Articles

Hypocholesterolemic Activity of Synthetic and Natural Tocotrienols

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Tocotrienols are farnesylated benzopyran natural products that exhibit hypocholesterolemic activity in vitro and in vivo. The mechanism of their hypolipidemic action involves posttranscriptional suppression of HMG-CoA reductase by a process distinct from other known inhibitors of cholesterol biosynthesis. An efficient synthetic route to tocotrienols and their isolation from palm oil distillate using an improved procedure is presented. γ-Tocotrienol exhibits a 30-fold greater activity toward cholesterol biosynthesis inhibition compared to α-tocotrienol in HepG2 cells in vitro. The synthetic (racemic) and natural (chiral) tocotrienols exhibit nearly identical cholesterol biosynthesis inhibition and HMG-CoA reductase suppression properties as demonstrated in vitro and in vivo.

Hypercholesterolemia is a recognized risk factor for atherosclerotic disease.[1] Studies have demonstrated that with very few exceptions, populations that consume large quantities of saturated fat and cholesterol have relatively high concentrations of serum cholesterol and correspondingly high mortality rates from coronary heart disease.[2] Conversely, populations that consume diets rich in cereal grains tend to have a lower prevalence of atherosclerosis.[3] The primary agent responsible for the cholesterol lowering effect of cereal grains is thought to be the water-soluble fiber β-glucan. The water-soluble fibers result in the sequestration of bile acids and/or inhibition of cholesterol absorption in the gut.[4] In addition to the hypocholesterolemic effects of plant fiber, other components such as plant sterols, and oxygenated terpenes may contribute to the hypocholesterolemic effect of certain cereal grains.[5]

Studies of cereal grains revealed that barley was particularly effective in lowering lipid levels in animal models.[6] The exceptional ability of barley extracts to lower lipids in vivo prompted the purification and identification of the chemical constituents responsible for cholesterol suppressive activity. α-Tocotrienol was recovered from barley extracts and was designated as one of the hypocholesterolemic components based on subsequent in vitro and in vivo evaluation.[7]

The tocotrienols are structurally related to the tocopherols (vitamin E) and differ only by possessing unsaturation in the isoprenoid side chain as shown in Chart I. The Greek letter prefix designates the degree and placement of methyl substitution on the chroman ring. The naturally occurring tocotrienols possess the (2*R*),3′-*trans*,7′-*trans* configuration.[8] Biosynthetically, the tocotrienols are thought to be derived from homogentistic acid and geranylgeranyl pyrophosphate, whereas the tocopherols incorporate phytyl pyrophosphate.[9] The richest sources of tocotrienols are cereals (such as barley, oats, rice, wheat, and rye), vegetable oils (such as palm oil or rice bran oil), and latex.[10]

The favorable lipid-lowering profile of α-tocotrienol was intriguing, and so a research effort in this minimally explored area was begun. An improved method for the isolation of tocotrienols from natural sources, the synthesis of racemic tocotrienols, and their preliminary biological evaluation are presented here.[12]

Suppression of HMG-CoA Reductase

Qureshi indicated that the hypocholesterolemic effect of α-tocotrienol was associated with lower levels of HMG-

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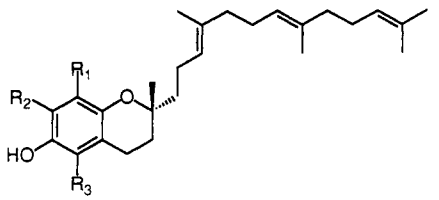
Chart I

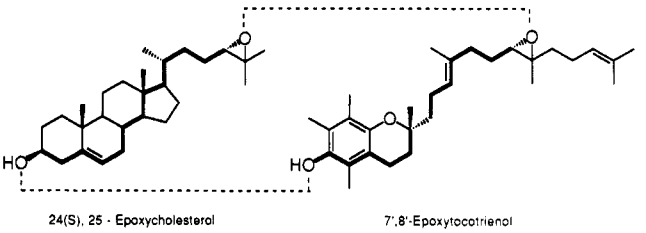


compound	R_1	R_2	R_3	ref
α-tocotrienol	Me	Me	Me	11a,b,e
β-tocotrienol	Me	H	Me	11a,e
γ-tocotrienol	Me	Me	H	11c,e
δ-tocotrienol	Me	H	H	11c,f
tocotrienol	H	H	H	11d

CoA reductase (HMGR) as measured by hepatic HMGR activity.[7] HMG-CoA reductase converts HMG-CoA into mevalonate and is the rate limiting enzyme in the cholesterol biosynthetic pathway.[13] Inhibition or suppression at the level of HMGR represents an attractive point of intervention since only early stage (water-soluble) products are accumulated. HMG-CoA reductase mass (activity) is diminished by tocotrienols through decreased synthesis and enhanced degradation of the reductase[12] and should be distinguished from competitive inhibitors such as mevinolin. The possibility to exploit a natural feedback mechanism of cholesterol regulation was apparent.[14]

In several repects, the tocotrienols appear to operate in a similar manner to oxysterols. Certain oxysterols have been shown to regulate cholesterol biosynthesis by a transcriptional down-regulation of the reductase gene.[15] It has been postulated that endogenously produced oxysterols are natural regulators of cholesterol biosynthesis. In particular, 24(*S*),25-epoxycholesterol and 25-hydroxycholesterol have been found in human liver, in vivo, in concentrations high enough for cholesterol regulation.[16] These oxysterols are potent repressors of HMG-CoA reductase and bind strongly to the cytosolic oxysterol binding protein.[17]

Early on it was apparent, that if oxysterols are indeed natural regulators of cholesterol biosynthesis, then the tocotrienols may have a similar function, since they appeared to cause the same effect (suppression of HMGR). In fact, Dreiding models[18] indicate that 24,25-epoxycholesterol and a 7′,8′-epoxy-α-tocotrienol share close structural resemblance. This relationship was confirmed by energy-minimized comparisons using MM2 calculations.[19]



Thus, α-tocotrienol or an oxygenated analogue might behave as an oxysterol surrogate. This comparison suggests that the terminal prenyl unit in tocotrienol may not be necessary for expression of biological activity.

Tocotrienol Synthetic Program

The initial objective was to extend the original findings of Qureshi et al.[7] by examining chromatographic fractions of tocotrienol-rich extracts in a cholesterol biosynthesis assay in primary rat hepatocytes. From high protein barley flour[20] extracts were obtained which were purified by silica gel chromatography. It was confirmed that one band (of multiple components) did exhibit the anticipated cholesterol suppressive activity in the rat hepatocyte.

A synthetic program was initiated to prepare *d,l*-α-tocotrienol and analogues based on the oxysterol hypothesis. The synthetic lot of α-tocotrienol was considerably less active (in the rat hepatocyte) than anticipated based on the activity of the barley extract (even taking into account the fact that it is a racemic mixture). A palm oil extract was received, which is a tocotrienol-rich fraction

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(TRF) obtained from palm oil by molecular distillation.[21] At the time, the detailed composition of the TRF was unknown, except that it was an enriched source of α-tocotrienol. The cholesterol suppressive activity of the TRF was, unexpectedly, several fold more active than pure α-tocotrienol. A portion of the TRF was subjected to chromatographic separation and the four major components were identified as being *d*-α-tocopherol (26%), *d*-α-tocotrienol (18%), *d*-γ-tocotrienol (27%), and *d*-δ-tocotrienol (7%). Biological evaluation of these components as inhibitors of cholesterol biosynthesis in rat hepatocytes revealed that γ- and δ-tocotrienols were at least 5-fold more active than α-tocotrienol, and that α-tocopherol was inactive. To provide further confirmation of this result, which was unpredicted based upon previous studies,[7] the effects of the tocotrienols on cholesterol synthesis in cultured human hepatoma HepG2 cells was examined. In the HepG2 cell line, which is a more sensitive assay, a 30-fold greater potency of γ- versus α-tocotrienol was revealed. Thus, tocotrienols lacking the 5-methyl substitution appeared to possess most of the biological activity.[22] Synthetic effort was then directed toward the development of 5-desmethyltocotrienol analogues.

Several tocotrienol analogues containing the oxygenation patterns found in the oxysterols were targeted for synthesis.[23] Emphasis was placed toward α-tocotrienol and γ-tocotrienol analogues lacking one prenyl unit, and incorporating oxygenation as found in 24,25-epoxycholesterol.

Tocotrienol Isolation from Natural Sources and the Synthesis of Tocotrienols and Related Benzopyran Analogues

Tocotrienols can be obtained from natural sources using published procedures.[24,11c,11d,21a] A more convenient source of enriched tocotrienol comes from molecular distilled palm oil, or "tocotrienol rich fraction" (TRF). TRF can be separated into the chromanol components using routine flash chromatography and HPLC, however this is tedious and not readily amenable to scaleup.[25] The phenolic components exhibit similar retention characteristics and are prone to air oxidation. A significant improvement in the ease of isolation of the various tocotrienols from TRF was achieved by derivatization. Of the phenolic components in TRF, γ- and δ-tocotrienols are less sterically hindered than α-tocopherol and α-tocotrienol, and could be selectively silylated in their presence. The much less polar silylated compounds can be easily separated from the α-tocopherol and α-tocotrienol by a quick column. The free phenols are then regenerated in quantitative yield by treatment of the silyl ethers with fluoride ion. The separation is reduced to only the γ- and δ-tocotrienols, which exhibit greater retention differences by chromatography.

Synthesis of α-tocotrienol was performed according to the literature method of Urano et al.[26] However, this method was not acceptable, since it provided a mixture of side-chain olefin isomers as determined by 300-MHz PMR[27] and HPLC. Other literature methods for the synthesis of α-tocotrienol are very lengthy and were not practical.[28,11f]

The polyprenyl carbon chains found in squalene and the various Q coenzymes have been synthesized by several investigators.[29] A general approach involves coupling of the prenyl groups through anion-alkylation reactions followed by reductive cleavage of the activating substituent. These alkylation products are different than that described earlier by Urano in that the products are unsymmetrical with regard to the allylic activating group. Thus, the reductive cleavage, when carried-out under proper conditions, is regio- and stereoselective. The synthesis of α-, γ-, and desmethyltocotrienols utilizes the basic ubiquinone synthetic strategy of Sato.[29e]

The synthesis of α-tocotrienol is outlined in Scheme I. Aldehyde 1 was prepared from the corresponding monoprenyl phenol described by Kato et al.,[30] following protection with (2-methoxyethoxy)methyl chloride, ozonolysis, and reductive workup. The addition of ethyl 2-(triphenylphosphoranylidene)propionate[31] (2) to aldehyde 1 afforded a 10:1 mixture of *E*/*Z* enoates which could be separated by chromatography. The aluminum hydride reduction[32] of ester 3 to the allylic alcohol 4 and its conversion to allylic chloride 5 proceeded smoothly.[33] The coupling of chloride 5 with sulfone 6[29b] provided tocotrienol

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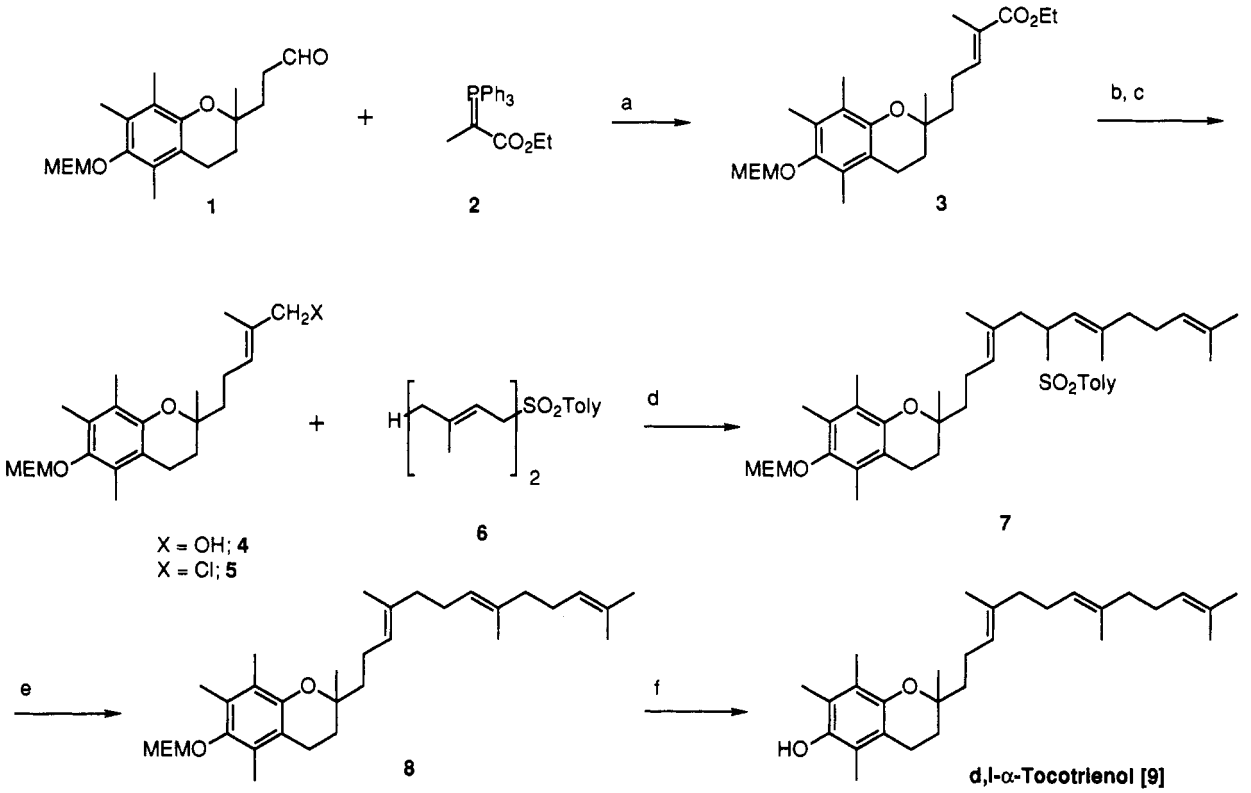
Scheme I. [a] Synthetic Route to α-Tocotrienol



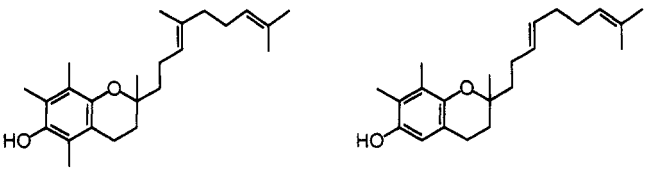
[a] (a) C_6H_6, Δ; (b) AlH_3, Et_2O, −5 °C; (c) NCS, Me_2S, CH_2Cl_2, −5 °C; (d) sulfone/*n*-BuLi/THF–HMPA and then chloride; (e) $PdCl_2$:dppb, $LiEt_3BH$, THF, −20 °C; (f) $C_2H_4BClS_2$, CH_2Cl_2, −20 °C.

derivative **7**. Reductive cleavage of **7** occurs with retention of olefin integrity to give **8**, using super-hydride catalyzed by palladium(0).[29g] Deprotection of the MEM ether occurs cleanly using 2-chloro-1,3,2-dithioborolan[34] to provide all *trans*-α-tocotrienol **9** of >95% purity (HPLC). Cleavage of the MEM ether with a number of other reagents was unsatisfactory.[35]

The synthesis of γ-tocotrienol is outlined in Scheme II. Synthesis of γ-tocotrienol begins with the known aldehyde **10**,[36] which is reacted with ethyl 2-(triphenylphosphoranylidene) propionate to provide the all *trans*-ester **11** contaminated by less than 3% of the *cis*-ester (^{1}H NMR). After removal of the tetrahydropyranyloxy protecting group, the allylic alcohol **12** was condensed with 2,3-dimethylhydroquinone to give the oxidatively unstable alkylated hydroquinone **13a**. The presence of the conjugated ester moiety prevents the electrophilic participation of the terminal olefin during the alkylation process. Electron-rich olefinic allylic alcohols (e.g. geraniol) led to complex mixtures under these alkylation conditions. The hydroquinone was immediately cyclized under the influence of catalytic *p*-toluenesulfonic acid to yield the benzopyranol **14a**, which was directly protected as its (2-methoxyethoxy)methyl ether. The oxidatively stable ether **15a** was purified by chromatography on silica gel. Completion of the synthesis of γ-tocotrienol **20** from ester **15a** proceeds as described for α-tocotrienol from the analogous ester **3**.

Tocotrienol **21**, lacking benzopyran ring methylation, was prepared in the same manner as γ-tocotrienol shown in Scheme II.

The α-tocodienol analogue **22** was prepared using the route shown in Scheme III. Attempts at achieving selective epoxidation of this compound at the terminal olefin were unsuccessful (results not shown). The corresponding 4′-desmethyl-γ-tocodienol **23** was prepared as shown in Scheme III. This compound was chosen in order to obtain better regioselectivity toward epoxidation, and to enhance activity with γ-substitution. Triethyl phosphonoacetate and aldehyde **10** were condensed using Rathke's conditions[37] to give the conjugated ester. The corresponding ester **24** was elaborated into the 4′-desmethyl-γ-tocodienol **23** using prenyl *p*-tolyl sulfone[29a] as shown in Scheme III. The selective epoxidation of this compound with *m*-chloroperbenzoic acid at the trisubstituted olefin was successful giving a pure sample of racemic 4′-desmethyl-7,8′-epoxy-γ-tocodienol **33**.



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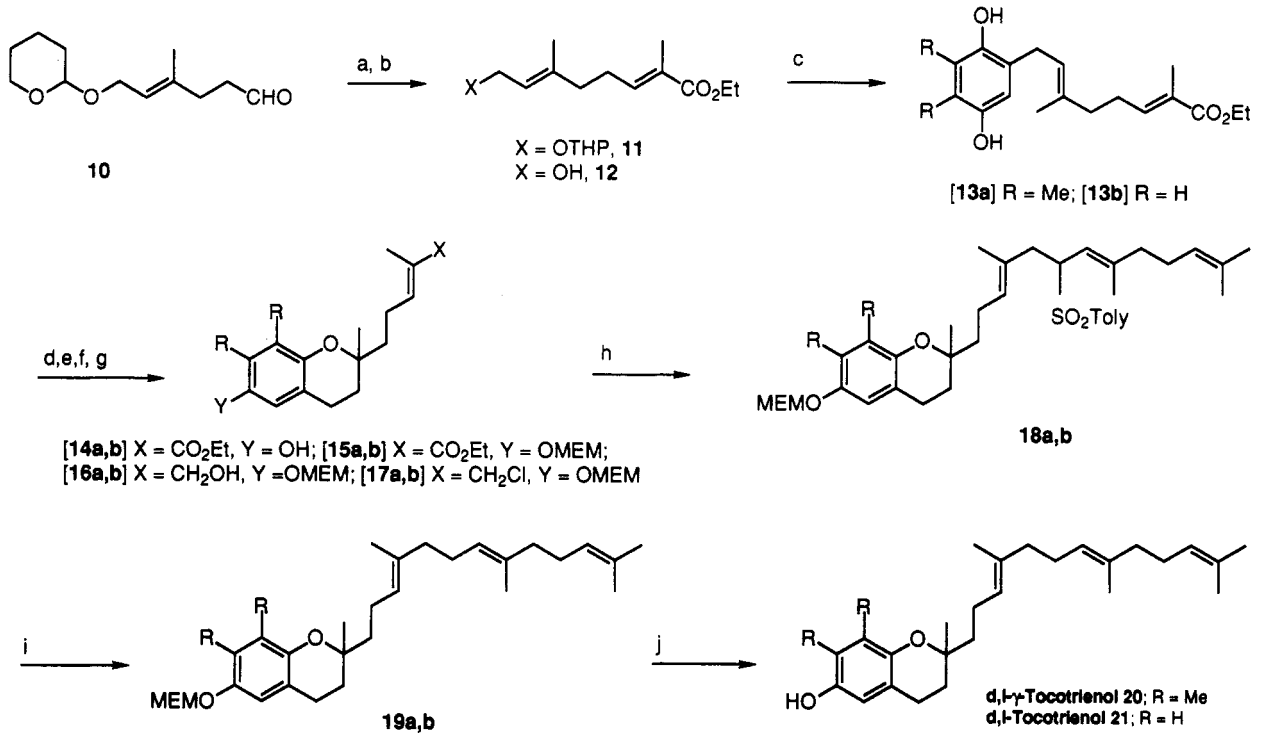
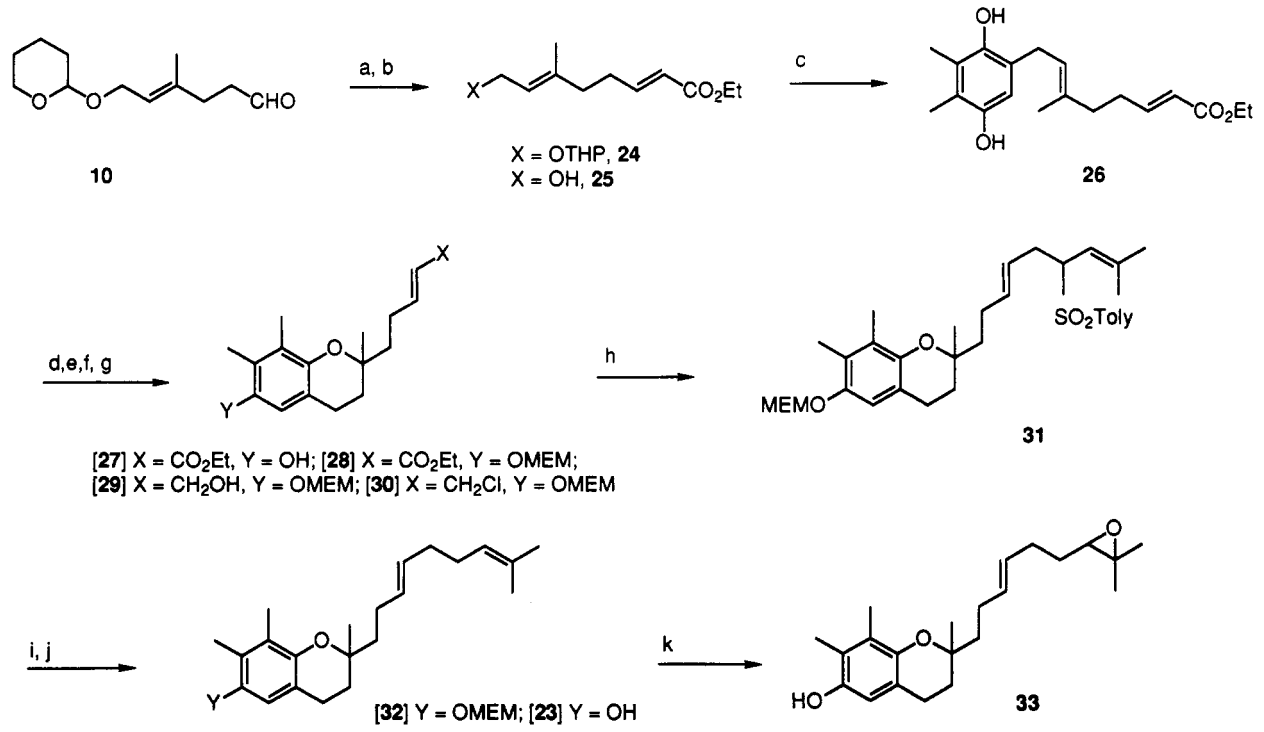
Scheme II. [a] Synthetic Route to γ-Tocotrienol and Tocotrienol



[a] (a) Ethyl 2-(triphenylphosphoranylidene)propionate, CH_2Cl_2, 23 °C; (b) PPTS, EtOH, Δ; (c) hydroquinone, BF_3, dioxane, Δ; (d) *p*TSA, C_6H_6, Δ; (e) MEMCl, NaH, THF; (f) AlH_3, Et_2O, −5 °C; (g) NCS, Me_2S, CH_2Cl_2, −5 °C; (h) geranyl *p*-tolyl sulfone, *n*-BuLi, THF–HMPA, −78 °C; (i) $PdCl_2$:dppb, $LiEt_3BH$, THF, −20 °C; (j) $C_2H_4BClS_2$, CH_2Cl_2, −20 °C.

Scheme III. [a] Synthetic Route to 4′-Desmethyl-γ-tocodienol



[a] (a) (Carbethoxymethylene)triphenylphosphorane, CH_2Cl_2, 23 °C; (b) PPTS, EtOH, Δ; (c) 2,3-dimethylhydroquinone, BF_3, dioxane, Δ; (d) *p*TSA, C_6H_6, Δ; (e) MEMCl, NaH, THF; (f) AlH_3, Et_2O, −5 °C; (g) NCS, Me_2S, CH_2Cl_2, −5 °C; (h) prenyl *p*-tolyl sulfone, *n*-BuLi, THF–HMPA, −78 °C; (i) $PdCl_2$:dppb, $LiEt_3BH$, THF, −20 °C; (j) $C_2H_4BClS_2$, CH_2Cl_2, −20 °C; (k) *m*CPBA, $NaHCO_3$, CH_2Cl_2/H_2O, 23 °C.

Results

Rat Primary Hepatocyte Model. A tocotrienol-rich fraction (TRF) of palm oil was tested for effects on cholesterol synthesis and on HMGCoA reductase activity in freshly isolated, primary rat hepatocytes. Cells were preincubated for 45 min with a series of concentrations of TRF in Tween-80 vehicle (0.5% v/v, final), followed by assay of sterol biosynthesis by a 45-min pulse incorporation of [2-^{14}C]acetate into cholesterol. As seen in Figure 1, a TRF concentration-dependent inhibition of cholesterol synthesis rate was observed. This was reflected in corresponding decreases in the level of microsomal HMG-CoA reductase total activity in parallel incubations of cells (Figure 1). The loss of HMGCoA reductase activity was

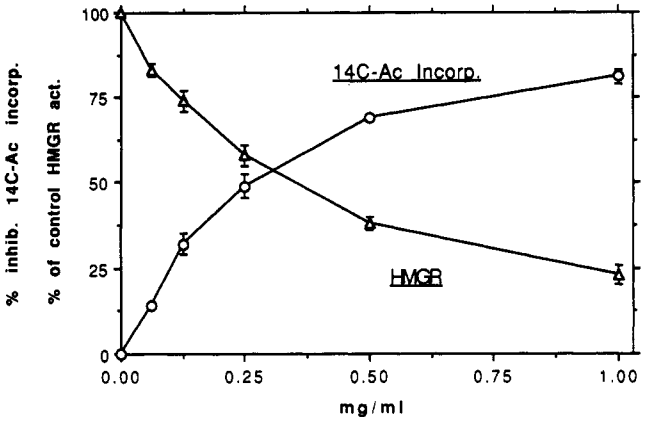


Figure 1. Inhibition of cholesterol synthesis concomitant with loss of microsomal HMGCoA reductase activity in rat hepatocytes incubated with a tocotrienol-rich fraction from palm oil. Two sets of cell incubations were conducted in parallel. Cells received the indicated levels of TRF in Tween-80 vehicle (0.5% v/v final concn) for a total of 90 min. To assay sterol synthesis, [2-^{14}C]-acetate incorporation over the final 45 min was determined. To assay HMGCoA reductase total activity levels, cells were harvested at 90 min, lysed, microsomes were isolated, and total enzymic activity was measured by radiochemical methods. Results are given as mean ($\pm^1/_2$ range of duplicates) percent inhibition of [^{14}C]acetate incorporation or percent of control HMGCoA reductase activity, compared to controls incubated with vehicle.

Table I. Synthetic α-Tocotrienol Is Less Effective Than a Mixture of Tocotrienols as an Inhibitor of Cholesterol Biosynthesis in Rat Hepatocytes

compound	concn μg/mL	% inhib[b]	IC_{50}, μg/mL
d-α-tocopherol	210[a]	0	–
d,l-α-tocotrienol	41[a]	4	
	210	17	
tocotrienol mixture (TRF)	138	31	600
	275	38	
	550	45	
	1100	60	
	2200	70	

[a] For α-tocopherol and α-tocotrienol, 41 μg/mL equals approximately 100 μM, and 210 μg/mL is approximately 500 μM. [b] Data is given as per cent inhibition of cholesterol synthesis vs controls receiving vehicle, and are average values from duplicate or triplicate determinations.

closely matched by loss of immunoreactive enzyme mass as measured by Western immunoblot analysis of the microsomal fractions using SDS-PAGE and a specific antireductase antibody (data not shown). These findings extend the original regults of Qureshi et al.[7] and suggest that one or more components in TRF decreases cholesterol synthesis by down-regulating HMGCoA reductase protein levels.

Primary rat hepatocytes were preincubated for 15 min in the presence of increasing amounts of the tocotrienol mixtures, followed by the assay of cholesterol synthesis by a 60-min incorporation of [^{14}C]acetate into total digitonin-precipitable sterols. As shown in Table I, synthetic α-tocotrienol proved to be only weakly active in this system. In contrast, unexpectedly greater activity was detected in this assay using mixtures of γ-tocotrienol and δ-tocotrienol (tocotrienol-rich fraction (TRF) from palm oil) than could be accounted for by α-tocotrienol.

In order to examine whether the cholesterol synthesis inhibitory activity present in tocotrienol mixtures derived from natural sources (TRF) actually resided in compounds other than α-tocotrienol, we chromatographically separated the α-tocotrienol, γ-tocotrienol, and δ-tocotrienol

Table II. Evaluation of α-, γ-, and δ-Tocotrienols Separated from a Natural Product Source as Inhibitors of Cholesterol Synthesis in Isolated Rat Hepatocytes

		% inhib at μg/mL concn			
compound	expt no.	125	250	500	IC_{50}, μg/mL
d-α-tocopherol	1	–	0	2	no inhib
	2	–	0	0	
d-α-tocotrienol	1	–	8	12	≫1000
	2	8	20	33	1300
d-γ-tocotrienol	1	–	48	60	270
	2	28	34	61	360
d-δ-tocotrienol	1	–	49	62	260
	2	26	33	63	340

Table III. Effects of Binary Mixtures on Cholesterol Synthesis in Rat Hepatocytes

		% inhibition at 250 μg/mL each[a]
α-tocopherol	+ α-tocotrienol	3
	+ γ-tocotrienol	42
	+ δ-tocotrienol	49
α-tocotrienol	+ γ-tocotrienol	49
	+ δ-tocotrienol	53
γ-tocotrienol	+ δ-tocotrienol	64

[a] Data represent average values from duplicate or triplicate determinations.

Table IV. IC_{50} for Cholesterol Synthesis Inhibition in HepG2 Cells by γ- and α-Tocotrienol

HepG2 cell incubation time, h	d,l-γ-tocotrienol IC_{50}, μM	d,l-α-tocotrienol	
		IC_{50}, μM	rel potency[a]
2	5.9	380	0.016
4	2.8	94	0.030
6	3.0	110	0.027

[a] Relative potency vs γ-tocotrienol at each time point. HepG2 Cells were preincubated with tocotrienols for the indicated times, and cholesterol synthesis was assayed by incorporation of [^{14}C]acetate over the final 60 min of the incubation. Each IC_{50} was determined by linear regression of at least four concentration points assayed in duplicate.

plus α-tocopherol from TRF and examined these compounds in the rat hepatocyte cholesterol synthesis assay. The primary rat hepatocyte assay for cholesterol biosynthesis inhibition was conducted. The indicated compounds were purified from the same natural product source as the mixture used in Table I. Data from two separate sets of experiments are given in Table II. The results indicate that natural γ-tocotrienol and δ-tocotrienol are approximately equipotent and are each at least 5 times more active than α-tocotrienol in the rat hepatocyte system (Table II). Binary combinations of the components were also examined to test for possible additivity which might reveal activity in α-tocotrienol. When assayed under the conditions of Table II, only the mixtures containing γ-tocotrienol or δ-tocotrienol were significantly active, and α-tocotrienol did not increase the apparent activity of any other component (Table III). These data suggest that as an inhibitor of cholesterol biosynthesis in rat liver cells, α-tocotrienol is significantly less active than γ-tocotrienol and δ-tocotrienol.

HepG2 Cell Culture Model. The human hepatoma HepG2 cell culture model was employed to further compare the intrinsic activities of the tocotrienols. HepG2 cells were incubated with the indicated compounds for 2, 4, and 6 h at 10 μM. Cholesterol synthesis was assayed by [^{14}C]acetate incorporation over the final hour of incubation. Time course studies (Table IV) indicated that 4-h preincubations provided maximal suppression of sterol synthesis for both compounds. These data show that the

Table V. Tocotrienols Examined in HepG2 Cells: Correlation of Inhibition of Cholesterol Synthesis from [^{14}C]Acetate with HMGCoA Reductase Suppression[a]

	% inhibition			
compound, 10 μM	n	[^{14}C]-Ac	n	HMGR
d-γ-tocopherol	2	3 ± 1	2	0
d-α-tocotrienol	2	32 ± 4	2	15 ± 1
d,l-α-tocotrienol	3	21 ● 10	2	19 ± 3
d-γ-tocotrienol	4	78 ± 3	4	64 ± 3
d,l-γ-tocotrienol	9	71 ± 4	9	62 ± 5
d-δ-tocotrienol	3	77 ± 3	3	65 ± 2
d,l-tocotrienol	4	63 ± 5	3	56 ● 8
23	2	38 ● 2	-	-
32	2	19 ± 4	-	-

[a] Cholesterol synthesis inhibition and HMGCoA reductase suppression were assayed in HepG2 cells incubated for 4 h with the compounds indicated at 10 μM. Values represent mean percent inhibition vs controls receiving DMSO vehicle; n = number of repeated experiments, each assayed in duplicate.

HepG2 cell model is at least 1 order of magnitude more sensitive than rat hepatocytes to the inhibitory effects of tocotrienols as cholesterol biosynthesis suppressors. As the data in Table IV indicate, γ-tocotrienol has an even greater relative potency compared to α-tocotrienol in the HepG2 cell model than in rat hepatocytes. It can be seen that the intrinsic potency of γ-tocotrienol (IC_{50} = 2.8 μM) is at least 30-fold greater than that of α-tocotrienol (IC_{50} = 94 μM).

The mechanism of cholesterol synthesis inhibition by tocotrienols appears to involve down-regulation of the rate-limiting enzyme of sterol synthesis, HMG-CoA reductase. γ-Tocotrienol did not inhibit HMGR activity when directly added to microsomal fractions derived from hepatocytes or HepG2 cells. In the HepG2 cell culture model, measurements of suppression of total HMG-CoA reductase activity showed that γ-tocotrienol and δ-tocotrienol were significantly more active than α-tocotrienol in suppressing HMG-CoA reductase. In these studies, HMG-CoA reductase suppression was assayed in the microsomal fraction isolated from HepG2 cultures at the end of the 4-h incubation. As seen in Table V, the suppression of HMGR total activity by tocotrienols closely correlated with inhibition of sterol synthesis from [^{14}C]acetate in parallel cell incubations. The suppression of HMG-CoA reductase protein expression by γ-tocotrienol and δ-tocotrienol in HepG2 cells and in rat liver was confirmed by immunoassay using the Western blot technique (data not shown). The magnitude of suppression of HMGR activity and of HMGR immunoreactive protein were directly correlated.

The tocodienol **23** exhibited somewhat lower activity relative to γ-tocotrienol in the HepG2 assay of cholesterol biosynthesis inhibition (Table V). Interestingly, the oxygenated derivative **33** exhibited an even lower cholesterol suppression activity, suggesting that this feature is not important.

In Vivo Evaluation of Synthetic and Natural Tocotrienols in Normocholesterolemic Chickens. Hypocholesterolemic activity was evaluated for natural α-tocopherol and α-, γ-, and δ-tocotrienols in normocholesterolemic chickens (see ref 7 for experimental details). Newborn male chicks (6–10 for each group) were raised on a standard corn–soybean-based control diet for 2 weeks and then were switched to either control or experimental diets for 4 weeks. Drug treatment consisted of the addition of test compound to the corn–soybean-based control diet. At the end of the feeding period, all the birds were fasted (36 h) and refed (48 h) to induce cholesterolgenic enzymes prior to sacrifice. The specific activity of HMG-CoA reductase, total serum cholesterol levels, HDL/LDL cholesterol pools, and triglyceride levels (data not shown) were examined (Table VI).

The drug treated birds exhibited significant reductions in hepatic HMG-CoA reductase and total serum cholesterol levels over control diet fed chickens. In addition, the drug-treated birds showed significant enhancements in their HDL/LDL cholesterol as a measure of atherogenic index.

As a means to further evaluate any differences between the natural (chiral) and synthetic (racemic) tocotrienols, these compounds were multiply-dosed, side-by-side in 6-week-old male chicks. The same experimental protocol described above was used. Natural and synthetic γ-tocotrienols were chosen for the study as shown in Table VII.

Discussion

Tocotrienols appear to reduce cellular cholesterol biosynthesis by a mechanism consistent with down-regulation of the rate limiting enzyme HMG-CoA reductase (Figure 1, Tables V–VII). Details of our present understanding of the mechanism of action of the cholesterol suppressive effects of the tocotrienols are forthcoming as a separate publication. As previously disclosed,[12] the tocotrienols inhibit incorporation of labeled acetate but not mevalonate into sterol. Analysis of the nonsaponifiable cellular components of HepG2 cells after tocotrienol treatment indicates that accumulation of pathway intermediates characteristic of several known cholesterol biosynthesis inhibitors trisnorsqualene alcohol[38] (inhibitor of squalene epoxidase), U18666A[39] (inhibitor of 2,3-oxidosqualene cyclase), and miconazole[40] (inhibitor of lanosterol 14-α-demethylase) are notably absent. A small increase in a peak coeluting with squalene is observed.

It was our initial hypothesis that tocotrienols may be acting as oxysterol surrogates. Our present understanding is that the tocotrienols operate in a distinctly different manner from the prototype oxysterol 25-hydroxycholesterol, which down-regulates HMGR at the transcriptional level. The promoter region of the HMGR gene contains a regulatory sequence shared by HMG-CoA synthetase and the LDL receptor, which also undergo coordinate regulation in response to oxysterols.[41] Studies from our laboratories suggest that tocotrienols do not down-regulate the HMGS or LDL receptor.[42] This data suggests a post-transcriptional point of intervention for tocotrienols. In addition, the synthetic analogues designed to mimic epoxycholesterol show no significant activity as cholesterol biosynthesis inhibitors. In fact, the oxygenated tocotrienol analogues are weaker than their parent molecules.

Side-chain unsaturation is important for the activity of tocotrienols. α-Tocopherol and γ-tocopherol are completely inactive as cholesterol biosynthesis inhibitors (Tables I–III, V, VI). In vitro models reveal that the intrinsic pharmacological activity of the tocotrienols (rat hepatocytes and HepG2 cell culture) are markedly dif-

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(39) Kagan, F. U.S. Patent 3 389 051, **1968**.
(40) Janssen, Pharmaceutica U.S. Patent 3 936 470, **1976**.
(41) (a) Clarke, C. F.; Tanaka, R. D.; Svenson, K.; Wamsley, M.; Fogelman, A. M.; Edwards, P. A. Molecular Cloning and Sequence of a Cholesterol-Repressible Enzyme Related to Prenyltransferase in the Isoprene Biosynthetic Pathway. *Mol. Cell. Biol.* **1987**, *7*, 3138–3146. (b) Sudhof, T. C.; Russell, D. W.; Brown, M. S.; Goldstein, J. L. 42 bp Element from LDL Receptor Gene Confers End-Product Repression by Sterols when Inserted into Viral TK Promoter. *Cell* **1987**, *48*, 1061–1069.
(42) Parker, R. A.; Gordon, D. A., unpublished results.

Table VI. Effects of α-Tocopherol and α-, γ-, and δ-Tocotrienols on Lipid Metabolism in 6-Week-Old Male Chickens[a]

test compound	concentration in the diet, ppm	total cholesterol	LDL cholesterol	HDL cholesterol	HMGR activity
control		197 ± 5	76 ± 5	107 ± 7	1086 ± 114
d-α-tocopherol	20	190 ± 7	72 ± 6	113 ± 8	1296 ± 61
d-α-tocotrienol	20	155 ± 3	55 ± 6	102 ± 7	670 ± 31
d-γ-tocotrienol	20	151 ± 4	41 ± 8	102 ± 5	494 ± 23
d-δ-tocotrienol	20	141 ± 3	41 ± 7	99 ± 8	550 ± 34

[a] Data expressed as means ± SD; n = 10 chickens per group; cholesterol values expressed as mg/100 mL; HMGR activity expressed as picomoles of mevalonic acid synthesized per minute per mg of microsomal protein.

Table VII. Effects of Natural and Synthetic γ-Tocotrienols on Lipid Parameters in Chickens[a]

	d-γ-tocotrienol				*d,l*-γ-tocotrienol			
dose, ppm	total cholesterol	LDL cholesterol	HDL cholesterol	HMGR activity	total cholesterol	LDL cholesterol	HDL cholesterol	HMGR activity
control	180 ± 2	72 ± 1	104 ± 3	658 ± 15	180 ± 2	72 ± 1	104 ± 3	658 ± 15
15	141 ± 2	42 ± 2	97 ± 1	606 ± 5	143 ± 1	44 ± 1	97 ± 2	603 ± 9
30	132 ± 2	35 ± 1	95 ± 2	561 ± 10	138 ± 1	39 ± 1	97 ± 2	561 ± 7
45	125 ± 2	29 ± 1	94 ± 1	546 ± 12	132 ± 1	32 ± 2	96 ± 2	551 ± 16

[a] Data expressed as means ± SD; n = 6 chickens per group; cholesterol values expressed as mg/100 mL; HMGR values expressed as picomoles of mevalonic acid synthesized per minute per mg of microsomal protein.

ferent and that tocotrienols lacking the 5-methyl substituent present in α-tocotrienol possess significantly greater cholesterol synthesis suppressive activity. Furthermore, the racemic synthetic tocotrienols exhibit comparable biological activity to the natural tocotrienols in the cholesterol suppression assays (Tables V, VII). The triprenylated (farnesyl) analogues are more active in vitro than the diprenylated (geranyl)-containing compounds tested.

The data presented are consistent with the concept that a specific interaction of tocotrienols with a component of the regulatory mechanism controlling HMGR protein levels occurs in vitro in cells and in vivo.

The structure–activity relationships in the compounds studied suggest that γ- and δ-tocotrienols are optimal structures for this interaction. The hypocholesterolemic action and associated structure–activity relationship data of an expanded series of farnesylated benzopyrans is the subject of a forthcoming publication. Further mechanistic studies will reveal the nature of the macromolecular interactions coupling tocotrienols to HMGR expression.

Experimental Section

Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. Boiling points are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 1800 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AM 300 spectrometer or a Varian Gemini 300 NMR spectrometer; nuclear magnetic resonance (NMR) spectral characteristics refer to chemical shifts (δ) expressed in parts per million (ppm) with tetramethylsilane as an internal standard. Mass spectra were measured on a Finnegan 4500 spectrometer (low resolution) or a kratos MS50 spectrometer (high resolution).

Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. (visualization with iodine or phosphomolybdic acid); flash chromatography[43] was performed on fine silica (EM Sciences, 230–400 mesh). HPLC analyses were performed on a Spectra-Physics apparatus. All reactions were run under dry nitrogen unless otherwise indicated. Dry solvents were purchased from Aldrich, Milwaukee, WI in sure/seal bottles and transferred by syringe under nitrogen. Most commercially available starting materials did not require further purification.

Purification of Tocotrienol-Rich Fraction (TRF) from Palm Oil. Palm oil TRF was fractionated by flash chromatography, and the compounds were isolated without delay by solvent evaporation under vacuum and stored under nitrogen at −20 °C. A 1.059-g sample of palm oil was chromatographed on a 60 × 90 mm column of 230–400 mesh silica gel (gradient 40:1 to 30:1 hexanes–ether) taking 50-mL aliquots. Nine major fractions were recovered. Fractions 1 (160 mg), 3 (153 mg), 6 (158 mg), and 8 (76 mg) were one spot by TLC and were evaluated by 300-MHz PMR, IR, MS, and HPLC analysis.

Fractions 1, 3, 6, and 8 were shown to be >90% pure by PMR and HPLC and were identified as *d*-α-tocopherol, *d*-α-tocotrienol, *d*-γ-tocotrienol and *d*-δ-tocotrienol, respectively. Identification of these components was made from literature comparison of physical and spectroscopic data.[11]

HPLC analysis of the TRF revealed its composition to be *d*-α-tocopherol (26%), *d*-α-tocotrienol (18%), *d*-γ-tocotrienol (27%), and *d*-δ-tocotrienol (7%) by integration methods. The chromatographic separation of these components is possible, but is very tedious requiring large quantities of solvents and is limited to small amounts of palm oil. Treatment of the palm oil extract (23.2 g) with approximately 0.75 equiv of *tert*-butyldimethylsilyl chloride (based on MW ≃ 424, 6.18 g) and imidazole (3.7 g) in dimethylformamide (30 mL) for 18 h at 60 °C, preferentially silylates the δ-T3 followed by the γ-T3. Only traces of the α-T and α-T3 derivatize. After an ether extraction from water, the γ- and δ-T3 were isolated by flash chromatography (1:200 ether–hexanes) as a colorless oil (12 g). The free phenols are then regenerated in quantitative yield by treatment of the silyl ethers with tetra-*n*-butylammonium fluoride (23 mL, 1.0 M). The crude phenols were purified by flash chromatography (gradient 45:1 to 30:1 hexanes–ether) to yield 5.4 g of pure *d*-γ-T3 and 2.0 g of pure *d*-δ-T3.

***d*-α-Tocotrienol.** Light brown oil: IR (film) 3480, 2930, 1453, 1380, 1260, 1085 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.58 (s, 3 H), 1.60 (s, 6 H), 1.69 (s, 3 H), 1.81 (m, 2 H), 1.95–2.10 (m, 12 H), 2.12 (s, 6 H), 2.17 (s, 3 H), 2.63 (t, J = 6.9 Hz, 2 H), 4.18 (s, 1 H), 5.09–5.14 (m, 3 H); MS m/e 424 (M^+); $[\alpha]^{20}_D$ −2.9° (c = 1.0, $CHCl_3$).

***d*-γ-Tocotrienol.** Yellow oil: IR (film) 3420, 2930, 1450, 1430, 1225, 1080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3 H), 1.60 (s, 3 H), 1.61 (s, 6 H), 1.69 (s, 3 H), 1.79 (m, 2 H), 1.95–2.10 (m, 12 H), 2.13 (s, 3 H), 2.14 (s, 3 H), 2.69 (t, J = 6.4 Hz, 2 H), 4.19 (s, 1 H), 5.08–5.14 (m, 3 H), 6.38 (s, 1 H); MS m/e 410 (M^+); $[\alpha]^{20}_D$ −5.2° (c = 1.0, $CHCl_3$).

***d*-δ-Tocotrienol.** Pale yellow oil: IR (film) 3370, 2920, 1473, 1375, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3 H), 1.61 (s, 9 H), 1.69 (s, 3 H), 1.75 (m, 2 H), 1.95–2.10 (m, 12 H), 2.14 (s, 3 H), 2.71 (t, J = 6.8 Hz, 2 H), 4.17 (s, 1 H), 5.08–5.17 (m, 3 H), 6.39 (d, J = 2.8 Hz, 1 H), 6.49 (d, J = 2.9 Hz, 1 H); MS m/e 396 (M^+); $[\alpha]^{20}_D$ −2.2° (c = 1.0, $CHCl_3$).

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-propanal (1). A mixture (about 60:40)[30] of 2,5,7,8-tetramethyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-6-ol and its cyclized isomer (42.5 g, 0.15 mol) dissolved

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in 50 mL of dry THF was added dropwise to a cooled (5 °C) oil-free suspension of sodium hydride [6.5 g (60%), 0.16 mol] in 200 mL of dry THF. (2-Methoxyethoxy)methyl chloride (18.5 ml, 0.16 mole) was added dropwise to the mixture, initiating hydrogen release. After hydrogen evolution ceased, the solution was warmed to 23 °C and stirred an additional 18 h. The solution was poured into 1 N NaOH and the product was extracted into ether. The organic layers were washed with brine and dried (K_2CO_3). Evaporation in vacuo yielded 60 g of a yellow oil.

The above oil was used directly in the ozonolysis step, whereby it was dissolved in 400 mL of CH_2Cl_2 containing 12 mL of MeOH. The solution was cooled to −78 °C while ozone was bubbled through the mixture. The reaction was followed by TLC (1:1 Et_2O–hexanes), and when the less polar mixture of ethers was reduced to about $^1/_2$, the process was stopped. The mixture was warmed to about −5 °C and dimethyl sulfide (12 mL) was added. After stirring for 12 h at 23 °C, the volatile components were stripped off in vacuo leaving a yellow oil which was directly purified by flash chromatography (gradient 9:1 to 1:1 hexanes–Et_2O) to give 1, 26.18 g, as a pale yellow oil: IR (film) 2925, 1725, 1450, 1250, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.7–2.0 (m, 4 H), 2.05 (s, 3 H), 2.13 (s, 3 H), 2.17 (s, 3 H), 2.62 (m, 4 H), 3.43 (s, 3 H), 3.61 (t, 2 H), 3.97 (t, 2 H), 4.92 (s, 2 H), 9.79 (s, 1 H); MS *m/e* 351 (MH+). Anal. ($C_{20}H_{30}O_5$) C, H.

Ethyl 5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-pentenoate (3). A benzene solution (100 mL) of aldehyde 1 (5.0 g, 14.3 mmol) and ethyl 2-(triphenylphosphoranylidene)propionate (7.76 g, 21.4 mmol) was heated to reflux for 30 min, at which time TLC (2:1 Et_2O–hexanes) indicated a trace of aldehyde remaining that did not react with further heating. An additional amount of phosphorane (1.56 g, 4.29 mmol) was added, and the mixture was heated for an additional 30 min at which time TLC analysis indicated the reaction to be complete. The benzene was stripped in vacuo and the solid material was triturated with hexanes and filtered. Concentration of the hexanes yielded 7.3 g of a yellow oil which was purified by flash chromatography (gradient 12:1 to 4:1 hexanes–Et_2O). A 1.14-g mixture of the *Z* (23%, PMR) and *E* isomers eluted first, followed by 3.10 g (7.14 mmol, 50%) of the pure *E*-ester **3**, which was purified for analysis by Kugelrohr distillation (bath 210 °C/0.1 mm) yielding a colorless oil: IR (film) 2978, 2931, 2878, 1710, 1459, 1399, 1252, 1098, 981 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.26 (t, *J* = 6.1 Hz, 3 H), 1.58–1.85 (m, 4 H), 1.80 (s, 3 H), 2.06 (s, 3 H), 2.12 (s, 3 H), 2.16 (s, 3 H), 2.27–2.34 (m, 2 H), 2.57 (t, *J* = 5.7 Hz, 2 H), 3.38 (s, 3 H), 3.59 (m, 2 H), 3.93 (m, 2 H), 4.15 (q, *J* = 6.1 Hz, 2 H), 4.92 (s, 2 H), 6.75 (t, *J* = 6.2 Hz, 1 H); MS *m/e* 435 (MH^+). Anal. ($C_{25}H_{38}O_6$) C, H.

5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-penten-1-ol (4). To a suspension of $LiAlH_4$ (1.14 g, 30 mmol) in 70 mL of dry ether cooled to −5 °C was added $AlCl_3$ (1.33 g, 10 mmol) portionwise. After stirring the slurry of alane for 0.5 h at −5 °C, ester **3** (2.85 g, 6.58 mmol) was added as an ether solution (20 mL) dropwise over a period of 0.5 h. After 0.25 h at −5 °C, TLC (1:1 hexanes–Et_2O) indicated complete reduction of the ester and the mixture was quenched by the slow addition of saturated Na_2SO_4 solution. The aluminum salts were filtered and washed well with methanol. The combined organic fractions were washed with water, extracted into fresh ether, and dried (brine, $MgSO_4$). Concentration in vacuo gave a colorless oil (2.39 g, 6.09 mmol, 93%) of **4**; an analytical sample was prepared by Kugelrohr distillation (TLC indicated trace decomposition): IR (film) 3460, 2940, 1465, 1260, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.25–1.85 (m, 4 H), 1.65 (s, 3 H), 2.06 (s, 3 H), 2.07–2.19 (m, 2 H), 2.12 (s, 3 H), 2.16 (s, 3 H), 2.56 (t, *J* = 5.6 Hz, 2 H), 3.38 (s, 3 H), 3.59 (m, 2 H), 3.94 (m, 4 H), 4.92 (s, 2 H), 5.40 (t, *J* = 5.02 Hz, 1 H); MS *m/e* 392 (M+). Anal. ($C_{23}H_{36}O_5$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2-[6-[(4-methylphenyl)sulfonyl]-4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl]-2*H*-1-benzopyran (7). Dimethyl sulfide (0.59 mL, 7.98 mmol) was added dropwise to a solution of *N*-chlorosuccinimide (977 mg, 7.32 mmol) in 25 mL of dry CH_2Cl_2 cooled to −5 °C. The white suspension was stirred for 0.25 h while the alcohol **4** (2.61 g, 6.66 mmol) was added as a CH_2Cl_2 solution (5 mL). The clear solution was stirred for 1.5 h at −5 °C at which time TLC (2:1 hexanes–Et_2O) indicated complete conversion to a less polar spot. The CH_2Cl_2 was removed in vacuo (<40 °C) leaving an oily solid. The material was triturated with hexanes, filtered, and concentrated in vacuo (<40 °C) to a pale yellow oil. The oil was immediately flushed through a pad of silica gel (gradient 8:1 to 4:1 hexanes–Et_2O) to give the unstable chloride **5** as a pale yellow oil (2.44 g, 5.95 mmol, 89%). **3,7-Dimethyl-1-(*p*-tolylsulfonyl)-2(*E*),6-octadiene[44] (6)** (2.09 g, 7.14 mmol) was dissolved in 15 mL of a dry THF/HMPA 4:1 mixture. The solution was cooled to −78 °C under nitrogen, and butyllithium [2.98 mL (2.5 M hexanes), 7.44 mmol] was added dropwise giving rise to a red-orange anion. After stirring for 2 h at −78 °C, chloride **5** (2.44 g, 5.95 mmole) was added dropwise as a THF solution (3 mL) to the anion and stirring was continued at −78 °C for 4 h. The mixture was quenched at −78 °C with pH 7.0 buffer solution, poured into water, and extracted into ether. The organic layers were dried (brine, $MgSO_4$) and concentrated in vacuo to give a yellow oil which was purified by flash chromatography (gradient 8:1 to 4:1 hexanes–Et_2O) to yield the sulfone **7** (3.72 g, 5.59 mmol, 94%) as pale yellow oil: IR (film) 2950, 1460, 1150, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16 (d, 3 H), 1.18 (s, 3 H), 1.47, 1.48 (s, 3 H), 1.4–1.5 (m, 4 H), 1.56 (s, 3 H), 1.66 (s, 3 H), 1.75 (m, 2 H), 1.89 (m, 4 H), 2.03 (s, 3 H), 2.10 (s, 3 H), 2.14 (s, 3 H), 2.21 (t, *J* = 9.98 Hz, 1 H), 2.41 (s, 3 H), 2.53 (t, *J* = 5.68 Hz, 2 H), 2.82 (d, *J* = 10.51 Hz, 1 H), 3.38 (s, 3 H), 3.58 (m, 2 H), 3.83 (m, 1 H), 3.93 (m, 2 H), 4.84 (d, *J* = 8.62 Hz, 1 H), 4.91 (s, 2 H), 4.99 (m, 1 H), 5.13 (t, *J* = 5.77 Hz, 1 H), 7.27 (d, *J* = 6.71 Hz, 2 H), 7.68 (d, *J* = 6.72 Hz, 2 H); MS *m/e* 666 (M+). Anal. ($C_{40}H_{58}O_6S_1$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl)-2*H*-1-benzopyran (8). Sulfone **7** (3.64 g, 5.47 mmol) was dissolved in 25 mL of dry THF and cooled to 0 °C under nitrogen. Palladium [1,4-bis(diphenylphosphino)butane] chloride (165 mg, 0.27 mmol) was added to the THF solution. Lithium triethylborohydride [10.9 mL (1.0 M THF), 10.9 mmol] was added dropwise to the suspension of $PdCl_2$-dppb over a period of 10 min. The yellow-tan heterogeneous mixture goes to a clear brown homogeneous solution upon addition of all the hydride. The mixture was stirred for 5 h at 0 °C and then at −20 °C for 12 h at which time TLC (1:1 hexanes–Et_2O) indicated the reaction to be approximately 80% complete. An additional amount of palladium catalyst (41 mg, 0.07 mmol) was added, and the mixture was stirred an additional 4 h at 0 °C at which time TLC indicated complete conversion. The reaction mixture was quenched at 0 °C with excess potassium cyanide in 1 N NaOH, and then poured into water and extracted with ether. The organic layers were washed with water, dried (brine, $MgSO_4$), and concentrated in vacuo to give a light brown oil. Purification by flash chromatography (gradient 20:1 to 10:1 hexanes–Et_2O) yielded **8** (2.68 g, 5.22 mmol, 96%) as a colorless oil: IR (film) 2960, 1460, 1063, 985 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 1.58 (s, 9 H), 1.66 (s, 3 H), 1.77 (m, 2 H), 1.90–2.2 (m, 12 H), 2.07 (s, 3 H), 2.12 (s, 3 H), 2.16 (s, 3 H), 2.56 (t, *J* = 6.77 Hz, 2 H), 3.39 (s, 3 H), 3.59 (m, 2 H), 3.94 (m, 2 H), 4.92 (s, 2 H), 5.10 (m, 3 H); MS *m/e* 512 (M+). Anal. ($C_{33}H_{52}O_4$) C, H.

3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl)-2*H*-1-benzopyran-6-ol [*d,l*-α-Tocotrienol] (9). MEM ether **8** (1.27 g, 2.48 mmol) was dissolved in 6 mL of dry CH_2Cl_2 and cooled to −78 °C under N_2. 2-Chloro-1,3,2-dithioborolan (0.47 mL, 4.96 mmol) was added to the CH_2Cl_2 solution, and the mixture was placed into a −20 °C freezer for 20 h. The solution was poured into saturated $NaHCO_3$ solution, extracted with fresh CH_2Cl_2, dried ($MgSO_4$), and concentrated in vacuo to give a foul-smelling oil. The oil was purified by flash chromatography (20:1 hexanes–Et_2O) to yield α-tocotrienol (1.07 g, 2.52 mmol) as a colorless oil which still smelled of sulfur but was one spot by TLC (2:1 hexanes–Et_2O). The product was further purified by crystallization (×3) from cold (−78 °C) pentane to give an odorless white solid: mp 25–28 °C; 716 mg, 1.69 mmole, 68%. HPLC analysis (IB-Sil C18; 91:9 MeCN–H_2O) indicated one major band of >94% purity by total integration. α-Tocotrienol (**9**): IR (film) 3460, 2940, 1460, 1380, 1260, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.60 (s, 3 H), 1.61 (s, 6 H), 1.69 (s, 3 H), 1.80 (m, 2 H), 1.95–2.12 (m, 12 H), 2.12

(44) Gosselin, P.; Maignan, C.; Rouessac, F. Stereospecific Synthesis of Homogeranic and Homoneric Acids. *Synthesis* **1984**, 876–881.

(s, 6 H), 2.17 (s, 3 H), 2.62 (t, J = 6.8 Hz, 2 H), 4.18 (s, 1 H), 5.11 (m, 3 H); MS m/e 424 (M+). Anal. ($C_{29}H_{44}O_2$) C, H.

Ethyl 2,6-Dimethyl-8-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2(*E*),6(*E*)-octadienoate (11). Aldehyde 10[37] (20.29 g, 0.1 mol) and ethyl 2-(triphenylphosphoranylidene)propionate (41.96 g, 0.12 mol) were dissolved, with cooling (0 °C, 30 min) in 300 mL of dry CH_2Cl_2. After 18 h at 23 °C, TLC (2:1 hexanes–Et_2O) indicated the reaction complete and the solvent was removed in vacuo. The oily yellow solid was triturated with 1:1 hexanes–ether, and the solid material was removed by filtration. The solvents were removed in vacuo to give a yellow oil which was purified by flash chromatography (gradient 15:1 to 7:1 hexanes–Et_2O) to give **11** (26.7 g, 0.09 mol, 93%) as a colorless oil: IR (film) 2952, 1715, 1272, 1120, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (t, J = 7.8 Hz, 3 H), 1.55 (m, 4 H), 1.73 (s, 3 H), 1.78 (m, 2 H), 1.85 (s, 3 H), 2.1–2.4 (m, 4 H), 3.52 (m, 1 H), 3.90 (M, 1 H), 4.07 (m, 2 H), 4.19 (q, J = 7.8 Hz, 2 H), 4.65 (t, J = 3 Hz, 1 H), 5.4 (t, J = 7.5 Hz, 1 H), 6.75 (t, J = 7 Hz, 1 H); MS m/e 297 (MH+). Anal. ($C_{17}H_{28}O_4$) C, H.

Ethyl 2,6-Dimethyl-8-hydroxy-2(*E*),6(*E*)-octadienoate (12). THP ether **11** (10.20 g, 0.034 mol) was dissolved in 300 mL of absolute ethanol containing pyridinium tosylate (700 mg, 2.79 mmol), and the mixture was heated under reflux for 2 h. The solution was poured into water, and the ester was extracted into ether. The ether layers were washed with water, dried (brine, $MgSO_4$), and concentrated in vacuo to an oil (7.1 g). The product was purified by flash chromatography (gradient 10:1 to 1:1 hexanes–ether) to yield 980 mg of an oil which contained the title compound contaminated by the less polar 2*E*,6*Z* isomer, followed by the all *trans*-ester **12** (6.06 g, 0.029 mol, 84%) as a colorless oil which was Kugelrohr-distilled for analytical purposes: IR (film) 3420, 2994, 2944, 1715, 1280, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, J = 6.5 Hz, 3 H), 2.71 (s, 3 H), 2.87 (s, 3 H), 2.08–2.20 (m, 4 H), 4.19 (m, 4 H), 5.44 (t, J = 6.5 Hz, 1 H), 6.73 (t, J = 6.5 Hz, 1 H); MS m/e 213 (MH^+). Anal. ($C_{12}H_{20}O_3$) C, H.

Ethyl 5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-pentenoate (15a). 2,3-Dimethylhydroquinone (4.18 g, 0.03 mol) and ester **12** (3.21 g, 0.015 mol) were dissolved in warm (65 °C) dry dioxane (50 mL) under nitrogen. Boron trifluoride etherate (0.47 mL, 3.8 mmol) was added, and the mixture was stirred under nitrogen for 3 h at 65 °C at which time TLC (3:2 hexanes–Et_2O) indicated complete reaction. The solvent was removed in vacuo, and the residue was dissolved in ether. The ether layers were washed successively with aqueous sodium bicarbonate and sodium hydrosulfite, then dried (brine, $MgSO_4$), and concentrated in vacuo to an oily solid. The residue was triturated with 3:1 (hexanes–Et_2O) to remove unreacted 2,3-dimethylhydroquinone by filtration. Concentration of the organic layers in vacuo yielded 4.93 g of crude **13a** isolated as an amber oil.

The above oil is very oxidatively unstable and was immediately dissolved in 200 mL of benzene containing *p*-toluenesulfonic acid monohydrate (500 mg, 2.63 mmol). The benzene solution was heated for 2 h under reflux, with water removal facilitated by a Dean–Stark apparatus. The cooled benzene mixture was washed with aqueous $NaHCO_3$, dried (brine, $MgSO_4$), and stripped in vacuo to give crude **14a** isolated as a dark gum (4.86 g).

The above gum was dissolved in 200 mL of dry THF and cooled to 5 °C under nitrogen. Sodium hydride (876 mg, 50%, 18.25 mmol) and (2-methoxyethoxy)methyl chloride (2.0 mL, 17.52 mmol) were added to the THF solution, and the mixture was stirred for 2 h at 5 °C and then for 1 h at 23 °C. The major portion of THF was removed in vacuo. The mixture was then poured into water and extracted into ether. The organic layers were dried (brine, $MgSO_4$) and concentrated in vacuo to give 6.34 g of a light brown oil which was purified by flash chromatography (gradient 9:1 to 6:1 hexanes–ether) to give **15a** (2.66 g, 6.33 mmol, 42%) as a colorless oil: IR (film) 2994, 2950, 1715, 1483, 1247, 1243, 1100, 745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 3 H), 1.26 (t, J = 7.08 Hz, 3 H), 1.58–1.80 (m, 4 H), 1.81 (s, 3 H), 2.08 (s, 3 H), 2.11 (s, 3 H), 2.30 (q, J = 8.0 Hz, 2 H), 2.71 (m, 2 H), 3.38 (s, 3 H), 3.56 (m, 2 H), 2.81 (m, 2 H), 4.15 (q, J = 7.08 Hz, 2 H), 5.17 (s, 2 H), 6.68 (s, 1 H), 6.75 (t, J = 7.35 Hz, 1 H); MS m/e 420 (M+). Anal. ($C_{24}H_{36}O_6$) C, H.

5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-penten-1-ol (16a). A suspension of aluminum hydride in ether (100 mL) was prepared from lithium aluminum hydride (1.56 g, 41 mmol) and aluminum chloride (1.82 g, 13.7 mmole). Ester **15a** (3.83 g, 9.11 mmol) was added to the alane slurry at −5 °C as an ether solution (30 mL) over 0.5 h, and stirring was continued an additional 20 minutes at −5 °C. The mixture was quenched at −5 °C by the careful addition of saturated sodium sulfate. The suspension was filtered, and the recovered aluminum salts were triturated with hot methanol and refiltered. The organic layers were combined, washed with water, dried (brine, $MgSO_4$), and concentrated in vacuo to a thick oil (2.80 g) which was one spot by TLC (1:1 hexanes–Et_2O). The recovered oil was passed through a pad of silica gel (1:1 hexanes–Et_2O) to yield (2.80 g, 7.41 mmol, 81%) **16a** as a colorless oil of which a portion was Kugelrohr-distilled for analysis (bath 200–220 °C/0.1mm): IR (film) 3420, 2932, 1483, 1235, 1100, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.50–1.82 (m, 4 H), 1.64 (s, 3 H), 2.09 (s, 3 H), 2.11 (s, 3 H), 2.16 (m, 2 H), 2.69 (m, 2 H), 3.37 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 3.96 (s, 2 H), 5.16 (s, 2 H), 5.40 (t, J = 7.19 Hz, 1 H), 7.68 (s, 1 H); MS m/e 378 (M+). Anal. ($C_{22}H_{34}O_5$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2-(5-chloro-4-methyl-3(*E*)-pentene)-2*H*-1-benzopyran (17a). The procedure for the preparation of chloride **17a** from alcohol **16a** (2.71 g, 7.17 mmol) follows that described for chloride **5**. The crude chloride **17a** was purified by flash chromatography (gradient 8:1 to 4:1 hexanes–Et_2O) to give 2.72 g (6.86 mmol, 96%) of a colorless oil: IR (film) 2940, 1482, 1238, 1103, 1065, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 1.50–1.82 (m, 4 H), 1.71 (s, 3 H), 2.08 (s, 3 H), 2.10 (s, 3 H), 2.16 (m, 2 H), 2.69 (m, 2 H), 3.37 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 3.98 (s, 2 H), 5.16 (s, 2 H), 5.52 (t, J = 7.0 Hz, 1 H), 6.67 (s, 1 H); MS m/e 396 (M+). Exact mass calcd for $C_{22}H_{33}Cl_1O_4$: 396.2067. Found: 396.2069.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2-[6-[(4-methylphenyl)sulfonyl]-4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl]-2*H*-1-benzopyran (18a). The procedure for the preparation of sulfone **18a** from chloride **17a** (2.72 g, 6.86 mmol) follows that described for sulfone **7**. The crude material was purified by flash chromatography (gradient 8:1 to 2:1 hexanes–Et_2O) to yield sulfone **18a** (4.03 g, 6.18 mmol, 90%) as a colorless oil: IR (film) 2980, 2930, 1600, 1480, 1455, 1145, 665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (d, J = 4.2 Hz, 3 H), 1.19 (s, 3 H), 1.40–1.73 (m, 4 H), 1.49 (s, 3 H), 1.56 (s, 3 H), 1.66 (s, 3 H), 1.90 (m, 4 H), 2.07 (m, 2 H), 2.05 (s, 3 H), 2.10 (s, 3 H), 2.21 (t, J = 11.96 Hz, 1 H), 2.41 (s, 3 H), 2.66 (m, 2 H), 2.83 (d, J = 13.0 Hz, 1 H), 3.37 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 3.85 (m, 1 H), 4.85 (d, J = 10.05 Hz, 1 H), 4.99 (m, 1 H), 5.13 (m, 1 H), 5.15 (s, 2 H), 6.66 (s, 1 H), 7.27 (d, J = 8.20 Hz, 2 H), 7.68 (d, J = 8.13 Hz, 2 H); MS m/e 652 (M^+). Anal. ($C_{39}H_{56}O_6S_1$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2-(4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl)-2*H*-1-benzopyran (19a). The procedure for the preparation of the MEM-protected γ-tocotrienol from sulfone **18a** (3.95 g, 6.05 mmol) follows that described for MEM-protected α-tocotrienol **8**. The crude tocotrienol was purified by flash chromatography (gradient 18:1 to 9:1 hexanes–Et_2O) to give **19a** (2.83 g, 5.68 mmol, 94%) as a colorless oil. A portion was Kugelrohr-distilled for analysis (bath 220 °C/0.1 mm): IR (film) 2950, 1482, 1450, 1103, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.50–1.82 (m, 4 H), 1.58 (s, 9 H), 1.66 (s, 3 H), 1.90–2.10 (m, 10 H), 2.09 (s, 3 H), 2.11 (s, 3 H), 2.69 (t, J = 6.29 Hz, 2 H), 3.38 (s, 3 H), 3.57 (m, 2 H), 3.82 (m, 2 H), 3.82 (m, 2 H), 5.08 (m, 3 H), 5.17 (s, 2 H), 6.68 (s, 1 H); MS m/e 498 (M+). Anal. ($C_{32}H_{50}O_4 \cdot 0.4\ H_2O$) C, H.

3,4-Dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltrideca-3-(*E*),7(*E*),11-trienyl)-2*H*-1-benzopyran-6-ol [γ-Tocotrienol] (20). The procedure for the preparation of *d,l*-γ-tocotrienol (**20**) from the MEM ether **19a** (2.75 g, 5.53 mmol) follows that described for α-tocotrienol. The crude tocotrienol was purified by flash chromatography (gradient 18:1 to 12:1 hexanes–Et_2O) to give a sample of γ-tocotrienol (**20**) (2.13 g, 5.2 mmol, 94%) which smelled of sulfur. The odiferous oil was rechromatographed (gradient 16:1 to 9:1 hexanes–Et_2O) to yield γ-tocotrienol (1.70 g, 4.15 mmol, 75%) as an odorless, pale yellow oil which was >94% pure as determined by HPLC (IB-Sil C18 91:9 acetonitrile–H_2O) integration: IR (film) 3420, 2980, 2940, 2860, 1440, 1215, 1081 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (s, 3 H), 1.59 (s, 3 H), 1.60 (s, 6 H), 1.69 (s, 3 H), 1.77 (m, 2 H), 1.95–2.10 (m, 12 H), 2.12 (s, 3 H), 2.13 (s, 3 H), 2.68 (t, J = 6.2 Hz, 2 H), 4.22 (s, 1 H), 5.07–5.15 (m, 3 H), 6.37 (s, 1 H); MS m/e 411 (MH+). Anal. ($C_{28}H_{42}O_2$) C, H.

Ethyl 5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2-methyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-pentenoate (15b). The sequence of steps for the preparation of ester **13b** from the allylic alcohol **12** (9.8 g, 0.046 mol) follows those described for ester **13a**. Hydroquinone ester **13b** (7.20 g, 0.023 mol, 52%) isolated as a thick yellow oil: IR (film) 3400, 2994, 2940, 1690, 1457, 1280, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.08 Hz, 3 H), 1.63 (s, 3 H), 1.84 (s, 3 H), 2.28–2.39 (m, 4 H), 3.28 (d, J = 7.54 Hz, 2 H), 4.21 (q, J = 7.08 Hz, 2 H), 4.46 (s, 1 H), 5.34 (t, J = 7.59 Hz, 1 H), 6.51 (s, 1 H), 6.53–6.67 (m, 2 H), 6.94 (t, J = 6.80 Hz, 1 H); MS m/e 304 (M+). Anal. ($C_{18}H_{24}O_4$·0.1 H_2O) C, H.

Benzopyranol 14b was (1.50 g, 4.93 mmol, 94%) isolated as a pale yellow oil: IR (film) 3420, 2980, 2950, 1715, 1690, 1500, 1290, 1220, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.12 Hz, 3 H), 1.26 (s, 3 H), 1.60–1.90 (m, 4 H), 1.80 (s, 3 H), 2.28 (q, J = 8.24 Hz, 2 H), 2.70 (t, J = 6.46 Hz, 2 H), 4.15 (q, J = 7.11 Hz, 2 H), 4.47 (s, 1 H), 6.52–6.65 (m, 3 H), 6.73 (t, J = 6.16 Hz, 1 H); MS m/e 304 (M+). Anal. ($C_{18}H_{24}O_4$) C, H.

MEM Ether 15b was (1.1 g, 2.81 mmol, 61%) isolated as a colorless oil: IR (film) 2990, 2946, 1710, 1500, 1100, 1020, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.11 Hz, 3 H), 1.27 (s, 3 H), 1.57–1.83 (m, 4 H), 1.80 (s, 3 H), 2.28 (q, J = 7.78 Hz, 2 H), 2.72 (t, J = 6.3 Hz, 2 H), 3.36 (s, 3 H), 3.55 (m, 2 H), 3.80 (m, 2 H), 4.15 (q, J = 7.09 Hz, 2 H), 5.16 (s, 2 H), 6.62–6.81 (m, 4 H); MS m/e 392 (M+). Anal. ($C_{22}H_{32}O_6$) C, H.

5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2-methyl-2*H*-1-benzopyran-2-yl]-2-methyl-2(*E*)-penten-1-ol (16b). The procedure for the preparation of allylic alcohol **16b** from the ester **15b** (1.0 g, 2.55 mmol) follows that described for alcohol **16a**. The alcohol **16b** (0.8 g, 2.29 mmol, 90%) was recovered as a colorless oil: IR (film) 3440, 2984, 2940, 1500, 1230, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 4 H), 1.50–1.86 (m, 4 H), 1.63 (s, 3 H), 2.13 (q, J = 7.91 Hz, 2 H), 2.71 (t, J = 6.73 Hz, 2 H), 3.36 (s, 3 H), 3.56 (m, 2 H), 3.80 (m, 2 H), 3.96 (d, J = 5.74 Hz, 2 H), 5.15 (s, 2 H), 5.38 (t, J = 7.07 Hz, 1 H), 6.62–6.81 (m, 3 H); MS m/e 350 (M+). Anal. ($C_{20}H_{30}O_5$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2-methyl-2-(5-chloro-4-methyl-3(*E*)-pentene)-2*H*-1-benzopyran (17b). The procedure for the preparation of allylic chloride **17b** from the alcohol **16b** (4.20 g, 12 mmol) follows that described for allylic chloride **17a**. The allylic chloride **17b** (3.1 g, 8.41 mmol, 70%) was isolated as a colorless oil: IR (film) 2990, 2950, 1500, 1230, 1107, 1020, 685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.50–1.82 (m, 4 H), 1.70 (s, 3 H), 2.13 (q, J = 7.22 Hz, 2 H), 2.69 (t, J = 6.46 Hz, 2 H), 3.35 (s, 3 H), 3.54 (m, 2 H), 3.78 (m, 2 H), 3.96 (s, 2 H), 5.14 (s, 2 H), 5.49 (t, J = 6.08, 1 H), 6.62–6.80 (m, 3 H); MS m/e 368 (M+). Anal. ($C_{20}H_{29}Cl_1O_4$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2-methyl-2-[6-[(4-methylphenyl)sulfonyl]-4,8,12-trimethyltrideca-3-(*E*),7(*E*),11-trienyl]-2*H*-1-benzopyran (18b). The procedure for the preparation of sulfone **18b** from the chloride **16b** (3.0 g, 8.14 mmol) follows that described for sulfone **18a**. The sulfone adduct **18b** (4.50 g, 7.21 mmol, 89%) was isolated as a colorless oil: IR (film) 2990, 2940, 1500, 1310, 1150, 820, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J < 5 Hz, 3 H), 1.20 (s, 3 H), 1.40–1.80 (m, 4 H), 1.49 (s, 3 H), 1.56 (s, 3 H), 1.66 (s, 3 H), 1.88 (m, 4 H), 2.00 (m, 2 H), 2.20 (d, J = 12.15 Hz, 1 H), 2.40 (s, 3 H), 2.67 (m, 2 H), 2.80 (d, J = 13.0 Hz, 1 H), 3.36 (s, 3 H), 3.56 (m, 2 H), 3.79 (m, 2 H), 3.84 (m, 1 H), 4.83 (d, J = 9.87 Hz, 1 H), 4.97 (m, 1 H), 5.09 (m, 1 H), 5.14 (s, 2 H), 6.58–6.79 (m, 3 H), 7.25 (d, J = 8.20 Hz, 2 H), 7.67 (d, J = 8.15 Hz, 2 H); MS m/e 624 (M+). Anal. ($C_{37}H_{52}O_6S_1$·0.2H_2O) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2-methyl-2-(4,8,12-trimethyltrideca-3(*E*),7(*E*),11-trienyl)-2*H*-1-benzopyran (19b). The procedure for the preparation of MEM ether **19b** from the sulfone **18b** (0.4 g, 6.41 mmol) follows that described for MEM ether **19a**. The MEM ether **19b** (2.0 g, 4.25 mmol, 66%) was isolated as a colorless oil: IR (film) 2990, 2940, 1500, 1225, 1110, 1025, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.50–1.84 (m, 4 H), 1.59 (s, 9 H), 1.66 (s, 3 H), 1.90–2.14 (m, 10 H), 2.71 (t, J = 6.7 Hz, 2 H), 3.37 (s, 3 H), 3.56 (m, 2 H), 3.81 (m, 2 H), 5.09 (m, 3 H), 5.16 (s, 2 H), 6.63–6.81 (m, 3 H); MS m/e 470 (M+). Anal. ($C_{30}H_{46}O_4$) C, H.

3,4-Dihydro-2-methyl-2-(4,8,12-trimethyltrideca-3(*E*),7-(*E*),11-trienyl)-2*H*-1-benzopyran-6-ol (Tocotrienol) (21). The procedure for the preparation of tocotrienol (**21**) from the MEM ether **19b** (700 mg, 1.49 mmol) follows that described for γ-tocotrienol. Tocotrienol (**21**) (400 mg, 1.05 mmol, 70%) was isolated as a colorless oil: IR (film) 3400, 2990, 2950, 2876, 1500, 1458, 1240, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.50–1.84 (m, 4 H), 1.58 (s, 9 H), 1.66 (s, 3 H), 1.90–2.15 (m, 10 H), 2.69 (t, J = 6.73 Hz, 2 H), 4.23 (s, 1 H), 5.07 (m, 3 H), 6.50–6.70 (m, 3 H); MS m/e 383 (MH+). Anal. ($C_{26}H_{38}O_2$·0.5 H_2O) C, H.

Ethyl 6-Methyl-8-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2(*E*),6-(*E*)-octadienoate (24). Aldehyde **10** (15.0 g, 0.071 mol) was added dropwise to a THF solution (50 mL) of triethyl phosphonoacetate (16.0 g, 0.071 mol), triethylamine (8.1 g, 0.08 mol), and lithium bromide (7.4 g, 0.08 mol). The mixture was stirred for 15 h and poured into water. The water solution was extracted with ether, and the organic layers were dried (brine, MgSO4) and concentrated in vacuo to an oil (20 g). The oil was purified by flash chromatography (gradient 20:1 to 10:1 hexanes–ether) to yield a mixture of *E* and *Z* esters (8.20 g, 0.029 mol) followed by a fraction of the pure *E*-ester **24** (8.20 g, 0.029 mol, 41%) as a colorless oil: IR (film) 2950, 1728, 1270, 1210, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.2 Hz, 3 H), 1.50 (m, 6 H), 1.65 (s, 3 H), 1.7–1.85 (m, 2 H), 2.15 (m, 2 H), 2.32 (m, 2 H), 3.47 (m, 1 H), 3.85 (m, 1 H), 4.0 (m, 1 H), 4.16 (m, 3 H), 4.57 (m, 1 H), 5.35 (t, J = 6.4 Hz, 1 H), 5.79 (d, J = 15.6 Hz, 1 H), 6.91 (d of t, J = 15.6, 6.70 Hz, 1 H); MS m/e 283 (MH+). Anal. ($C_{16}H_{26}O_4$) C, H.

Ethyl 6-Methyl-8-hydroxy-2(*E*),6(*E*)-octadienoate (25). The THP ether **24** (8.20 g, 29 mmol) was cleaved to the alcohol using the same procedure as that described for alcohol **12**. Alcohol **25** was obtained as a colorless oil (4.30 g, 22 mmol, 75%): IR (film) 3430, 2940, 1725, 1280, 1207, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3 H), 1.65 (s, 3 H), 2.14 (m, 2 H), 2.32 (m, 2 H), 4.14 (m, 5 H), 5.40 (t, J = 5.7 Hz, 1 H), 5.79 (d, J = 15.5 Hz, 1 H), 6.91 (d of t, J = 15.5, 6.8 Hz, 1 H); MS m/e 198 (M+). Anal. ($C_{11}H_{18}O_3$) C, H.

Ethyl 5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2*H*-1-benzopyran-2-yl]-2(*E*)-pentenoate (28). The sequence of events leading to **28** from the allylic alcohol **25** (4.0 g, 20.2 mmol) follows that for **15** (Scheme II). The alkylated hydroquinone **26** (4.4 g, 13.8 mmol, 68%) was isolated as an unstable oil. The phenolic cyclization adduct **27** (3.8 g, 11.9 mmol, 86%) was isolated as a thick colorless oil: IR (film) 3440, 2940, 1728, 1706, 1435, 1230, 1085 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.26 (t, J = 7.1 Hz, 3 H), 1.58–1.84 (m, 4 H), 2.08 (s, 3 H), 2.12 (s, 3 H), 2.32 (q, J = 8.4 Hz, 2 H), 2.67 (m, 2 H), 4.16 (q, J = 7.1 Hz, 2 H), 5.80 (d, J = 15.6 Hz, 1 H), 6.36 (s, 1 H), 6.98 (d of t, J = 15.6, 6.8 Hz, 1 H); MS m/e 318 (M+). Anal. ($C_{19}H_{26}O_4$) C, H.

The phenol **27** (3.5 g, 11.0 mmol) was protected using the same procedure as that described for **15**. The MEM ether **28** (4.0 g, 9.85 mol, 90%) was isolated as a colorless oil: IR (film) 2940, 1725, 1485, 1108, 1065, 990 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 3 H), 1.26 (t, J = 7.1 Hz, 3 H), 1.60–1.86 (m, 4 H), 2.07 (s, 3 H), 2.11 (s, 3 H), 2.35 (q, J = 7.3 Hz, 2 H), 2.71 (m, 2 H), 3.38 (s, 3 H), 3.56 (m, 2 H), 3.81 (m, 2 H), 4.15 (q, J = 7.1 Hz, 2 H), 5.17 (s, 2 H), 5.81 (d, J = 15.7 Hz, 1 H), 6.68 (s, 1 H), 6.98 (d of t, J = 15.7, 6.8 Hz, 1 H); MS m/e 406 (M+). Anal. ($C_{23}H_{34}O_6$) C, H.

5-[3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2*H*-1-benzopyran-2-yl]-2(*E*)-penten-1-ol (29). The title compound was prepared from the ester **28** (3.4 g, 8.37 mmol) in the same manner as that described for allylic alcohol **16** (Scheme II). The allylic alcohol **29** (2.8 g, 7.69 mmol, 92%) was isolated as a colorless oil: IR (film) 3440, 2940, 1485, 1245, 1100, 1065, 1005, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 1.45 (br s, 1 H), 1.53–1.80 (m, 4 H), 2.07 (s, 3 H), 2.11 (s, 3 H), 2.18 (m, 2 H), 2.70 (m, 2 H), 3.37 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 4.05 (d, J = 5.0 Hz, 2 H), 5.16 (s, 2 H), 5.67 (m, 2 H), 6.67 (s, 1 H); MS m/e 364 (M+). Anal. ($C_{21}H_{32}O_5$) C, H.

3,4-Dihydro-6-[(2-methoxyethoxy)methoxy]-2,7,8-trimethyl-2-[6-[(4-methylphenyl)sulfonyl]-8-methyl-3(*E*),7-nonadienyl]-2*H*-1-benzopyran (31). The route to the sulfone **31** proceeds as described for **18** (Scheme II), starting with the alcohol **29** (2.50 g, 6.87 mmol). The allylic chloride **30** was isolated as a pale yellow oil (1.70 g, 4.45 mmol, 65%). The sulfone **31** (2.2 g, 3.86 mmol, 87%) was isolated as a yellow oil: IR (film) 2940, 1450, 1310, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.16 (m, 3 H), 1.19 (s, 3 H), 1.46–1.76 (m, 4 H), 1.65 (s, 3 H), 2.05 (s, 3 H), 2.10 (s, 3 H), 2.29 (m, 1 H), 2.42 (s, 3 H), 2.66 (m, 2 H), 2.76 (m, 1 H), 3.37 (s,

3 H), 3.45 (q, J = 7.0 Hz, 1 H), 3.55 (m, 2 H), 3.67 (m, 1 H), 3.82 (m, 2 H), 4.91 (d, J = 9.0 Hz, 1 H), 5.15 (s, 2 H), 5.22 (m, 1 H), 5.47 (m, 1 H), 6.66 (s, 1 H), 7.27 (d, J = 8.1 Hz, 2 H), 7.66 (d, J = 8.1 Hz, 2 H); MS m/e 570 (M+). Anal. ($C_{33}H_{46}O_6S_1$) C, H.

6-[(2-Methoxyethoxy)methoxy]-2,7,8-trimethyl-2-(8-methyl-3(*E*),7-nonadienyl)-2*H*-1-benzopyran (32). The title compound was prepared from the sulfone **31** (2.10 g, 2.68 mmol) using the same procedure as that described for **19** (Scheme II). The MEM ether **32** (750 mg, 1.80 mmol, 67%) was isolated as a colorless oil: IR (film) 2940, 1485, 1105, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 1.57 (s, 3 H), 1.66 (s, 3 H), 1.50–1.72 (m, 4 H), 1.98 (m, 2 H), 2.08 (s, 3 H), 2.10 (m, 4 H), 2.11 (s, 3 H), 2.68 (m, 2 H), 3.38 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 5.09 (m, 1 H), 5.16 (s, 2 H), 5.41 (m, 2 H), 6.67 (s, 1 H); MS m/e 416 (M+). Anal. ($C_{26}H_{40}O_4$) C, H.

2,7,8-Trimethyl-2-(8-methyl-3,7-nonadienyl)-2*H*-1-benzopyran-6-ol (23). The MEM ether **32** (1.20 g, 2.88 mmol) was deprotected as described for **19**. The phenol **23** (750 mg, 2.29 mmol, 79%) was isolated as a yellow oil which was distilled in a Kugelrohr oven (bath 185 °C/0.1 mm) for analysis: IR (film) 3420, 2940, 1430, 1225, 1090, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (s, 3 H), 1.57 (s, 3 H), 1.66 (s, 3 H), 1.50–1.70 (m, 4 H), 1.98 (m, 2 H), 2.09 (s, 3 H), 2.10 (m, 4 H), 2.11 (s, 3 H), 2.65 (t, J = 6.6 Hz, 2 H), 4.17 (s, 1 H), 5.08 (m, 1 H), 5.41 (m, 2 H), 6.35 (s, 1 H); MS m/e 328 (M+). Anal. ($C_{22}H_{32}O_2 \cdot 0.4H_2O$) C, H.

2-[6-(3,3-Dimethyl-2-oxiranyl)-3-hexenyl]-3,4-dihydro-2,7,8-trimethyl-2*H*-1-benzopyran-6-ol (33). To a biphasic mixture of diene **23** (600 mg, 1.83 mmol) in methylene chloride (20 mL) and saturated $NaHCO_3$ (20 mL) was added *m*-chloroperbenzoic acid (452 mg, 2.64 mol) giving rise to a yellow color. The mixture was stirred at 23 °C for 18 h. The mixture was poured into water and extracted with ether. The organic layers were dried (brine, $MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography (10:1 hexanes–ether) to give the pure epoxide **33** (100 mg, 0.29 mmol, 16%) as a pale yellow oil: IR (film) 3400, 2940, 1425, 1225, 1085 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (s, 6 H), 1.27 (s, 3 H), 1.55 (m, 4 H), 1.73 (m, 2 H), 2.08 (s, 3 H), 2.11 (s, 3 H), 2.13 (m, 4 H), 2.67 (m, 3 H), 4.23 (br s, 1 H), 5.43 (m, 2 H), 6.35 (s, 1 H); MS m/e 344 (M+). Anal. ($C_{22}H_{32}O_3 \cdot 0.6H_2O$) C, H.

Rat Hepatocyte Cholesterol Biosynthesis Model. Primary rat parenchymal hepatocytes were prepared from male Wistar rats (180–220 g) at mid-dark in the diurnal cycle by standard collagenase perfusion method as described previously.[45] Aliquots of cells were suspended (100 mg in 2 mL) in Eagle's minimum essential medium (MEM) supplemented with 2% bovine albumin and preincubated in a shaking water bath at 37 °C under 95% O_2/5% CO_2 with compounds added as given in the data. Tween-80 was employed to aid solubilization of the tocotrienols at 0.2% final concentration as suggested by Qureshi et al.;[7] in separate studies dimethyl sulfoxide vehicle (0.5% v/v) gave results similar to the Tween vehicle data. Following a preincubation of 15 or 45 min as indicated, cholesterol synthesis in intact cells was assayed by [2-^{14}C]acetate (1.8 mCi/mmol, 0.5 μCi/mL) incorporation for 60, 45, or 30 min as indicated in the legends into total digitonin-precipitable sterols. Previous studies established time linearity of incorporation. The isolation of this total sterol fraction followed standard methods as described previously.[45,46] Briefly, samples were precipitated and washed with perchloric acid, saponified in 90% methanol/0.30 N NaOH, and then quantitatively extracted in hexanes to obtain the nonsaponifiable lipids. From this fraction, the digitonin-precipitable sterols were obtained. Greater than 98% of the ^{14}C content in this fraction was shown by HPLC to coelute with authentic cholesterol standard. Percent inhibition was calculated from the average of duplicates vs triplicate vehicle controls conducted simultaneously.

HepG2 Cell Culture Model. [^{14}C]Acetate Incorporation Assay. HepG2 cells obtained from the American Type Culture Collection were routinely passaged in RPMI-1640 plus 10% fetal bovine serum (FBS) and were subcultured into 35-mm diameter wells for experiments. At approximately 70% confluence, the medium was changed to 2.0 mL of RPMI-1640 plus 7% lipid-depleted serum (LDS) to induce cholesterogenesis as suggested by Burki et al.[47] The LDS medium supplement was prepared according to Cham et al.[48] After 16 h in LDS-containing media, test compounds were added in dimethyl sulfoxide vehicle (0.5% v/v final concentration) for the period of time indicated in the data (generally 4 h). Cholesterol synthesis was then directly determined by a 60-min pulse incorporation of [2-^{14}C]acetate (1.8–3.0 mCi/mmol) into digitonin-precipitable sterols essentially as described for the rat hepatocyte method above. Greater than 97% of the radiolabeled sterol isolated by this procedure was cholesterol as judged by HPLC. Percent inhibition was calculated from the average of duplicates or triplicates vs controls receiving vehicle.

HepG2 Cell Culture Model. HMG-CoA Reductase Suppression Assay. HMG-CoA reductase suppression in HepG2 cells was conducted with growing cells in RPMT-1640 plus 10% FBS on 100-mm plates, and when cells reached approximately 70% confluency they were induced with LDS (as described above) for about 16 h prior to assays. Compounds were added using dimethyl sulfoxide vehicle (0.5% v/v, final), and after 4 h of incubation at 37 °C, cells were harvested by scraping. Cell pellets were rinsed and lysed by sonication in 1.7 mL of cold 50 mM imidazole–HCl, pH 7.2, 50 mM NaCl, 10 mM EDTA, 10 mM EGTA, 5 mM DTT, and 40 μM leupeptin). Lysates were centrifuged at 150g, and the supernatant was centrifuged at 100000g in a Beckman airfuge to isolate the postnuclear total membrane fraction. The membranes were resuspended in 50 mM imidazole–HCl, pH 7.2, 250 mM NaCl, 5 mM DTT, and 20 μM leupeptin and used for the assay of HMG-CoA reductase activity by the radiochemical procedure as described previously by Parker et al.[49] Values were normalized for protein content by the Lowry method.[49] HMG-CoA reductase percent suppression was calculated as the decrease in specific activity of HMG-CoA reductase for treated cells vs controls receiving vehicle. Averages of duplicate cell determinations assayed in duplicate were taken.

Registry No. (±)-**1**, 143172-79-6; **2**, 5717-37-3; (±)-**3**, 143172-80-9; (±)-(*Z*)-**3**, 143191-21-3; (±)-**4**, 143172-81-0; (±)-**5**, 143172-82-1; **6**, 53254-60-7; **7**, 135897-88-0; (±)-**8**, 143172-83-2; (±)-**9**, 90242-77-6; (*R*)-**9**, 58864-81-6; (±)-**10**, 143172-84-3; (±)-**11**, 143172-85-4; **12**, 135897-92-6; **13a**, 143172-86-5; **13b**, 143172-97-8; (±)-**14a**, 143172-87-6; (±)-**14b**, 143172-98-9; (±)-**15a**, 143172-88-7; (±)-**15b**, 143172-99-0; (±)-(*Z*)-**11**, 143173-01-7; (±)-**16a**, 143172-89-8; (±)-**16b**, 143191-19-9; (±)-**17a**, 143172-90-1; (±)-**17b**, 143191-20-2; **18a**, 135897-98-2; **18b**, 135898-05-4; (±)-**19a**, 143172-91-2; (±)-**19b**, 143173-00-6; (±)-**20**, 16772-32-0; (*R*)-**20**, 14101-61-2; (±)-**21**, 136774-62-4; (*R*)-**21**, 136774-61-3; (±)-**22**, 143172-92-3; (±)-**22**, 143191-10-0; (±)-**24**, 143172-93-4; (±)-(*Z*)-**24**, 143173-02-8; **25**, 143172-94-5; **26**, 143191-11-1; (±)-**27**, 143172-95-6; (±)-**28**, 143191-12-2; (±)-**29**, 143191-13-3; (±)-**30**, 143191-14-4; **31**, 143191-15-5; (±)-**32**, 143191-16-6; **33**, 143191-17-7; $(EtO)_2P(O)CH_2CO_2Et$, 867-13-0; hydroquinone, 608-43-5; 2,3-dimethylhydroquinone, 608-43-5; prenyl *p*-tolyl sulfone, 15543-64-3; *d*-α-tocopherol, 59-02-9; *d*-β-tocotrienol, 490-23-3; *d*-δ-tocotrienol, 25012-59-3; HMG-CoA reductase, 9028-35-7; cholesterol, 57-88-5; (±)-3,4-dihydro-2,5,7,8-tetramethyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-6-ol, 90164-57-1; (±)-3,4-dihydro-6-[(2-methoxyethoxy)methoxy]-2,5,7,8-tetramethyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran, 143191-18-8; 5,6,7,8,8a,10a-hexahydro-1,3,4,8,8,10a-hexamethylxanthen-2-ol, 143172-96-7.

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