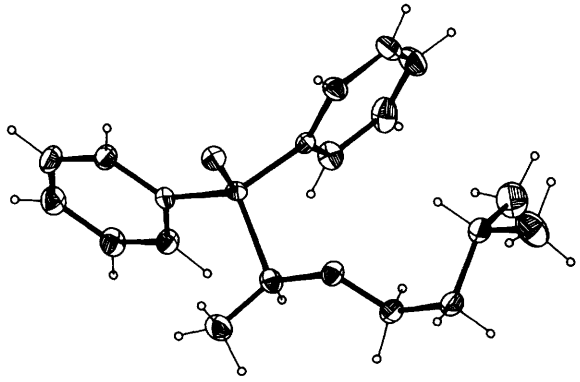
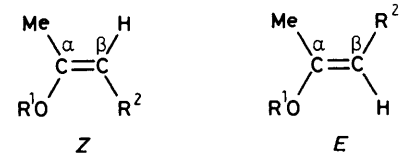


Figure 1. Drawing of the molecular structure of (1-isopentyloxyethyl)-diphenylphosphine oxide (**24**), as determined by *X*-ray analysis



The test for parallelism gave highly significant results ($p < 0.01$). K_i Values, calculated from Dixon analysis [40 and 60 μM for (**5**) and (**27**) respectively], in the presence of increasing concentrations of SO, were similar to K_m values (30 ± 7 μM), indicating that affinity of the inhibitors for the enzyme is of the same order as that of the substrate. Similar results were obtained on the SO-β-amyrin synthetase of *Pisum sativum*. In this system the I_{50} of compounds (**5**) and (**27**) was 300 μM and the K_i = 250 μM, compared with a K_m = 250 μM.

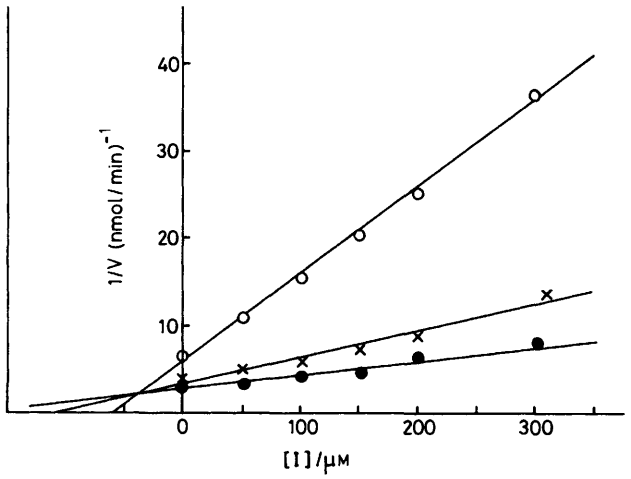


Figure 2. Dixon analysis of inhibition by compound (**5**) of rat liver SO cyclase in the presence of increasing concentration of substrate. SO cyclase activity was determined in the presence of various concentrations of (**5**), with 10 μM (○), 30 μM (×), and 80 μM (●) SO, as substrate. The points are the average of two replicate experiments. [I] = Concentration of inhibitor (**5**)

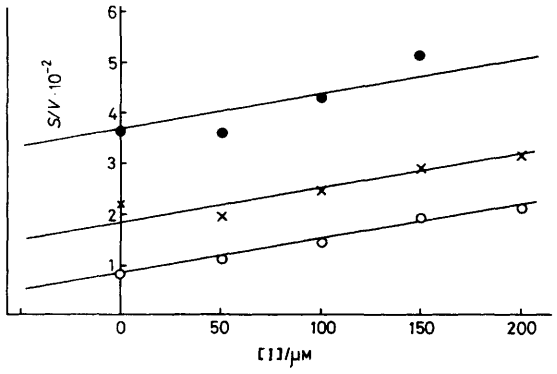


Figure 3. Cornish-Bowden analysis of inhibition by compound (**5**) of rat liver SO cyclase in the presence of increasing concentrations of (**5**), with 10 μM (○), 40 μM (×), and 80 μM (●) SO, as substrate. The points are the average of two replicate experiments. Rates are expressed as mmol of lanosterol/min. [I] = Concentration of inhibitor (**5**)

Experimental

^{1}H N.m.r. spectra were recorded either on a Jeol GX 270 or on a Varian T-60 spectrometer, with $SiMe_4$ as internal standard. ^{13}C N.m.r. spectra were recorded on a Jeol GX 270 instrument. Mass spectra were performed either on a Kratos MS 80 or on a VG Analytical 7070 EQ-HF spectrometer by electron impact: high resolution (6 000), electron energy 70 eV, trap current 100 μA, spring temperature 230 °C. I.r. spectra were recorded either on a Perkin-Elmer 267 or on a Perkin-Elmer 781 instrument. Microanalyses were performed on an Elemental Analyser 1106 (Carlo Erba Strumentazione), except in the case of P, analysed according to the method of Schôniger.

For isolation, purification, and identification, the following techniques were used: (a) column 'flash chromatography'[65]: an air-pressure column chromatography which has been perfected for rapid separations. A column of appropriate diameter is selected and filled with 15—30 cm of the appropriate silica gel (230—400 mesh), the sample is introduced and the column is eluted at a high flow rate; (b) thin layer chromatography (t.l.c.): Merck silica gel 60 F_{254}, 0.2 mm coated plates, for analytical purposes. After development, the plates were exposed to iodine vapours.

Squalene Epoxides (*as a Mixture of the two* trans *Internal Monoepoxides*): (6E,10E,14E)-*trans*-18,19-*Epoxy*-2,6,10,15,19,23-*hexamethyltetracosa*-2,6,10,14,22-*pentaene* (**10**) *and* (6E,10E,18E)-trans-14,15-*Epoxy*-2,6,10,15,19,23-*hexamethyltetracosa*-2,6,10,18,22-*pentaene* (**11**).—A solution of squalene (**8**) (10 g, 24.3 mmol) dissolved in CH_2Cl_2 (250 ml) at 0 °C was stirred whilst 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 (Na_2SO_4), and evaporated to dryness to give a mixture of products. The resulting oil was purified by flash chromatography (light petroleum–diethyl ether, 95:5) to give a mixture of the two *trans* internal monoepoxides (**10**) and (**11**) (3.0 g, 29% yield) (lit.,[24]) and then the external monoepoxide (**9**) (1.5 g, 14% yield) (lit.,[66]), as colourless oils; (**10**) and (**11**) $\nu_{max.}$ (liq. film) 2 980, 2 910, 2 850, 1 450, 1 385, 1 250, 1 110, and 985 cm^{-1}; δ_H($CDCl_3$) 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), and 5.06—5.15 (m, 5 H, vinylic CH) (Found: M^+, 426.3867. $C_{30}H_{50}O$ requires M, 426.3861), 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), and 109 (100); (**9**) δ_H($CDCl_3$) 1.24 and 1.28 (two peaks, 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), 2.69 (t, 1 H, J 6.2 Hz, oxirane CH), and 5.06—5.17 (m, 5 H, vinylic CH).

C_{22} *Squalenoid Aldehyde* (4E,8E,12E)-4,9,13,17-*Tetramethyloctadeca*-4,8,12,16-*tetraenal* (**12**) *and* C_{17} *Squalenoid Aldehyde:* (4E,8E)-5,9,13-*Trimethyltetradeca*-4,8,12-*trienal* (**13**).—HIO_4·$2H_2O$ (1.5 equiv., 1.60 g, 7.04 mmol) was added to ether (250 ml) with vigorous stirring and, when dissolution was almost complete, the squalene epoxides (**10**) and (**11**) (2.0 g, 4.69 mmol) in ether (5 ml) were added. Stirring was continued for 15 min after which the reaction mixture was washed with saturated brine (100 ml × 3), dried (Na_2SO_4), and evaporated to dryness. The resulting oil was purified by flash chromatography with various eluants (light petroleum–CH_2Cl_2, 90:10, 80:20) to give a mixture of C_{22} and C_{17} aldehydes (**12**) and (**13**) (1.16 g). The mixture was separated by a 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 the C_{17} aldehyde (**13**) [472 mg, 40% yield from (**10**) + (**11**)] and (lit.,[23]) the C_{22} aldehyde (**12**) [610 mg, 41% yield from (**10**) + (**11**)]; (**12**) $\nu_{max.}$ (liq. film) 2 980, 2 910, 2 850, 1 730 (CO), 1 450, and 1 385; δ_H($CDCl_3$) 1.58—1.70 (m, 15 H, allylic Me), 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), and 9.70 (m, 1 H, CHO); (**13**) $\nu_{max.}$ (liq. film) 2 980, 2 910, 2 850, 1 725 (CO), 1 445, and 1 385 (Found: C, 82.05; H, 11.3. $C_{17}H_{28}O$ requires C, 82.20; H, 11.36%); δ_H($CDCl_3$) 1.60—1.71 (m, 12 H, allylic Me), 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), and 9.71 (m, 1 H, CHO).

C_{22} *Aldehyde 'Vicinal' Bromohydrin:* (8E,12E)-5-*Bromo*-4-*hydroxy*-4,9,13,17-*tetramethyloctadeca*-8,12,16-*trienal* (**14**) *and* C_{22} *Aldehyde External Bromohydrin:* (4E,8E,12E)-16-*Bromo*-17-*hydroxy*-4,9,13,17-*tetramethyloctadeca*-4,8,12-*trienal* (**15**): *Method A.*—The C_{22} squalenoid aldehyde (**12**) (500 mg, 1.58 mmol) was dissolved in THF (15 ml) and the solution cooled to 0 °C; it was then diluted with water until it became opalescent and then with a small amount of THF until it cleared again.

NBS (1.1 equiv., 308 mg, 1.73 mmol) was added over a period of 10 min, at 0 °C and the stirred mixture then left for 30 min at room temperature. The mixture was diluted with water (50 ml) and extracted with light petroleum (50 ml × 3). The combined organic layers were washed with saturated brine (150 ml × 2), dried (Na_2SO_4) and evaporated to dryness. The resulting oil was purified by a long flash chromatography (light petroleum–diethyl ether, 98:2; 95:5; 97:7; 90:10; 80:20) to give unchanged compound (**12**) (102 mg, 20% recovery), the C_{22} aldehyde internal bromohydrin (**14**) (188 mg, 29%) and the desired C_{22} aldehyde external bromohydrin (**15**) (25 mg, 4%) (lit.,[23]); (**14**) (Found: C, 64.0; H, 8.9. $C_{22}H_{37}BrO_2$ requires C, 63.91; H, 9.02%); $\delta_H(CDCl_3)$ 1.25 (s, 3 H, non-allylic CH_3), 1.56—1.70 [m, 16 H, allylic CH_3 and $C(CH_3)(OH)CH_2CH_2$], 1.89—2.23 (m, 12 H, allylic CH_2 and CH_2CHBr), 3.50 (m, 1 H, CHBr), and 4.98—5.22 [m, 4 H, vinylic CH and CHO(OH)]; (**15**) $\nu_{max.}$ (liq. film) 3 400—3 500, 2 960, 2 920, 2 860, 1 725 (CO), 1 450, 1 390, and 1 110 cm^{-1}; $\delta_H(CDCl_3)$ 1.28 [s, 6 H, $(CH_3)_2COH$], 1.48—1.62 (m, 9 H, allylic CH_3), 1.85—2.20 (m, 14 H, allylic CH_2 and CH_2CHBr), 2.35—2.40 (m, 2 H, CH_2CHO), 3.84 (m, 1 H, CHBr), 4.98—5.23 (m, 3 H, vinylic CH), and 9.78 (m, 1 H, CHO) (Found: M^+, 412.1962. $C_{22}H_{37}BrO_2$ requires M, 412.1977), m/z 414 (0.5), 412 (0.5), 332 (3), 316 (1), 247 (1), 153 (6), 135 (15), 111 (16), 93 (38), 81 (90), and 43 (100).

C_{22} *Aldehyde 'Vicinal' Epoxide:* (8E,12E)-4,5-*Epoxy*-4,9,13,17-*tetramethyloctadeca*-8,12,16-*trienal* (**16**).—C_{22} Aldehyde 'vicinal' bromohydrin (**14**) (50 mg, 0.12 mmol) was dissolved in anhydrous THF (10 ml) and BuLi (1.6M solution in hexane; 1 ml) added; the mixture was then stirred for 1 h at room temperature. The reaction mixture was diluted with water (50 ml), extracted with ether (50 ml × 3), and the combined extracts were dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by flash chromatography (light petroleum–diethyl ether, 95:5; 90:10; 85:15), to give compound (**16**) (31 mg, 77% yield) as a colourless oil (Found: C, 79.3; H, 10.7. $C_{22}H_{36}O_2$ requires C, 79.47; H, 10.91%); $\delta_H(CDCl_3)$ 1.26 (s, 3 H, oxirane CH_3), 1.58—1.70 (m, 16 H, allylic CH_3 and CH_2-oxirane-CH_2), 1.95—2.06 (m, 10 H, allylic CH_2), 2.42 (m, 2 H, CH_2CHO), 2.70 (t, 1 H, oxirane CH), 5.03—5.15 (m, 3 H, vinylic CH), and 9.75 (m, 1 H, CHO).

C_{22} *Aldehyde External Epoxide:* (4E,8E,12E)-16,17-*Epoxy*-4,9,13,17-*tetramethyloctadeca*-4,8,12-*trienal* (**17**).—K_2CO_3 (2 equiv., 13.4 mg, 0.097 mmol) was dissolved in methanol (5 ml), and the C_{22} aldehyde external bromohydrin (**15**) (20 mg, 0.048 mmol) was added. The mixture was stirred for 1 h at room temperature after which it was diluted with water (50 ml), extracted with ether (50 ml × 3), and the combined extracts were dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by flash chromatography (light petroleum–diethyl ether, 95:5; 90:10; 80:20), to give compound (**17**) (13.2 mg, 83%), as a colourless oil (Found: C, 79.45; H, 11.05. $C_{22}H_{36}O_2$ requires C, 79.47; H, 10.91%); $\delta_H(CDCl_3)$ 1.24 and 1.28 (two peaks, 6 H, oxirane CH_3), 1.59—1.70 (m, 11 H, m, allylic CH_3 and oxirane CH_2), 1.96—2.08 (m, 12 H, allylic CH_2), 2.40 (m, 2 H, CH_2CHO), 2.70 (t, 1 H, oxirane CH), 5.05—5.15 (m, 3 H, vinylic CH), and 9.73 (m, 1 H, CHO).

Squalene Monobromohydrin: (6E,10E,14E,18E)-3-*Bromo*-2,6,10,15,19,23-*hexamethyltetracosa*-6,10,14,18,22-*pentaen*-2-*ol* (**18**).—Squalene (**8**) (41.1 g, 0.10 mol) was dissolved in THF (250 ml) and the solution cooled to 0 °C. It was then diluted with water until the solution became opalescent followed by a small amount of THF until it cleared. NBS (1.2 equiv., 21.4 g, 0.12 mol) was added, over a period of 30 min, at 0 °C, and the stirred mixture then left for 1 h at room temperature. The product was extracted with light petroleum (150 ml × 3) and the combined organic layers were washed with saturated brine (150 ml × 2), dried (Na_2SO_4) and evaporated to dryness. The resulting oil was purified by flash chromatography (light petroleum to remove unchanged squalene, then light petroleum–diethyl ether, 95:5) to give (**18**) (17.8 g, 35%) (lit.,[66]), as a pale yellow oil; $\delta_H(CDCl_3)$ 1.34 [s, 6 H, $(CH_3)_2COH$], 1.61—1.65 (m, 20 H, allylic CH_3 and CH_2CHBr), 1.97—2.10 (m, 18 H, allylic CH_2), 3.92 (m, 1 H, CHBr), and 5.01—5.28 (m, 5 H, vinylic CH).

Squalene Bromohydrin Epoxides: (6E,10E,14E)-3-*Bromo*-trans-18,19-*epoxy*-2,6,10,15,19,23-*hexamethyltetracosa*-6,10,14,22-*tetraen*-2-*ol* (**19**) *and* (6E,10E,18E)-3-*Bromo*-trans-14,15-*epoxy*-2,6,10,15,19,23-*hexamethyltetracosa*-6,10,18,22-*tetraen*-2-*ol* (**20**).—Squalene monobromohydrin (**18**) (15 g, 29.5 mmol) was dissolved in CH_2Cl_2 (150 ml), at 0 °C, with stirring, and MCPBA (85% purity; 6.0 g, 29.5 mmol) was added over a period of 30 min. After a further 30 min the reaction mixture was washed with 20% aqueous $NaHCO_3$ (100 ml × 3) and saturated brine (100 ml × 2), dried (Na_2SO_4) and evaporated to dryness to give a mixture of products. The resulting oil was purified by flash chromatography (light petroleum–diethyl ether, 95:5, 90:10, 85:15, 80:20, 75:25) to give unchanged (**18**) followed by the desired product (**19**) and (**20**) together (3.1 g, 20% yield) as a pale yellow oil (Found: C, 68.65; H, 9.95. $C_{30}H_{51}BrO_2$ requires C, 68.81; H, 9.82%); $\nu_{max.}$ (liq. film) 3 400—3 500, 2 960, 2 920, 2 860, 1 450, 1 390, 1 250, and 1 110 cm^{-1}; $\delta_H(CDCl_3)$ 1.22 (s, 3 H, oxirane CH_3), 1.29 [s, 6 H, $(CH_3)_2COH$], 1.50—1.65 (m, 19 H, allylic CH_3 and CH_2-oxirane-CH_2), 1.95—2.20 (m, 16 H, allylic CH_2 and CH_2CHBr), 2.68 (t, 1 H, oxirane CH), 3.92 (m, 1 H, CHBr), and 4.98—5.25 (m, 4 H, vinylic CH).

C_{22} *Aldehyde External Bromohydrin:* (4E,8E,12E)-16-*Bromo*-17-*hydroxy*-4,9,13,17-*tetramethyloctadeca*-4,8,12-*trienal* (**15**) *and* C_{17} *Aldehyde External Bromohydrin:* (4E,8E)-12-*Bromo*-13-*hydroxy*-5,9,13-*trimethyltetradeca*-4,8-*dienal* (**21**): *Method B.*—$HIO_4 \cdot 2H_2O$ (1.5 equiv., 1.96 g, 8.6 mmol) was added to ether (250 ml), with vigorous stirring and, when dissolution was almost complete, the squalene bromohydrin epoxides (**19**) and (**20**) (3.0 g, 5.7 mmol) in ether (5 ml) were added. Stirring was continued for 15 min after which the reaction mixture was washed with saturated brine (100 ml × 3), dried (Na_2SO_4), and evaporated to dryness. The resulting oil was purified by flash chromatography (light petroleum–diethyl ether, 90:10; 85:15; 80:20) to give compound (**15**) (0.82 g, 35%) (lit.,[23]) and compound (**21**) (0.53 g, 27%) as colourless oils. Compound (**15**), spectroscopic data: see method A; compound (**21**) (Found: C, 59.0; H, 8.6. $C_{17}H_{29}BrO_2$ requires C, 59.13; H, 8.46%); $\nu_{max.}$ 3 400—3 500, 2 970, 2 920, 2 850, 1 725 (CO), 1 445, 1 385, and 1 115 cm^{-1}; $\delta_H(CDCl_3)$ 1.31 [s, 6 H, $(CH_3)_2COH$], 1.52—1.67 (m, 6 H, allylic CH_3), 1.98—2.23 (m, 10 H, allylic CH_2 and CH_2CHBr), 2.34—2.41 (m, 2 H, CH_2CHO), 3.91 (m, 1 H, CHBr), 5.05—5.23 (m, 2 H, vinylic CH), and 9.73 (m, 1 H, CHO) (Found: M^+, 344.1347. $C_{17}H_{29}BrO_2$ requires M, 344.1351); m/z 344 (3), 346 (3), 328 (3), 326 (3), 302 (1), 300 (1), 264 (5), 243 (4), 229 (2), 203 (1), 135 (32), 107 (15), 93 (35), and 81 (100).

1-(1-*Chloroethoxy*)-3-*methylbutane* (**22**).—Isopentyl alcohol (8.8 g, 0.1 mol) was cooled at −20 °C and the MeCHO (4.4 g, 0.1 mol) was added, with stirring. The mixture was then flushed for 5 h with HCl, at −20 °C to give two phases, the upper layer being the α-chloro ether. Anhydrous N_2 was flushed over the mixture for a period of 30 min to eliminate excess of HCl after which the system was dried ($CaCl_2$). The upper layer of the α-chloro ether was sufficiently pure (^1H n.m.r. analysis) to be used directly in the next step: $\delta_H(CDCl_3)$ 0.86 [(d, 6 H, $(CH_3)_2CH$)], 1.42—1.61 (m, 3 H, CH_2CH), 1.75 (d, 3 H, CH_3CHCl), 3.45—3.85 (m, 2 H, CH_2O), and 5.55—5.81 (q, 1 H, CHCl).

(1-*Isopentyloxyethyl*)*triphenylphosphonium Chloride* (**23**).—Triphenylphosphine (22.6 g, 86 mmol) was dissolved in anhydrous benzene. As the solution cleared, the crude α-chloro ether (**22**) (1.1 equiv., 14.6 g, 97 mmol) was added and the mixture left for 20 h at 50 °C with stirring. The solvent was evaporated and the crude product (**23**), a viscous oil, was used directly in the next step.

(1-*Isopentyloxyethyl*)*diphenylphosphine Oxide* (**24**).—The crude phosphonium salt (**23**) was added to 30% aqueous NaOH (50 ml) and the mixture was heated with removal of the benzene formed under reduced pressure (water pump). The reaction mixture became dark red and was evaporated to give a red oil, which was purified by flash chromatography (light petroleum–ethyl acetate, 90:10, to remove triphenylphosphine and then ethyl acetate); this gave compound (**24**) as a white crystalline solid (9.8 g, 31% from isopentyl alcohol), m.p. 102—103 °C (from ethyl acetate) (Found: C, 72.35; H, 8.0; P, 9.65. $C_{19}H_{25}O_2P$ requires C, 72.13; H, 7.96; P, 9.79%); $\nu_{max.}$(KBr) 3 070, 3 050, 3 015, 2 950, 2 940, 2 910, 2 860, 1 590, 1 480, 1 465, 1 440, 1 385, 1 370, 1 190, 1 120, 1 090, 990, 980, 725, and 700 cm^{-1}; $\delta_H(CDCl_3)$ 0.76 [two d, 6 H, $(CH_3)_2CH$], 1.28—1.53 [m, 6 H, $CH_2CH(CH_3)_2$ and $CHCH_3$], 3.17 and 3.57 (two m, 2 H, OCH_2), 4.13 (m, 1 H, OCH), 7.45 (m, 6 H, *meta* and *para* to P aromatic H), and 7.77—8.01 (m, 4 H, *ortho* to P aromatic H) (Found: M^+, 316.1605. $C_{19}H_{25}O_2P$ requires *M*, 316.1592); *m/z* 316 (12), 245 (12), 230 (15), 202 (100), 183 (10), 155 (8), 125 (9), 108 (14), and 90 (45).

Squalenoid Alcohol Diastereoisomers: (6E,10E,14E)-2-*Diphenylphosphinoyl*-18,19-*epoxy*-2-*isopentyloxy*-6,11,15,19-*tetramethylicosa*-6,10,14-*trien*-3-*ol* (**25**) *and* (**26**).—(1-Isopentyloxyethyl)diphenylphosphine oxide (**24**) (1.2 equiv., 918 mg, 2.9 mmol) was dissolved in anhydrous THF (10 ml), at 0 °C with stirring. LDA (2.4 equiv., 621 mg, 5.8 mmol) in anhydrous THF (10 ml) was added and the mixture stirred for 10 min during which time it became dark red. The mixture was cooled to −78 °C and the C_{22} aldehyde external bromohydrin (**15**) (1.0 g, 2.4 mmol) in anhydrous THF (10 ml) was added dropwise; the mixture was left for 10 min at −78 °C and then allowed to warm to room temperature. It was then poured into aqueous saturated NH_4Cl (50 ml)–diethyl ether (50 ml) and extracted with ether (50 ml × 3). The combined organic layers were washed with saturated brine (50 ml × 3), dried (Na_2SO_4), and evaporated to dryness. The crude oil was purified by flash chromatography (hexane–diethyl ether, 40:60; 30:70; 20:80; 10:90; pure diethyl ether; diethyl ether–ethyl acetate, 80:20) to separate the diastereoisomers. The first diastereoisomer to be eluted from the column (HR_F) was the (2*RS*,3*RS*)-adduct, *erythro*-(**25**) (575 mg, 37% yield) (Found: C, 75.65; H, 9.6; P, 4.95. $C_{41}H_{61}O_4P$ requires C, 75.89; H, 9.47; P, 4.77%); $\nu_{max.}$ (liq. film) 3 400—3 300, 2 960, 2 920, 2 860, 1 440, 1 390, 1 370, and 1 115 cm^{-1}; $\delta_H(CDCl_2)$ 0.91 [two d, 6 H, *J* 6.4 Hz, $(CH_3)_2CH$], 1.25 and 1.30 (two peaks, 6 H, oxirane CH_3), 1.39—1.67 [m, 19 H, CH_2CH_2O, $CH(CH_3)_2$, CH_2CHOH, oxirane-CH_2, CH_3CP and allylic CH_3], 1.95—2.16 (m, 12 H, allylic CH_2), 2.70 (t, 1 H, *J* 6.2 Hz, oxirane CH), 3.40 (t, 2 H, *J* 6.7 Hz, CH_2O), 3.73 (m, 1 H, CHOH), 4.90—5.19 (m, 3 H, vinylic CH), 7.47 (m, 6 H, *meta* and *para* to P aromatic H), and 7.93—8.22 (m, 4 H, *ortho* to P aromatic H) (Found: M^+, 648.4320. $C_{41}H_{61}O_4P$ requires *M*, 648.4307); *m/z* 648 (0.4), 559 (0.5), 496 (1), 495 (4), 446 (3), 429 (1), 428 (2), 427 (2), 359 (1), 341 (3), 316 (27), 245 (100), and 43 (81). The second diastereoisomer to be eluted from the column (LR_F) was the (2*RS*,3*SR*)-adduct, *threo*-(**26**) (607 mg, 39% yield) (Found: C, 75.85; H, 9.55; P, 4.9. $C_{41}H_{61}O_4P$ requires C, 75.89; H, 9.47; P, 4.77%); $\nu_{max.}$ (liq. film) 3 400—3 300, 2 960, 2 920, 2 860, 1 440, 1 390, 1 170, and 1 115 cm^{-1}; $\delta_H(CDCl_3)$ 0.88 [two d, 6 H, *J* 7.4 Hz, $(CH_3)_2CH$], 1.25 and 1.29 (two peaks, 6 H, oxirane CH_3), 1.40—1.68 [m, 19 H, CH_2CH_2O, $CH(CH_3)_2$, CH_2CHOH, oxirane-CH_2, CH_3CP and allylic CH_3], 1.92—2.25 (m, 12 H, allylic CH_2), 2.70 (t, 1 H, *J* 6.2 Hz, oxirane CH), 3.31 and 3.46 (two m, 2 H, CH_2O), 3.94 (t, 1 H, *J* 9.8 Hz, CHOH), 5.05—5.18 (m, 3 H, vinylic CH), 7.49 (m, 6 H, *meta* and *para* to P aromatic H), and 7.90—8.19 (m, 4 H, *ortho* to P aromatic H) (Found: M^+, 648.4320. $C_{41}H_{61}O_4P$ requires *M*, 648.4307); *m/z* 648 (0.9), 559 (1), 496 (1), 495 (3), 446 (3), 429 (1), 428 (1), 427 (2), 359 (2), 341 (3), 316 (31), 245 (88), and 43 (100).

Squalenoid Epoxide Vinyl Ether: (2E,6E,10E,14E)-18,19-*Epoxy*-2-*isopentyloxy*-6,11,15,19-*tetramethylicosa*-2,6,10,14-*tetraene* (**5**).—The squalenoid alcohol LR_F (**26**) (100 mg, 0.154 mmol) was dissolved in anhydrous THF (10 ml) and then NaH (50% suspension in oil, washed with pentane, × 4; 14.8 mg, 0.616 mmol) was added, with stirring. After 4 h, the reaction mixture was filtered to remove sodium diphenylphosphinite; the residue was washed with ether and the combined organic fractions were evaporated to dryness, at 30 °C. The crude oil was purified by flash chromatography (light petroleum–isopropylamine, 99.5:0.5) on silica gel previously deactivated by elution with light petroleum–isopropylamine (99:1) to basicity, to give compound (**5**) (56 mg, 85%), as a colourless oil (Found: C, 80.7; H, 11.5. $C_{29}H_{50}O_2$ requires C, 80.87; H, 11.70%); $\nu_{max.}$ (liq. film) 2 960, 2 920, 2 865, 1 665, 1 450, 1 380, 1 320, 1 230, 1 160 and 1 080 cm^{-1}; $\delta_H(CDCl_3)$ 0.92 [d, 6 H, *J* 6.5 Hz, $(CH_3)_2CH$], 1.26 and 1.30 (two peaks, 6 H, oxirane CH_3), 1.43—1.73 [m, 14 H, allylic CH_3, CH_2CH_2O, oxirane-CH_2 and $(CH_3)_2CH$], 1.76 [s, 3 H, CH=C(CH_3)(OR)], 1.97—2.17 (m, 14 H, allylic CH_2), 2.70 (t, 1 H, *J* 6.3 Hz, oxirane CH), 3.60 (t, 2 H, *J* 6.7 Hz, CH_2O), 4.33 [t, 1 H, *J* 6.4 Hz, CH=C(CH_3)(OR)], and 5.08—5.21 (m, 3 H, vinylic CH); $\delta_C(CDCl_3)$ 15.88, 16.11, 18.61 (q), 22.50, 24.74, 25.09, 25.65, 26.54, 27.38, 28.14, 29.20, 36.20 (q), 37.92 (q), 39.54 (q), 40.74 (q), 58.04 (s), 63.98 (d), 64.61 (t, CH_2O), 96.57 (d, C=CO), 124.27, 124.35, 124.80, 133.80, 134.74, 134.79, and 152.29 (s, C=CO) (Found: M^+, 430.3805. $C_{29}H_{50}O_2$ requires *M*, 430.3811), *m/z* 430 (15), 342 (3), 277 (7), 189 (14), 153 (12), 149 (9), 142 (86), 141 (100), 135 (26), and 121 (35).

Squalenoid Epoxide Vinyl Ether: (2Z,6E,10E,14E)-18,19-*Epoxy*-2-*isopentyloxy*-6,11,15,19-*tetramethylicosa*-2,6,10,14-*tetraene* (**27**).—This compound was prepared and purified in the same way as (**5**), starting from the squalenoid alcohol HR_F (**25**) and obtained in 88% yield, as a colourless oil (Found: C, 80.7; H, 11.6. $C_{29}H_{50}O_2$ requires C, 80.87; H, 11.70%); $\nu_{max.}$ (liq. film) 2 960, 2 920, 2 865, 1 665, 1 450, 1 380, 1 320, 1 250, and 1 070 cm^{-1}; $\delta_H(CDCl_3)$ 0.92 [d, 2 H, *J* 6.4 Hz, $(CH_3)_2CH$], 1.25 and 1.29 (two peaks, 6 H, oxirane CH_3), 1.43—1.72 [m, 14 H, allylic CH_3, CH_2CH_2O, oxirane-CH_2 and $(CH_3)_2CH$], 1.79 [s, 3 H, CH=C(CH_3)(OR)], 1.98—2.17 (m, 14 H, allylic CH_2), 2.69 (t, 1 H, *J* 6.4 Hz, oxirane CH), 3.71 (t, 2 H, *J* 6.5 Hz, CH_2O), 4.41 [t, 1 H, *J* 6.4 Hz, CH=C(CH_3)(OR)], and 5.06—5.20 (m, 3 H, vinylic CH); $\delta_C(CDCl_3)$ 1.590 (q), 17.88 (q), 18.64 (q), 22.52, 23.39, 24.78, 24.85, 26.59, 27.41, 28.16, 28.19, 36.23 (t), 38.75, 39.57, 39.88, 52.12 (s), 64.04 (d), 66.10 (t, CH_2O), 108.94 (d, C=CO), 124.07, 124.36, 124.85, 133.83, 134.74, 135.08, and 149.92 (s, C=CO) (Found: M^+, 430.3795. $C_{29}H_{30}O_2$ requires *M*, 430.3811); *m/z* 430 (18), 342 (3), 277 (8), 189 (14), 153 (12), 149 (8), 142 (54), 141 (100), 135 (21), and 121 (26).

Biological Assays.—I_{50} and inhibition kinetics of SO cyclase were determined in microsomal preparations of rat liver. Male Wistar rats (250—300 g) were decapitated and the livers were perfused with cold 0.15M KCl and homogenized in 0.1M Na/K phosphate buffer (pH = 7.4), containing KCl 150 mM and EDTA 0.1 mM (1.5 ml × g of liver), in a loose-fitting (0.4 mm clearance) Teflon-glass potter. The homogenate was centrifuged for 10 min at 10 000 g. The supernatant was centrifuged for 1 h

at 100 000 g. Supernatant S_{105} was removed and stored at 0 °C in ice. The pellet was resuspended in phosphate buffer and centrifuged again (35 min at 100 000 g). The supernatant was removed and the pellet resuspended in buffer (10 ml/liver). Protein determination was carried out according to the method of Lowry *et al.*[67]

2,3-*Oxidosqualene–Lanosterol Cyclase Assay.*—The substrate, insoluble inhibitors, and Tween-80 were added to the test tubes as organic solutions and the solvent was evaporated under nitrogen. The products were then emulsified with 100 μl of Na/K phosphate buffer 0.1M (pH = 7.4). The reaction mixture contained in a volume of 1 ml: [3-^{3}H](*R*,*S*)-2,3-oxidosqualene (100 000 c.p.m., 0.5 nmol) diluted with (*R*,*S*)-2,3-oxidosqualene (final concentration = 50 μM), Tween-80 (final concentration = 0.05% w/v), 0.1M buffer pH 7.4, rat liver microsomal suspension (2 mg proteins) and supernatant fraction S_{105} (5 mg proteins). The reaction mixture was incubated for 60 min at 37 °C. A boiled enzyme preparation served as control. The reaction was stopped by addition of 1 ml of 10% KOH in methanol (w/v) and saponification for 30 min at 70 °C. The mixture was extracted with an equivalent volume of light petroleum (× 2). The combined extracts were evaporated to dryness under nitrogen and authentic lanosterol and SO in CH_2Cl_2 were added as carriers, before application of the extracts to silica t.l.c. T.l.c. plates were developed in cyclohexane–ethyl acetate (85 : 15) and sprayed with berberine to visualize the bands of squalene and lanosterol. The areas corresponding to SO and to lanosterol were scraped and counted for radioactivity in a Beckman LS-100 liquid scintillator. The nmol of lanosterol formed were calculated according to the formula: (nmol of SO incubated X counts in lanosterol band):(counts in lanosterol band + counts in SO band). Determinations of I_{50} (inhibitor concentration which reduces the observed reaction rate by 50%) were carried out under standard conditions in the presence of variable amounts of inhibitors. The amount of conversion was expressed as % of conversion obtained in absence of inhibitors. The values obtained are the average of at least 2 experiments. K_i Was determined graphically, under initial rate conditions (conversion ≤10%), using different substrate and inhibitor concentrations, according to the Dixon method.[68]

Cornish-Bowden plots were used to establish the inhibition pattern.[69] Linear regression analysis was used to estimate the fit of experimental data to theoretical straight lines in both Dixon and Cornish-Bowden plots. In the Cornish-Bowden plot, parallelism was tested by the method Snedecor and Cochran.[70]

Acknowledgements

We thank the researchers of the Laboratorio di Gascromatografia-Spettrometria di Massa della Provincia—Università di Torino and the researchers of the Dipartimento di Chimica Organica e Industriale—Università di Milano for the mass spectra; the researchers of the Istituto di Chimica Generale e Inorganica della Facoltà di Scienze dell'Università di Torino, for the 270 MHz n.m.r. spectra and CSI Computing Centre for free allowance of CPU time.

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Received 29*th January* 1987; *Paper* 7/152