Stereocontrolled synthesis of fluorosqualenes and fluoroepoxysqualenes as inhibitors of squalene epoxidase and 2,3-oxidosqualene cyclase

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Z-Fluorosqualene derivatives having one or more fluorine atoms at the terminal methyls of the squalene skeleton have been synthesized. A highly stereoselective synthesis, based on a Wittig reaction, was developed together with a new method for obtaining bifunctional derivatives of squalene. The compounds tested showed poor inhibitory activity on squalene epoxidase and 2,3-oxidosqualene cyclase from microsomes of *Saccharomyces cerevisiae*, *Candida albicans* and rat liver, and on *S. cerevisiae* and 3T3 fibroblast cell cultures.

Squalene epoxidase (EC 1.14.99.7) catalyses the stereospecific epoxidation of squalene to (3*S*)-2,3-oxidosqualene, the acyclic precursor of animal, fungal and plant sterols.[1] Little is known about this enzyme except that it is not cytochrome P450 dependent,[2,3] and it requires O_2, NADPH and FAD for activity. 2,3-Oxidosqualene cyclase (OSC) (EC 5.4.99.7) catalyses the conversion of (3*S*)-2,3-oxidosqualene (OS) into lanosterol in mammals and fungi,[4-7] while in algae and higher plants OS is cyclized into cycloartenol.

In recent years many reversible squalene epoxidase[5,8-16] and 2,3-oxidosqualene cyclase[5,7-11,17-23] inhibitors, often acting as antifungal or hypocholesterolemic agents, have been described. Bisnordifluorosqualene, having two fluorine atoms instead of the terminal two methyls of squalene has been shown to be a potent time-dependent inhibitor of rat liver squalene epoxidase.[24] *E*-Terminal monofluoromethyl- and difluoromethylsqualene are poor inhibitors of this enzyme, as well as of five fungal plant pathogens.[25,26]

On the basis of these different results, our aim was to better evaluate the structure–activity relationships, by preparing novel classes of fluorosqualenes and epoxyfluorosqualenes. In the present work we aimed to obtain *Z*-fluorosqualene derivatives to establish whether the poor activity was due to the *E* geometry.

In these compounds the electronic properties of the terminal double bond have been greatly changed, having a much lower electron density. These substances, especially the hexafluorosqualene derivatives, should inhibit squalene epoxidase if it acts by an electrophilic oxidative mechanism. The epoxy fluorosqualenes should be less susceptible to the nucleophilic cyclization process catalysed by OSC.

We have prepared terminal fluorine derivatives of squalene possessing a *Z* geometry. In order to diminish the electron density considerably, we obtained bis(trifluoromethyl)- and bis(monofluoromethyl)-squalenes that do not have geometrical isomers at the terminals of the squalene skeleton.

As it is well known that squalene and squalene epoxide are both good substrates for squalene epoxidase,[27] we have also synthesized bis(trifluoromethyl)oxidosqualene.

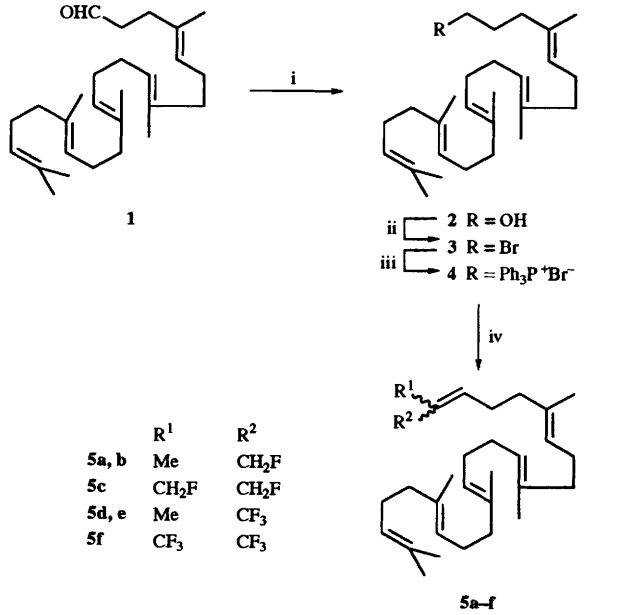
Finally, we aimed to develop methods to obtain terminal monofunctional and bifunctional fluorine derivatives of squalene and oxidosqualene, and in general squalene derivatives modified at the two ends of the molecule.

Results and discussion

Chemistry

E-Monofluoro- and *E*-difluorosqualene were obtained by a method that is *E* specific.[25,26] For our purposes, we needed to develop a method for obtaining *Z*-fluorosqualene derivatives,[28] also generally applicable to symmetrically substituted fluorosqualene derivatives.

The aldehyde **1** was obtained according to a method developed by us involving initial formation of the terminal epoxide, followed by treatment with periodic acid in diethyl ether.[19,20] The aldehyde **1** was then reduced with sodium boranuide ($NaBH_4$) in methanol to give the alcohol **2** (Scheme 1). The alcohol **2** was converted into the bromide **3** with tetrabromomethane and triphenylphosphine in dry diethyl ether, which was treated with triphenylphosphine in dimethylformamide to give the triphenylphosphonium bromide

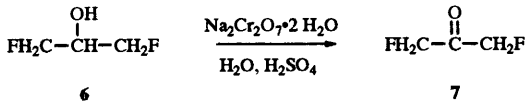


	R^1	R^2
5a, b	Me	CH_2F
5c	CH_2F	CH_2F
5d, e	Me	CF_3
5f	CF_3	CF_3

Scheme 1 Synthesis of fluorosqualenes. *Reagents:* i, $NaBH_4$, MeOH; ii, CBr_4, Ph_3P, Et_2O; iii, Ph_3P, DMF; iv, $R^1R^2C{=}O$, MeLi or BuLi, Et_2O or THF

4. Hydrolysis of **4** with aqueous NaOH afforded the corresponding diphenylphosphine oxide, which was treated with a suitable fluoro ketone under Wittig–Horner reaction conditions,[20] but this reaction did not give the expected fluorosqualenes.

We therefore turned our attention to the Wittig reaction in which the squalenoid phosphonium salts and fluoro ketones were not reactive. After experimenting with various conditions and bases, the ylide was prepared with methyllithium in diethyl ether or butyllithium in THF and then treated at −80 °C with the required fluoro ketone. These Wittig reactions show high stereoselectivity, as asymmetric fluorosqualenes were obtained with *Z*/*E* ratios 93 : 7–91 : 9, as determined by ^{19}F and ^{1}H NMR analysis (Schemes 1 and 2). The high *Z* stereoselectivity may be



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Scheme 2 Synthesis of 1,3-difluoroacetone

due to the fact that the ylide gives the *Z* isomer preferentially by kinetic control.

The second part of the work concerned the synthesis of epoxyfluorosqualenes. For the first time, a general method for obtaining squalenoid derivatives bifunctionalized at the two ends of the squalene moiety was developed.

Since squalene adopts a coiled conformation in polar media,[19,20,29] selective bromohydrin formation at one terminal of squalene is usually achieved in aqueous tetrahydrofuran. With squalene derivatives, the added polar head, together with the molecular asymmetry radically altered the coiling, so that the internal double bonds also became reactive towards aqueous *N*-bromosuccinimide (NBS). As an example, reaction of a C_{27}- or C_{22}-squalenoid aldehyde with NBS in aqueous THF gave mainly the internal bromohydrin adjacent to the aldehyde group.[20] Similar reactions with 2-aza-2,3-dihydrosqualene or its *N*-oxide gave a mixture of products, mainly internal derivatives.

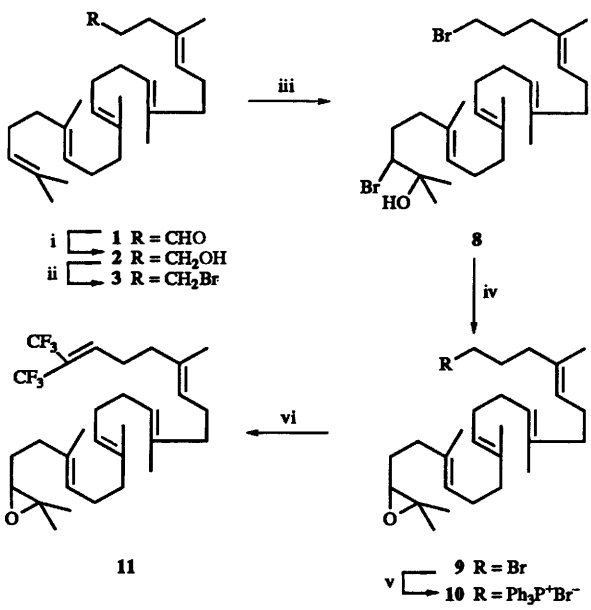
We found that the bromide **3** can be selectively transformed to the terminal bromohydrin **8**, by reaction with NBS in aqueous THF at 0 °C (Scheme 3). The yields can be improved by slowly adding NBS to a slightly opalescent solution of **3** in aqueous THF. During the first additions of NBS, the mixture became clear, due to the formation of the more polar bromohydrin **8**. So, a few drops of water were added following each addition of NBS to maintain a light opalescent mixture. In this way, only traces of internal derivatives were formed.

The bromohydrin **8** was converted into the epoxide **9** with K_2CO_3 in methanol and treated with triphenylphosphine in DMF to give the triphenylphosphonium bromide **10**. The ylide of **10**, prepared at 0 °C with BuLi in diethyl ether was flushed at −80 °C with hexafluoroacetone until the orange colour had disappeared, affording the hexafluorosqualene epoxide **11**.

Biological activity

The fluorosqualenes **5a–f** and hexafluorosqualene epoxide **11** were tested for inhibitory activity on squalene epoxidase and 2,3-oxidosqualene cyclase on microsomes from *Saccharomyces cerevisiae*, *Candida albicans* and rat and pig liver.

The compounds tested were poorly active: up to 150 μmol dm^{-3}, only the hexafluorosqualene epoxide **11** was a modest inhibitor of OSC from *S. cerevisiae* and *C. albicans* (IC_{50} = 150 μmol dm^{-3}). Moreover, they did not reduce the radioactivity accumulated in the C_{27} sterol fraction after incubation of [2-



Scheme 3 Synthesis of hexafluorosqualene epoxide. *Reagents:* i, $NaBH_4$, MeOH; ii, CBr_4, Ph_3P, Et_2O; iii, NBS, THF/H_2O; iv, K_2CO_3, MeOH; v, Ph_3P, DMF; vi, $F_3CC(O)CF_3$, MeLi or BuLi, Et_2O or THF

^{14}C]acetate either in *S. cerevisiae* or 3T3 fibroblast cell cultures, up to 50 μmol dm^{-3}.

Time-dependent inactivation experiments on OSC solubilized from *S. cerevisiae* were performed by pre-incubating with the hexafluorosqualene **7f** (0.5 and 5 mmol dm^{-3}) and the hexafluorosqualene epoxide **11** (0.5 and 5 mmol dm^{-3}), following a 10-fold dilution of the enzyme preparation. The results showed lack of irreversibility at the concentrations tested.

We do not believe, in contrast to an earlier hypothesis,[26] that the low biological activity of the fluorosqualene derivatives is due to a lack of penetration of these lipophilic derivatives to the active site of the enzyme. In fact, we synthesized similar lipophilic acetylenic and allenic squalenoid derivatives which possessed good squalene epoxidase activity.[12] It is possible that only the fluorine directly attached to the double bond can strongly influence the electron density of the terminal squalenic double bond.[24] It is also clear that squalene epoxidase or OSC does not act through a mechanism similar to that postulated for 5-fluorouracil and derivatives for thymidylate synthase.[30]

Experimental

^{1}H NMR spectra were recorded either on a Jeol EX-400, a Bruker AM 360 (also used for ^{19}F NMR spectra at 338.8 MHz), a Bruker AC 200 or a Jeol JNM-PMX 60 instrument in $CDCl_3$ solution at room temperature, with $SiMe_4$ as internal standard. Mass spectra were obtained on a VG Analytical 7070 EQ-HF or a VG ZAB 2F spectrometer, by electron impact or chemical ionization. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. Microanalyses were performed on an Elemental Analyser 1106 (Carlo Erba Strumentazione), except in the case of P, which was analysed according to the method of Schôniger.

The reactions were monitored by TLC on F_{254} silica gel precoated sheets (Merck); after development, the sheets were exposed to iodine vapour. Flash column chromatography was performed on 230–400 mesh silica gel (Merck). Light petroleum refers to the fraction boiling in the range 40–60 °C. THF was dried over sodium benzophenone ketyl, diethyl ether was dried over $LiAlH_4$.

(4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-Pentamethyldocosa-4,8,12,16,20-pentaen-1-ol 2

To a solution of the aldehyde **1** (10 g, 26 mmol) in methanol (200 cm^3) was added $NaBH_4$ (1.47 g, 39 mmol) and the mixture was stirred for 1 h. It was then evaporated to a volume of 50 cm^3, water (100 cm^3) was added, and the aqueous layer extracted with diethyl ether (80 cm^3 × 3). The dried combined organic layers were dried (Na_2SO_4) and evaporated and the residue was flash chromatographed eluting with light petroleum–diethyl ether (90:10) to give the title alcohol **2** (8.34 g, 83%) as a colourless oil,[13] $\delta_H(CDCl_3)$ 1.58–1.72 (m, 20 H, allylic CH_3 and CH_2CH_2OH), 1.96–2.10 (m, 18 H, allylic CH_2), 3.65 (t, 2 H, CH_2OH) and 5.01–5.17 (m, 5 H, vinylic CH); ν_{max}(thin film)/cm^{-1} 3330, 2925 and 1665.

(6*E*,10*E*,14*E*,18*E*)-22-Bromo-2,6,10,15,19-pentamethyldocosa-2,6,10,14,18-pentaene 3

The alcohol **2** (8 g, 20.69 mmol) and CBr_4 (13.72 g, 41.38 mmol) were dissolved in dry diethyl ether (200 cm^3) with stirring at 30 °C. Triphenylphosphine (10.85 g, 41.38 mmol) in ether (50 cm^3) was then added slowly and stirring was continued for 12 h at 30 °C, during which time a white solid formed. The precipitate was filtered off and the solution evaporated to give a viscous oil that was taken up in methanol–water (90:10, 100 cm^3) and light petroleum (100 cm^3). The layers were separated and the aqueous phase was then extracted with light petroleum (80 cm^3 × 2), the combined organic phases were washed with methanol–water; (90:10; 50 cm^3 × 2), dried (Na_2SO_4) and evaporated. The residue was flash chromatographed eluting with hexane to give the title bromide **3** (8.74 g, 94%) as a light yellow oil, $\delta_H(CDCl_3)$ 1.54–1.70 (m, 20 H, allylic CH_3 and CH_2CH_2Br), 1.97–2.16 (m, 18 H, allylic CH_2), 3.35 (t, 2 H, CH_2Br) and 5.02–5.24 (m, 5 H, vinylic CH); ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1440 and 1380; *m/z* (EI) 450 (3%), 448 (3), 407 (2), 405 (2), 381 (5), 379 (5), 368 (1), 366 (1), 339 (2), 337 (2), 311 (2), 299 (1), 273 (3), 243 (7), 177 (25), 175 (28), 137 (40) and 81 (100) (Found: C, 72.2; H, 10.1; Br, 17.7%; 448.2709. $C_{27}H_{45}Br$ requires C, 72.14; H, 10.09; Br, 17.77%; *M*, 448.2705).

(4*E*,8*E*,12*E*,16*E*)-(4,8,13,17,21-Pentamethyldocosa-4,8,12,16,20-pentaenyl)triphenylphosphonium bromide 4

The bromide **3** (8.0 g, 17.79 mmol) and triphenylphosphine (14 g, 53.37 mmol) were dissolved in dry DMF (200 cm^3) and the mixture was stirred for 3 d at 80 °C, when it was extracted with dichloromethane (80 cm^3 × 3), dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The crude product was flash chromatographed eluting with ethyl acetate to remove triphenylphosphine and impurities, then ethyl acetate–methanol (80:20) to give the triphenylphosphonium bromide **4** (5.2 g, 41%) as a viscous oil, $\delta_H(CDCl_3)$ 1.55–1.71 (m, 20 H, allylic CH_3 and CH_2CH_2P), 1.92–2.12 (m, 18 H, allylic CH_2), 3.67 (br t, 2 H, CH_2P), 5.04–5.15 (m, 5 H, vinylic CH) and 7.71–7.89 (m, 15 H, ArH); *m/z* (FAB+) 631 (40%), 562 (4), 494 (4), 425 (4), 356 (10), 288 (30), 374 (60) and 261 (100) (Found: C, 75.9; H, 8.5; Br, 11.2; P, 4.3%; M^+, 710.3621. $C_{45}H_{60}BrP$ requires C, 75.93; H, 8.50; Br, 11.22; P, 4.35%; *M*, 710.3616).

1,3-Difluoroacetone 7

To a mixture of 1,3-difluoropropanol **6** (10 g, 104 mmol), $Na_2Cr_2O_7 \cdot 2H_2O$ (15.5 g, 52 mmol) and water (10 cm^3) aqueous H_2SO_4 (1:1; 20 cm^3) was added slowly with stirring and ice–water cooling. The mixture was stirred for 2 h at room temperature, during which time the colour had changed to dark brown. Water was then added to it and the colour turned to olive green. The aqueous mixture was distilled to give an azeotropic mixture (bp 97–98 °C) to which anhydrous $CaCl_2$ was added with cooling until the mixture became turbid with some undissolved $CaCl_2$. Then diethyl ether (10 cm^3) was added to it and the upper layer decanted; this operation was repeated 5 times. The ethereal solution was evaporated at 50 °C at atmospheric pressure and the residue distilled at the same pressure. The first fraction (bp = 75–90 °C) contained a mixture of the alcohol **6** and the ketone **7**; the main fraction (bp = 120–124 °C) contained the ketone **7** (3.61 g) with traces of the alcohol **6** (37% yield),[31] $\delta_H(CDCl_3)$ 4.80 and 5.55 (d, 4 H, CH_2F); *m/z* (EI) 94 (58%) and 61 (100) (Found M^+, 94.0232. $C_3H_4F_2O$ requires *M*, 94.0230).

General procedure for the preparation of the fluorosqualenes 5a–f

Method A. The triphenylphosphonium bromide **4** (200 mg, 0.28 mmol) in dry diethyl ether (10 cm^3) was cooled to −20 °C under a nitrogen atmosphere with stirring. Methyllithium (1.5 mol dm^{-3} solution in diethyl ether; 280 mm^3, 0.42 mmol) was slowly added to it, during which time an orange–red solution formed. After 30 min, the mixture was cooled to −80 °C and the appropriate fluoro ketone (0.42 mmol) was added. In the case of compound **5f**, hexafluoroacetone was slowly bubbled through the solution until the red colour had disappeared. The reaction mixture was then slowly cooled to −20 °C over a period of *ca.* 2.5 h, when it was poured into 100 cm^3 of an ice-cooled biphasic system (diethyl ether–water; 50:50), washed with brine (50 cm^3 × 2) and evaporated to dryness. The crude product was purified by flash chromatography eluting with hexane. When the purification was not complete, further purification by preparative TLC (PTLC) eluting with light petroleum–diethyl ether (99.8:0.2) afforded the pure fluorosqualene.

Method B. The triphenylphosphonium bromide **4** (200 mg, 0.28 mmol) in dry THF (10 cm^3) was cooled to −20 °C under a nitrogen atmosphere with stirring. Butyllithium (1.6 mol dm^{-3} solution in hexane; 350 mm^3, 0.56 mmol) was slowly added to it, during which time an orange–red solution formed. After 30 min this was cooled to −80 °C and the appropriate fluoro ketone (0.42 mmol) was added. In the case of compound **7f**, hexafluoroacetone was slowly bubbled through the solution until the red colour had disappeared. The reaction mixture was slowly warmed to 0 °C over a period of 3–4 h, when it was poured into 100 cm^3 of an ice-cooled biphasic system (light petroleum–20% aqueous NH_4Cl; 50:50), washed with water (50 cm^3 × 2) and evaporated to dryness. The crude product was purified by flash chromatography eluting with hexane. When the purification was not complete, further purification by PTLC eluting with light petroleum–diethyl ether (99.8:0.2) afforded the pure fluorosqualene.

(2*Z*,6*E*,10*E*,14*E*,18*E*)-1-Fluoro-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 5a and (2*E*,6*E*,10*E*,14*E*,18*E*)-1-fluoro-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 5b. Yield 31% using method B (*Z*:*E*, 93:7), $\delta_H(CDCl_3)$ 1.55–1.72 (m, 21 H, allylic CH_3), 1.98–2.20 (m, 20 H, allylic CH_2), 4.88 (*Z* isomer, d, CH_2F) and 4.73 (*E* isomer, d, CH_2F) (2 H in total), 5.04–5.16 (m, 5 H, vinylic CH), 5.39 (*Z* isomer, m, CH=$CMeCH_2F$) and 5.54 (*E* isomer, m, CH=$CMeCH_2F$) (1 H in total); $\delta_F(CDCl_3)$ −54.64 (*Z* isomer) and −48.55 (*E* isomer); ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1445 and 1380; *m/z* (CI) 429 (12%), 409 (9), 391 (10), 385 (3), 373 (2), 359 (7), 347 (3), 341 (3), 327 (2), 305 (2), 299 (2), 291 (2), 285 (2), 273 (3), 259 (3), 220 (30) and 81 (100) (Found: C, 84.1; H, 11.5; F, 4.4%; M^+, 428.3816. $C_{30}H_{49}F$ requires C, 84.05; H, 11.52; F, 4.43%; *M*, 428.3818).

(6*E*,10*E*,14*E*,18*E*)-1-Fluoro-2-fluoromethyl-6,10,15,19,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene 5c. Yield 28% using method B, $\delta_H(CDCl_3)$ 1.55–1.72 (m, 18 H, allylic CH_3), 1.98–2.15 (m, 20 H, allylic CH_2), 4.95 [m, 4 H, $C(CH_2F)_2$], 5.05–5.22 (m, 5 H, vinylic CH) and 5.85 [m, 1 H, CH=$C(CH_2F)_2$]; ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1445 and 1380; *m/z* (EI)

446 (3%), 431 (1), 404 (2), 377 (3), 254 (2), 241 (3), 173 (20) 81 (78) and 69 (100) (Found: C, 80.7; H, 10.8; F, 8.5%; M^+, 446.3720. $C_{30}H_{48}F_2$ requires C, 80.66; H, 10.83; F, 8.51%; *M*, 446.3724).

(2*Z*,6*E*,10*E*,14*E*,18*E*)-1,1,1-Trifluoro-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 5d and (2*E*,6*E*,10*E*,14*E*,18*E*)-1,1,1-trifluoro-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 5e. Yield 35% using method B (*Z*:*E*; 91:9), $\delta_H(CDCl_3)$ 1.53–1.80 (m, 21 H, allylic CH_3), 1.94–2.16 (m, 20 H, allylic CH_2), 5.02–5.15 (m, 5 H, vinylic CH), 5.60 (*Z* isomer, t, C*H*=CMeCF$_3$) and 6.00 (*E* isomer, t, C*H*=CMeCF$_3$) (1 H in total); ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1670, 1450 and 1380; *m*/*z* (EI) 464 (9%), 450 (3), 445 (2), 421 (4), 395 (6), 341 (5), 285 (6), 191 (20) and 81 (100) (Found: C, 77.6; H, 10.2; F, 12.3%; M^+, 464.3627. $C_{30}H_{47}F_3$ requires C, 77.54; H, 10.20; F, 12.27%; *M*, 464.3630).

(6*E*,10*E*,14*E*,18*E*)-1,1,1-Trifluoro-2-trifluoromethyl-6,10,15,19,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene 5f. Yield 36% using method B, $\delta_H(CDCl_3)$ 1.58–1.75 (m, 18 H, allylic CH_3), 1.95–2.20 (m, 20 H, allylic CH_2), 5.04–5.15 (m, 5 H, vinylic CH) and 5.74 [t, 1 H, CH=C(CF$_3$)$_2$]; ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1670, 1450 and 1380; *m*/*z* (CI) 519 (18%), 503 (2), 491 (2), 475 (2), 451 (6), 435 (4), 395 (12), 381 (10), 367 (8), 339 (10), 327 (8), 313 (12), 299 (10), 285 (12) and 137 (100) (Found: C, 69.5; H, 8.5; F, 21.9%; M^+, 518.3344. $C_{30}H_{44}F_6$ requires C, 69.47; H, 8.55; F, 21.98%; *M*, 518.3347).

(6*E*,10*E*,14*E*,18*E*)-3,22-Dibromo-2,6,10,15,19-pentamethyldocosa-6,10,14,18-tetraen-2-ol 8

The bromide **3** (1 g, 2.22 mmol) was dissolved in THF (30 cm^3) and cooled to 0 °C. Water was then added to it until the mixture became lightly turbid. Then *N*-bromosuccinimide (474 mg, 2.66 mmol) was added to it over a period of 15 min at 0 °C and allowed to react for 1.5 h at 0 °C. Water was then added (50 cm^3) and the reaction mixture was extracted with diethyl ether (50 cm^3 × 3). The combined extracts were washed with brine (50 cm^3 × 2), dried (Na_2SO_4) and evaporated to give a yellow oil. The crude product was purified by flash chromatography eluting with light petroleum–diethyl ether (99:1) to remove unchanged starting material **3** (150 mg, 0.33 mmol), then light petroleum–diethyl ether (95:5) to give the bromohydrin **8** (631 mg, 52%) as a pale yellow oil, $\delta_H(CDCl_3)$ 1.34 (d, 6 H, Me_2COH), 1.60–1.70 (m, 16 H, allylic CH_3, CH_2CHBr and CH_2CH$_2$Br), 1.98–2.12 (m, 16 H, allylic CH_2), 3.37 (t, 2 H, CH_2Br), 4.01 (m, 1 H, CHBr) and 5.05–5.21 (m, 4 H, vinylic CH); ν_{max}(thin film)/cm^{-1} 3430, 2960, 2920, 2850, 1440 and 1380; *m*/*z* (EI) 548 (14%), 547 (12), 546 (29), 545 (13), 544 (21), 529 (6), 522 (2), 509 (2), 504 (2), 494 (3), 327 (10), 203 (48), 163 (32), 95 (72) and 79 (100) (Found: C, 59.4; H, 8.5; Br, 29.2%; M^+, 544.1919. $C_{27}H_{46}Br_2O$ requires C, 59.34; H, 8.48; Br, 29.24%; *M*, 544.1916).

(4*E*,8*E*,12*E*,16*E*)-1-Bromo-20,21-epoxy-4,8,13,17,21-pentamethyldocosa-4,8,12,16-tetraene 9

The bromohydrin **8** (600 mg, 1.10 mmol) and K_2CO_3 (456 mg, 3.30 mmol) were dissolved in methanol (20 cm^3) and allowed to react for 2 h with stirring. Water was then added to it and the reaction mixture extracted with diethyl ether (50 cm^3 × 3) and the organic extracts dried (Na_2SO_4) and evaporated. The residue was flash chromatographed with light petroleum–diethyl ether (99:1 then 98:2) to give the epoxide **9** (451 mg, 88%) as a colourless oil, $\delta_H(CDCl_3)$ 1.24 and 1.28 (two peaks, 6 H, epoxidic CH_3), 1.62–1.72 (m, 16 H, allylic CH_3, CH_2CHO and CH_2CH$_2$Br), 1.98–2.10 (m, 16 H, allylic CH_2), 2.69 (t, 1 H, 20-H), 3.36 (t, 2 H, CH_2Br) and 5.04–5.21 (m, 4 H, vinylic CH); ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1440 and 1380; *m*/*z* (EI) 467 (4%), 466 (12), 465 (4), 464 (12), 339 (22), 337 (24), 311 (44), 309 (40), 289 (40), 243 (80) and 203 (100) (Found: C, 69.7; H, 9.8; Br, 17.2%; M^+, 464.2651. $C_{27}H_{45}BrO$ requires C, 69.66; H, 9.74; Br, 17.16%; *M*, 464.2654).

(4*E*,8*E*,12*E*,16*E*)-(20,21-Epoxy-4,8,13,17,21-pentamethyldocosa-4,8,12,16-tetraenyl)triphenylphosphonium bromide 10

The bromide **9** (400 mg, 0.859 mmol) and triphenylphosphine (676 mg, 2.58 mmol) were dissolved in dry DMF (15 cm^3) and the mixture was stirred at 60 °C for 18 h under a nitrogen atmosphere. The resulting orange–yellow oil was cooled, poured into a biphasic system (water–dichloromethane; 1:1, 100 cm^3) and extracted with dichloromethane (50 cm^3 × 3). The combined extracts were washed with water (50 cm^3 × 4) to eliminate DMF, dried (Na_2SO_4) and evaporated. The crude oil was purified by flash chromatography eluting with dichloromethane to remove impurities and unchanged starting material, then dichloromethane–ethanol (95:5) to give the triphenylphosphonium bromide **10** (450 mg, 72%) as a viscous oil, $\delta_H(CDCl_3)$ 1.26 and 1.30 (two peaks, 6 H, epoxidic CH_3), 1.56–1.71 (m, 16 H, allylic CH_3, CH_2CHO and CH_2CH$_2$P), 1.95–2.10 (m, 16 H, allylic CH_2), 2.70 (t, 1 H, 20-H), 3.65 (br t, 2 H, CH_2P), 5.03–5.17 (m, 4 H, vinylic CH) and 7.72–7.87 (m, 15 H, ArH); *m*/*z* (FAB+) 728 (1%), 726 (1), 647 (90), 492 (5), 424 (7), 356 (10), 288 (32) and 261 (100) (Found: C, 74.3; H, 8.3; Br, 11.0; P, 4.2%; M^+, 726.3564. $C_{45}H_{60}BrOP$ requires C, 74.26; H, 8.31; Br, 10.98; P, 4.26%; *M*, 726.3565).

(6*E*,10*E*,14*E*,18*E*)-22,23-Epoxy-1,1,1-trifluoro-2-trifluoromethyl-6,10,15,19,23-pentamethyltetracosa-2,6,10,14,18-pentaene 11

The triphenylphosphonium bromide **10** (200 mg, 0.275 mmol) was added to diethyl ether (10 cm^3) and cooled to 0 °C under nitrogen with stirring. Butyllithium (1.6 mol dm^{-3} solution in hexane; 520 mm^3, 0.825 mmol) was added to it and the mixture was stirred for 15 min to give a yellow–orange solution, when it was cooled to −80 °C and hexafluoroacetone was bubbled through the solution until the orange colour had disappeared. It was then warmed to room temperature, poured into a biphasic system (100 cm^3 of water–diethyl ether; 50:50) and extracted with diethyl ether (50 cm^3 × 3). The combined extracts were washed with brine, dried (Na_2SO_4) and evaporated. The crude product was purified by flash chromatography eluting with light petroleum, then light petroleum–diethyl ether (99:1; then 98:2; finally 95:5) to give the epoxide **11** (45 mg, 31%) as a colourless oil, $\delta_H(CDCl_3)$ 1.267 and 1.309 (two peaks, 6 H, epoxidic CH_3), 1.56–1.70 (m, 14 H, allylic CH_3 and 21-H$_2$), 1.98–2.16 (m, 18 H, allylic CH_2), 2.71 (t, *J* 6.35, 1 H, 22-H), 5.07–5.18 (m, 4 H, vinylic CH) and 6.73 [m, 1 H, C*H*=C(CF$_3$)$_2$]; ν_{max}(thin film)/cm^{-1} 2960, 2920, 2850, 1670, 1450 and 1380; *m*/*z* (CI) 535 (1%), 521 (2), 509 (1), 495 (2), 477 (3), 457 (5), 439 (10), 419 (5), 397 (3), 391 (10), 383 (5), 371 (7), 357 (8), 303 (12), 285 (15) and 167 (100) (Found: C, 67.4; H, 8.3; F, 21.3%; M^+, 534.3292. $C_{30}H_{44}F_6O$ requires C, 67.39; H, 8.29; F, 21.32%; *M*, 534.3296).

Biological assays

IC_{50} (the concentration of inhibitor reducing the enzymatic conversion of OS into lanosterol by 50%) values on OSC activity were determined in microsomal preparations from rat and pig liver, *S. cerevisiae* and *C. albicans*. IC_{50} on squalene epoxidase activity were determined in microsomal preparations from rat and pig liver.

Microsomes of rat and pig liver and of *S. cerevisiae* were prepared according to known methods.[9,20–22] *C. albicans* microsomes were kindly provided by LEPETIT.

Assays of mammalian[20,21] and yeast[4] OSC activity were performed as described as were the isotope counting and activity calculations,[32] assays of mammalian squalene epoxidase activity[22] and inhibition of cholesterol biosynthesis in

3T3 fibroblast cell cultures.[33] Time-dependent inactivation on OSC solubilized from *S cerevisiae* was determined as reported.[34]

Acknowledgements

This work was supported by grants from the 'Ministero della Ricerca Scientifica e Tecnologica' (40% and 60%) and from 'CNR, Progetto Finalizzato Chimica Fine'.

We thank the NMR group of the 'Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino'; the 'Dipartimento di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Università di Torino'; the mass group of the 'Dipartimento di Chimica Organica e Industriale, Università di Milano' and the mass group of the 'CNR, Area della Ricerca di Padova'.

References

1 S. Yamamoto and K. Bloch, *J. Biol. Chem.*, 1970, **245**, 1670.
2 T. Ono, K. Nakazono and H. Kosaka, *Biochim. Biophys. Acta*, 1982, **709**, 84.
3 M. Bard, R. A. Woods and J. M. Haslam, *Biochem. Biophys. Res. Commun.*, 1974, **56**, 324.
4 G. Balliano, F. Viola, M. Ceruti and L. Cattel, *Arch. Biochem. Biophys.*, 1992, **293**, 122.
5 L. Cattel and M. Ceruti, *Physiology and Biochemistry of Sterols*, eds. G. W. Patterson and W. D. Nes, American Oil Chemists' Society, Champaign, IL, cap 3, 1992, pp. 50–82.
6 E. E. van Tamelen, *J. Am. Chem. Soc.*, 1982, **104**, 6480.
7 I. Abe, M. Rohmer and G. D. Prestwich, *Chem. Rev.*, 1993, **93**, 2189.
8 L. Cattel, M. Ceruti, G. Balliano, F. Viola, G. Grosa and F. Schuber, *Steroids*, 1989, **53**, 363.
9 L. Cattel, M. Ceruti, G. Balliano and F. Viola, *Regulation of Isopentenoid Metabolism*. eds. W. D. Nes, E. J. Parish and J. M. Trzaskos, American Chemical Society, Washington, 1992, pp. 174–191.
10 I. Abe, J. C. Tomesch, S. Wattanasin and G. D. Prestwich, *Nat. Prod. Rep.*, 1994, 279.
11 N. S. Ryder, M.-C. Dupont and I. Frank, *FEBS Lett.*, 1986, **204**, 239.
12 M. Ceruti, F. Viola, G. Grosa, G. Balliano, L. Delprino and L. Cattel, *J. Chem. Res.* (*S*), 1986, 18; (*M*) 239.
13 S. E. Sen and G. D. Prestwich, *J. Am. Chem. Soc.*, 1989, **111**, 1508.
14 S. E. Sen and G. D. Prestwich, *J. Am. Chem. Soc.*, 1989, **111**, 8761.
15 A. Stütz, *Antifungal Drugs*, 1988, **544**, 46.
16 Y. Nagata, M. Horie, Y. Hidaka, M. Yonemoto, M. Hayashi, H. Watanabe, F. Ishida and T. Kamei, *Chem. Pharm. Bull.*, 1992, **40**, 436.
17 L. Cattel, M. Ceruti, F. Viola, L. Delprino, G. Balliano, A Duriatti and P. Bouvier-Navé, *Lipids*, 1986, **21**, 31.
18 L. Cattel, M. Ceruti, G. Balliano, F. Viola, G. Grosa, F. Rocco and P. Brusa, *Lipids*, in the press.
19 M. Ceruti, G. Balliano, F. Viola, L. Cattel, N. Gerst and F. Schuber, *Eur. J. Med. Chem.*, 1987, **22**, 199.
20 M. Ceruti, F. Viola, F. Dosio, L. Cattel, P. Bouvier-Navé and P. Ugliengo, *J. Chem. Soc., Perkin Trans. 1*, 1988, 461.
21 F. Viola, M. Ceruti, G. Balliano, O. Caputo and L. Cattel, *Il Farmaco*, 1990, **45**, 965.
22 M. Ceruti, G. Balliano, F. Viola, G. Grosa, F. Rocco and L. Cattel, *J. Med. Chem.*, 1992, **35**, 3050.
23 M. Ceruti, F. Rocco, F. Viola, G. Balliano, G. Grosa, F. Dosio and L. Cattel, *Eur. J. Med. Chem.*, 1993, **28**, 675.
24 W. R. Moore, G. L. Schatzman, E. T. Jarvi, R. S. Gross and J. R. McCarthy, *J. Am. Chem. Soc.*, 1992, **114**, 360.
25 J. Mann and G. P. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2884.
26 A. Duriatti, M. Jung, J. Mann and G. P. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1993, 3073.
27 M. Bai, X.-Y. Xiao and G. D. Prestwich, *Biochem. Biophys. Res. Commun.*, 1992, **185**, 323.
28 F. Camps, F.-J. Sanchez and A. Messeguer, *Synthesis*, 1988, 823.
29 E. E. van Tamelen and K. B. Sharpless, *Tetrahedron Lett.*, 1967, **28**, 2655.
30 G. R. Stark and P. A. Bartlett, *Pharmacol. Ther.*, 1983, **23**, 45.
31 B. L. Shapiro, W. A. Thomas, J. L. McClanahan and M. D. Johnston, Jr., *J. Magn. Reson.*, 1973, **11**, 355.
32 G. Balliano, F. Viola, M. Ceruti and L. Cattel, *Biochim. Biophys. Acta*, 1988, **959**, 9.
33 M. Ceruti, F. Viola, G. Balliano, G. Grosa, F. Rocco, G. Biglino and L. Cattel, *Atti Accad. Sci. Torino*, 1992, **126**, 131.
34 G. Balliano, G. Grosa, P. Milla, F. Viola and L. Cattel, *Lipids*, 1993, **28**, 903.

Paper 4/05871B
Received 26*th September* 1994
Accepted 29*th November* 1994