146. *Tocopherols. Part VIII.*[1] *Structural and Synthetic Studies of ε-Tocopherol.*

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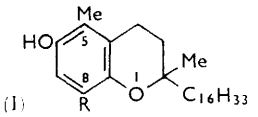
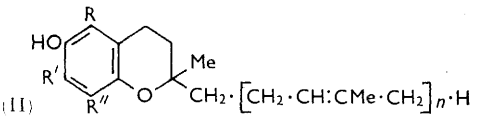
Evidence is presented in favour of the structure 2,5,8-trimethyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol for ε-tocopherol. Attempts to synthesise 2-methyl-2-[(poly-)3-methylbut-2-enyl]methylchroman-6-ols from quinols and unsaturated isoprenoid alcohols are complicated by the tendency of the side chain to undergo cyclisation. Conversion of poly-(3-methylbut-2-enyl)quinols into chromanols catalysed by hydrogen fluoride is accompanied by side-chain cyclisation. The degree of cyclisation appears to vary with the nature of the substitution of the quinol, and least cyclisation occurs when the polymethylbutenyl group has no substituent *para* to it. An attempt to synthesise 2,5,8-trimethyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol by a two-stage synthesis from 2,5-dimethylquinol and all-*trans*-geranyl-linaloöl gave a product differing slightly from ε-tocopherol in infrared spectrum and nuclear magnetic resonance spectrum.

In Part V [2] of this series ε-tocopherol was shown to differ from 5-methyltocol (I; R = H), with which it had previously been identified,[3] and an alternative dimethylchroman-6-ol structure differing from the tocopherols by altered substitution at position 2 was suggested. Green, Mamalis, Marcinkiewicz, and McHale later proposed [4] that ε-tocopherol was 2,5,8-trimethyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol (II; R′ = H, R = R″ = Me, n = 3). The evidence for this structure and certain synthetic studies on 2-methyl-2-[(poly-)3-methylbut-2-enyl]methylchroman-6-ols (II) are now presented.

ε-Tocopherol was isolated from the unsaponifiable fraction of wheat-bran oil and freed from other tocopherols by adsorption chromatography on alumina. After further purification through the 4-phenylazobenzoate, molecular distillation gave a pale yellow oil which was used for degradative studies.



(I)



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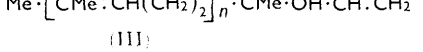

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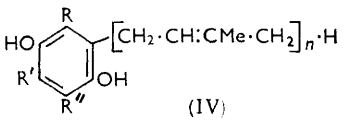

(IV)

Elementary analyses indicated an empirical formula of $C_{27-28}H_{40-42}O_2$ and an approximate molecular weight of 400, which is in keeping with a migration similar to that of the tocopherols on a partition chromatogram.[2] Three double bonds were shown to be present by the method of McHale, Green, and Marcinkiewicz,[5] and this was substantiated by hydrogenation. The hydrogenated material was identical with 5,8-dimethyltocol (β-tocopherol) (I; R = Me) in infrared spectrum, two-dimensional paper chromatography,[6] and migration of the nitroso-derivative.[7] Thus ε-tocopherol differed from β-tocopherol only in possessing three more double bonds. The ultraviolet absorption spectrum excluded a chromenol structure,[8] leaving the side chain as the only site for the double bonds. Ozonolysis of

[1] Part VII, Marcinkiewicz, McHale, Mamalis, and Green, *J.*, 1959, 3377.
[2] Green, McHale, Marcinkiewicz, Mamalis, and Watt, *J.*, 1959, 3362.
[3] Eggitt and Ward, *J. Sci. Food Agric.*, 1953, **4**, 569.
[4] Green, Mamalis, Marcinkiewicz, and McHale, *Chem. and Ind.*, 1960, 73.
[5] McHale, Green, and Marcinkiewicz, *Chem. and Ind.*, 1961, 555.
[6] Green, Marcinkiewicz, and Watt, *J. Sci. Food Agric.*, 1955, **6**, 274.
[7] Marcinkiewicz and Green, *Analyst*, 1959, **84**, 304.
[8] Rowland, *J. Amer. Chem. Soc.*, 1958, **80**, 6130.

ε-tocopheryl acetate and reductive cleavage gave acetone and lævulaldehyde, the expected products from a poly-(3-methylbut-2-enyl) side chain.

The nuclear magnetic resonance spectra of ε-tocopherol and its 4-phenylazobenzoate, when interpreted in comparison with the spectra of 5,8-dimethyltocol and its 4-phenylazobenzoate and ubiquinone 50, confirm the proposed structure. The chemical-shift data and assignments are assembled in the Table. The lack of high-field regions ($\tau > 8{\cdot}7$) in ε-tocopherol indicates absence of paraffinic hydrogen in the side chain, suggesting that the CH_2 and CH_3 groups are on doubly-bonded carbon atoms, as in a poly-(3-methylbut-2-enyl) chain. The presence of the following bands supports this: 5·0 (:C*H*), 7·87 (:C·C*H*$_2$·C*H*$_2$·C:), and 8·3 (C*H*$_3$·C:). On the assumption that the intensities of the aromatic *H* in the 4-phenylazobenzoates of ε-tocopherol and 5,8-dimethyltocol are the same, a comparison of the intensities of the other bands indicates that ε-tocopherol contains one hydroxylic, 3 olefinic, and 36 other hydrogen atoms. Neither 5,8-dimethyltocol nor ε-tocopherol shows a low-field band for the single aromatic hydrogen present, a behaviour not unknown with pentasubstituted benzene derivatives.[9] It is also interesting that the two non-equivalent ring-methyl groups appear as a single band in the spectra of ε-tocopherol and 5,8-dimethyltocol but as a doublet in the phenylazobenzoates.

Chemical shift data (τ) (p.p.m.).

Assignment	5,8-Dimethyltocol	5,8-Dimethyltocol phenylazobenzoate	ε-Tocopherol	ε-Tocopherol phenylazobenzoate	Ubiquinone 50
Aromatic *H*	—	1·45, 1·66, 1·8, 2·06, 2·5, 3·15	—	1·5, 1·6, 1·88, 2·13, 2·4, 2·51, 2·96	—
O*H*	3·77	—	3·7	—	—
·C*H*:.....	—	—	5·0	4·85	4·9
—	—	—	5·35	—	—
C*H*$_3$·O	—	—	—	—	5·97
—	—	—	—	—	6·12, 6·32
:C·C*H*$_2$·CH:.....	—	—	—	—	6·67, 6·82
O·C(CH$_3$)·C*H*$_2$·C*H*$_2$	7·42, 7·5	7·42	7·37, 7·57	7·3, 7·47	—
:C·C*H*$_2$·C*H*$_2$·C: ...	—	—	7·87	7·82	7·92
Aromatic C*H*$_3$ ...	7·92	7·8, 7·95	7·95	7·9, 7·97	7·92
—	8·25	—	8·15	—	—
C*H*$_3$·C:.....	—	—	8·3	8·35	8·37
—	—	—	—	—	8·67
C*H*$_3$·C·O	8·75	8·72	8·67	8·67	—
·C*H*$_2$·	8·75	8·72	—	—	—
C*H*$_3$·CH	9·0	9·0	—	—	—
(C*H*$_3$)$_2$CH	9·12	9·12	—	—	—

The esters and ubiquinone were examined in chloroform, and ε-tocopherol and 5,8-dimethyltocol in carbon tetrachloride. The latter spectra were transferred to the same scale as the esters by using the C*H*$_3$·C·O band as reference.

Although 5,8-dimethyltocol is obtained in good yield from 2,5-dimethylquinol and phytol under a variety of condensation conditions, the literature indicated that the synthesis of ε-tocopherol by a corresponding reaction from the more highly unsaturated geranylgeraniol or its isomer geranyl-linaloöl (III; $n = 3$) would not necessarily go as smoothly. For instance, Smith, Ungnade, Hoehn, and Wawzonek [10] failed to isolate a chromanol after reaction of geranyl bromide with trimethylquinol, and Karrer and Rentschler [11] reported that condensation of geranylgeranyl bromide with trimethylquinol gave a chromanol with a monounsaturated bicyclic side chain. In view of these results, and because only a limited quantity of geranyl-linaloöl was available, some preliminary studies were carried out with nerolidol (III; $n = 2$). Although paper partition chromatography fails to distinguish between an unsaturated and a cyclic structure, a saturated compound migrates more slowly than its unsaturated analogue [5] in a reversed-phase system. Thus it is possible to assess whether side-chain cyclisation accompanies chromanol formation by comparison of the migration of product and hydrogenated product.

[9] Feeney and Sutcliffe, unpublished work.
[10] Smith, Ungnade, Hoehn, and Wawzonek, *J. Org. Chem.*, 1939, **4**, 305.
[11] Karrer and Rentschler, *Helv. Chim. Acta*, 1944, **27**, 1296.

The condensation of 2,5-dimethylquinol with nerolidol proceeded less favourably than the corresponding reaction with phytol and led to chromanols with cyclised side chains. The work of Shunk, Erickson, Wong, and Folkers [12] indicated that under milder conditions this condensation would yield 3-farnesyl-2,5-dimethylquinol (IV; R′ = H, R = R″ = Me, $n = 3$) rather than a chromanol. Although unsuccessful attempts [13] had been made to cyclise quinols of type (IV) to chromanols without effecting cyclisation of the side chain, the results were not conclusive enough to preclude further attempts.

The condensation of nerolidol with 2,5-dimethylquinol in the presence of boron trifluoride–ether complex at room temperature yielded, besides 3-farnesyl-2,5-dimethylquinol, a second compound that reduced Emmerie and Engel's reagent.[14] The migration of this compound in a reversed-phase system was close to that expected for 2-(4,8-dimethylnona-3,7-dienyl)-2,5,8-trimethylchroman-6-ol but the brown coupling colour obtained with diazotised *o*-dianisidine under alkaline conditions [15] was darker than that given by 5,8-dimethylchroman-6-ols. Since chromanol formation was not expected under these conditions, it was helpful to examine the corresponding reaction with 2,3-dimethylquinol as 7,8-dimethylchroman-6-ols derived from this compound give a characteristic purple coupling colour with diazotised *o*-dianisidine. This reaction gave 5-farnesyl-2,3-dimethylquinol (IV; R = H, R′ = R″ = Me, $n = 3$) and a second product, which failed to give the purple coupling reaction but gave a brown colour similar to that given by *p*-alkoxyphenols. The second product was unstable at 200° and part underwent thermal cleavage to 2,3-dimethylquinol, behaviour indicative of a γ-substituted allyl ether. As boron trifluoride is known to catalyse the formation of ethers from olefins,[16] the presence of some *p*-alkoxyphenol in the reaction mixture is not unexpected.

A chance observation that, if the 2,3-dimethylquinol–nerolidol reaction product was left for several days before working-up, chromanol could be detected, prompted successful attempts to cyclise the reaction mixture with hydrogen fluoride. The chromanols derived in this way from 2,3-dimethylquinol, 2,6-dimethylquinol, and 2,3,6-trimethylquinol were mixtures with cyclised side chains, whilst that from 2,5-dimethylquinol contained little of the cyclised compound. The use of anhydrous hydrogen fluoride in place of boron trifluoride–ether gave disubstituted products, which failed to couple with diazotised *o*-dianisidine.

As a result of the foregoing experiments, an attempt was made to synthesise ε-tocopherol by boron trifluoride–ether-catalysed condensation of 2,5-dimethylquinol and all-*trans*-geranyl-linaloöl and cyclisation of the resulting 3-geranylgeranyl-2,5-dimethylquinol (IV; R′ = H, R = R″ = Me, $n = 4$) with anhydrous hydrogen fluoride. The course of the reaction was followed by paper chromatography and no attempt made to isolate the geranylgeranylquinol. Chromatography of the product on alumina removed hydrocarbon impurities and gave a fraction containing 41% of ε-tocopherol which appeared to be free from chromanols with cyclised side chains. The 4-phenylazobenzoate prepared from this concentrate could not be obtained solid and showed slight differences in infrared spectrum from that of the solid derivative prepared from natural ε-tocopherol: the latter has two additional weak bands at 1800 and 1175 cm.$^{-1}$. Hydrolysis of the phenylazobenzoate gave an oil that was purified by adsorption chromatography and molecular distillation. The infrared spectrum of the distilled oil differed from that of natural ε-tocopherol only in the absence of a weak band at 807 cm.$^{-1}$. The ultraviolet spectra were similar but the synthetic compound had a lower extinction coefficient at the absorption maximum. The hydrogen absorption of the synthetic compound, which was equivalent

[12] Shunk, Erickson, Wong, and Folkers, *J. Amer. Chem. Soc.*, 1959, **81**, 5000.
[13] Laidman, Morton, Paterson, and Pennock, *Biochem. J.*, 1960, **74**, 541; Shunk, Trenner, Hoffman, and Folkers, *Biochem. Biophys. Res. Com.*, 1960, **2**, 427.
[14] Emmerie and Engel, *Rec. Trav. chim.*, 1939, **58**, 283.
[15] Green and Marcinkiewicz, *Analyst*, 1959, **84**, 297.
[16] Kastner, "Newer Methods of Preparative Organic Chemistry," Interscience, Publ., Inc., New York, 1948, p. 275.

to 2·3 double bonds, was also less than that of the natural compound. The hydrogenated product and 5,8-dimethyltocol had almost identical infrared spectra and their nitroso-derivatives migrated to the same position on a reversed-phase chromatogram, as did the nitroso-derivatives of synthetic and natural ε-tocopherol.

It was evident from the nuclear magnetic resonance spectrum that the synthetic sample was not pure. The spectrum contained a band in the high field region (τ 9·12) attributable to paraffinic hydrogens in the side chain, and intensity measurements indicated 2—2·5 olefinic hydrogen atoms. With the exception of a small olefinic hydrogen peak, due to incomplete reduction, the spectrum of the hydrogenated product was similar to that of 5,8-dimethyltocol.

Both the nuclear magnetic resonance spectrum and the low hydrogen uptake of the synthetic sample suggested that the impurities were chromanols with cyclised or partially cyclised side chains. This was confirmed on rechromatography of the hydrogenated product at a heavier loading in the reversed-phase system, when two bands migrating between ε-tocopherol and 5,8-dimethyltocol were detected on the chromatogram.

An attempt to synthesise ζ_1-tocopherol (II; R = R′ = R″ = Me, $n = 3$) by condensing 2,3,5-trimethylquinol with geranyl-linaloöl gave a complex mixture of products, which was shown by gas-chromatography to contain some ζ_1-tocopherol.

The ε-tocopherol fraction from bran oil contained a second reducing material, which migrated with ε-tocopherol on an adsorption chromatogram but migrated much more slowly on a reversed-phase partition chromatogram. This compound had an absorption maximum at 291 mμ, gave a weak purple coupling colour with diazotised *o*-dianisidine, and was probably Eggitt and Ward's origin-spot material.[3]

Another reducing compound was separated from the α-tocopherol fraction by reversed-phase partition chromatography. This compound had an absorption maximum at 295 and a minimum at 265 mμ. Its infrared spectrum, chromatographic behaviour, and failure to couple with diazotised *o*-dianisidine were consistent with a chromanol or alkoxyphenol structure, fully substituted *ortho* to the hydroxyl group. It was unsaturated and the difference in migration from that of α-tocopherol suggested one less isoprenoid group than is present in the tocopherols. This material may have been an artifact formed during the processing of the bran oil or a seasonal component; for, when other bran samples were examined, it could not be detected.

An alcohol was also isolated from the α-tocopherol fraction. Preliminary studies on this compound suggested an empirical formula of $C_{16}H_{32}O$ and a molecular weight of the order of 300. The latter was obtained by a comparison of the molecular extinction of the 4-phenylazobenzoate with that of known 4-phenylazobenzoates, according to the method of Pennock, Hemming, and Morton.[17] The infrared spectrum showed a band at 1660 cm.$^{-1}$ characteristic of unconjugated double bonds and a band at 1005 cm.$^{-1}$ characteristic of an allylic alcohol.

Experimental

Ultraviolet light absorption was measured for ethanol solutions with a Uvispek spectrophotometer. Infrared spectra were measured with a Grubb-Parsons DB 1/34 spectrometer fitted with a sodium chloride prism; oils were measured as liquid films and solids as the potassium bromide discs. The nuclear magnetic resonance spectra were determined with a Varian 4300 spectrometer with a 40 Mc./sec.$^{-1}$ oscillator. Unless otherwise stated, the measurements were made in carbon tetrachloride solution, and tetramethylsilane was used as internal reference. The shielding values are expressed as τ as defined by Tiers.[18] Pressures cited for short-path distillations are Pirani-gauge measurements. Tocopherol analyses were by the method of Green *et al.*[6] Light petroleum refers to the fraction of b. p. 40—60°. Alumina is Peter Spence's type O.

[17] Pennock, Hemming, and Morton, *Nature*, 1960, **186**, 470.
[18] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.

Paper Chromatography.—Adsorption chromatograms were run on paper treated with zinc carbonate.[6] Partition chromatograms were of the reversed-phase type, with liquid paraffin as stationary phase and aqueous ethanol as mobile phase; alcohol strengths are given in parentheses. The ascending method of chromatography was used and spots were made visible by spraying them with Emmerie and Engel's reagent [14] (reducing reaction) or first with 5% aqueous sodium carbonate and then with diazotised *o*-dianisidine.[15]

Detection of Side-chain Cyclisation.—The compound (~1 mg.) was hydrogenated over platinum oxide in acetic acid. After dilution with light petroleum and filtration, the solution was washed and evaporated. Original and hydrogenated products were chromatographed side by side in a partition system for which ΔR_M(C:C) was known.[5] The number of rings in side chain = (expected number of double bonds) − (observed difference in migration)/ΔR_M(C:C).

Isolation of ε-Tocopherol.—Freshly milled wheat bran (4 cwt.) consisting of Manitoba 60%, Plate Red Winter 20%, and English 20% was extracted with light petroleum (b. p. 80—100°; 3 × 500 l.). The combined extracts on evaporation gave a brown viscous semi-solid oil (4·7 kg.) which contained ε-tocopherol (1·4 mg./g.), α-tocopherol (0·3 mg./g.), ξ_1-tocopherol (0·4 mg./g.), and a trace of β-tocopherol. This oil was dissolved in light petroleum (b. p. 80—100°; 50 l.), adsorbed on alumina (30 kg.), and eluted with light petroleum (b. p. 80—100°; 33 l.) followed by methanol (68 l.). On evaporation the light petroleum fraction gave a non-reducing oil (100 g.). The methanol fraction gave a reducing oil (2070 g.), which was dissolved in light petroleum (b. p. 80—100°; 3 l.), filtered from insoluble residue (54 g.), adsorbed on alumina (6 kg.), and eluted successively with light petroleum (b. p. 80—100°; 8 l. and 8·5 l.), 5% v/v ethanol–light petroleum (b. p. 80—100°; 20 l.) and methanol (12 l.). The first light petroleum eluate on evaporation gave a non-reducing oil (424 g.). The second eluate contained a reducing oil (550 g.) that assayed as the original concentrate. The ethanol–light petroleum eluate gave a strongly reducing oil (570 g.) that contained ε-tocopherol (7·3 mg./g.) and α- and ξ_1-tocopherol (total 2 mg./g.). The methanol eluate contained an oil (112 g.) that gave a strong purple coupling colour with alkaline diazotised *o*-dianisidine. The second light-petroleum eluate was reduced to 4 l., re-adsorbed on alumina (6 kg.), and eluted as above. The semi-solid oil (135 g.) from the ethanol–light petroleum eluate was combined with the main ε-tocopherol concentrate, dissolved in boiling methanol (5 l.), and cooled to −20°. The solid was collected and re-extracted with boiling methanol (2 × 1 l.); the residual solid (54 g.) had m. p. 120°. On evaporation, the combined methanol filtrate gave a reducing oil (600 g.). A well-stirred mixture of this oil (300 g.), pyrogallol (50 g.), and 95% ethanol (1 l.) was heated to the b. p., treated with 32% w/w aqueous potassium hydroxide (250 ml.), refluxed for 15 min., and then cooled rapidly under nitrogen by addition of ice (5 kg.). The aqueous layer was extracted three times with ether (3, 2½, 1½ l.); each extraction required the addition of a little ethanol to break the emulsion. The combined extract was washed with water and combined with the ether extract from the saponification of the second batch of oil (300 g.) and evaporated. The resulting mixture of oil and solid was extracted with boiling methanol (3 × 200 ml.), after which a small amount of insoluble gum remained. The combined methanol extract was cooled to −20° and the solid (37 g.) that separated was collected. Evaporation of the methanol gave a concentrate (34 g.) containing ε-tocopherol (10%), α-tocopherol (0·8%), and ξ_1-tocopherol (0·5%) that was dissolved in light petroleum (30 ml.) and adsorbed on alumina (300 g.). Elution gave the following fractions: (1) 0·45 g. of non-reducing oil, eluted by light petroleum; (2) 10·9 g., containing α- and ξ_1-tocopherol and a third reducing material that migrated faster than ε-tocopherol in the partition system (total 13·7% calc. as α-tocopherol), and ε-tocopherol (1·4%), eluted by benzene; (3) 1·1 g., containing α- and ξ_1-tocopherol, reducing fast-running material (total 2·7%), and ε-tocopherol (9·5%), eluted by 10% v/v chloroform in benzene; (4) 12·9 g., containing ε-tocopherol (23·5%), eluted by chloroform; (5) 1·4 g., containing ε-tocopherol (8·9%), eluted by 5% v/v ethanol–benzene. Fractions 4 and 5 also contained a reducing material that migrated more slowly than ε-tocopherol in the partition system. Fraction 4 was rechromatographed on alumina (500 g.), and the column was developed with light petroleum and benzene. 1:3 v/v Chloroform–benzene eluted an oil (11·1 g.) that, after being freed from sterols, gave a concentrate (8·4 g.) containing ε-tocopherol (2 g.), traces of α- and ξ_1-tocopherol and a reducing substance having R_F 0 in the partition system (75% ethanol). The latter substance, which gave a faint purple coupling reaction with diazotised *o*-dianisidine, was extracted from the paper with ethanol and showed an extinction maximum at 291 mμ.

The ε-tocopherol concentrate (8·3 g.), 4-phenylazobenzoyl chloride (8 g.), pyridine (16 ml.),

and ethylene dichloride (100 ml.) were refluxed for 8 hr. and then poured into an excess of 2N-hydrochloric acid and filtered. The filtrate was diluted with benzene, and the aqueous layer separated. The organic layer, after being washed with 2N-hydrochloric acid (2 × 50 ml.) and water (2 × 50 ml.), deposited crystals (1·5 g.), m. p. 170°. The filtrate was evaporated and the red oil (12·1 g.) dissolved in light petroleum (10 ml.) and adsorbed on alumina (350 g.). Elution with benzene gave a red oil (5·1 g.) that crystallised. 5% v/v Ethanol–benzene eluted a red oil (4·5 g.), which gave a positive reducing reaction. Solid (0·55 g.), m. p. 170°, remained when the benzene eluate was dissolved in light petroleum. Evaporation of the solvent and crystallisation of the resulting red mass from propan-2-ol gave a sticky solid (2·8 g.), m. p. 55°, which did not dissolve completely on recrystallisation but left a pink solid (0·23 g.), m. p. 155°. The solution, on cooling, deposited ε-*tocopheryl* 4-*phenylazobenzoate* as orange crystals (1·4 g.), which, after being twice recrystallised, had m. p. 70—71° (Found: C, 79·1; H, 8·0; N, 4·2. $C_{41}H_{50}N_2O_3$ requires C, 79·6; H, 8·1; N, 4·5%), $\nu_{max.}$ 2900s, 1720vs, 1595w, 1550w, 1465m, 1445w, 1405m, 1375m, 1265vs, 1230vs, 1165m, 1145w, 1105s, 1065m, 1055m, 1010w, 917w, 885w, 861m, 774s, 693s cm.$^{-1}$.

The azobenzoate (1·1 g.) was refluxed in 1 : 1 ethanol–propan-2-ol (40 ml.), and potassium hydroxide (2 g.) was added. After 20 min., 2N-hydrochloric acid (20 ml.) was added to the refluxing solution. The inorganic salt was dissolved in water, and the organic layer diluted with light petroleum. The aqueous layer was separated and the organic layer washed several times with water, filtered from 4-phenylazobenzoic acid, and evaporated to a yellow oil (0·65 g.). Short-path distillation [130°(bath)/5 × 10^{-3} mm.] gave ε-tocopherol as a pale yellow oil (Found: C, 81·8; H, 10·3. $C_{28}H_{42}O_2$ requires C, 81·9; H, 10·3%), $\lambda_{max.}$ 296 mμ ($E^{1\%}_{1cm.}$ 87), $\lambda_{min.}$ 255 mμ, $\nu_{max.}$ 3390m, 2905vs, 1450s, 1410s, 1375s, 1345m, 1305m, 1230vs, 1165s, 1095s, 1060s, 1005m, 971m, 926m, 860m, 807m, 723w, 696m cm.$^{-1}$ (liquid film), R_F 0·50 (70% ethanol).

Hydrogenation of ε-*Tocopherol.*—ε-Tocopherol (49·3 mg.) was hydrogenated over pre-reduced platinum oxide (5 mg.) in acetic acid (3 ml.). The uptake on the basis of a molecular weight of 410 corresponded to 2·9 double bonds. The hydrogenated product was diluted with light petroleum and filtered, and the filtrate washed free from acetic acid with water. Evaporation and short-path distillation [140°(bath)/5 × 10^{-3} mm.] gave a yellow oil (Found: C, 80·9; H, 11·3. Calc. for $C_{28}H_{48}O_2$: C, 80·5; H, 11·5%), R_F 0·33 (70% ethanol) (cf. 5,8-dimethyltocol, R_F 0·33).

The nitroso-derivative of the hydrogenated material and that of 5,8-dimethyltocol were prepared as previously described [7] and had R_F 0·42 (93% ethanol).

The hydrogenated material and 5,8-dimethyltocol had identical infrared spectra and migrated to the same position on a two-dimensional chromatogram.[6]

Ozonolysis of ε-*Tocopheryl Acetate.*—ε-Tocopherol (0·1 g.), sodium acetate (0·05 g.), and acetic anhydride (5 ml.) were heated on a steam-bath for 1 hr. and the product was cooled, diluted with light petroleum, and poured on ice. The organic layer was separated, washed with water, and evaporated. The resulting oil in acetic acid (3 ml.) was ozonised [ozone (0·1 g.) in oxygen (6 l./hr.)] at 20° for 1 hr. The product was diluted with ether (15 ml.), reduced with zinc dust (4 × 0·25 g.), and left overnight. The reduced product was cooled to −10° and filtered. The filtrate was distilled at atmospheric pressure from a steam-bath, and the volatile fraction collected in 10% sodium metabisulphite solution (5 ml.). The ether layer was separated and extracted with sodium metabisulphite solution (2 × 5 ml.). The combined aqueous layer was treated with potassium hydroxide (1·5 g.) in water (8 ml.) and distilled to half bulk, volatile material being collected in a cooled solution of 2,4-dinitrophenylhydrazine (0·03 g.) in 6N-sulphuric acid (12 ml.). The precipitated solid had m. p. 120° (alone or mixed with acetone 2,4-dinitrophenylhydrazone, m. p. 126°) and migrated to the same position as the authentic sample in the partition system (methanol).

The non-volatile portion was dissolved in ether and extracted with water (5 × 5 ml.). The combined aqueous extracts were added to 2,4-dinitrophenylhydrazine (0·2 g.) in 6N-sulphuric acid (60 ml.), and the precipitated solid was collected and boiled with methanol. After cooling, the solid was filtered off and dissolved in dioxan (40 ml.). Addition of ether (500 ml.) gave the derivative, m. p. 227—229°, undepressed by and identical in infrared spectrum with authentic lævulaldehyde bis-2,4-dinitrophenylhydrazone, m. p. 232—235°.

Condensation of 2,5- *and* 2,3-*Dimethylquinol with Nerolidol.*—2,5-Dimethylquinol (2·3 g.), nerolidol (3·6 g.), and boron trifluoride–ether complex (2 ml.) in dry ether (100 ml.) were kept at room temperature for 72 hr. with occasional shaking. The resulting solution was washed with N-sodium hydroxide and water and evaporated to a brown oil (4·3 g.), which contained,

besides 3-farnesyl-2,5-dimethylquinol, R_F 0·86 (55% ethanol), a second reducing compound, R_F 0·62, which gave a brown coupling reaction with diazotised *o*-dianisidine.

2,3-Dimethylquinol gave 5-farnesyl-2,3-dimethylquinol, R_F 0·86 and a compound, R_F 0·62, which also showed a brown coupling reaction. This condensation product was heated at 200° for 2 hr. under nitrogen. Addition of light petroleum precipitated a white solid, m. p. 234° (undepressed by 2,3-dimethylquinol, m. p. 234—235°). The band, R_F 0·62, was not present in the filtrate.

Cyclisation of 5-Farnesyl-2,3-dimethylquinol with Hydrogen Fluoride.—2,3-Dimethylquinol (0·5 g.), nerolidol (0·8 g.), and boron trifluoride–ether complex (1·0 ml.) in dry ether (25 ml.) were left for 72 hr. at room temperature in a Polythene bottle and then treated with hydrogen fluoride (0·4 g.) in dry ether (4 ml.). After 72 hr. the solution, which now gave a strong purple coupling reaction, was diluted with light petroleum and washed with water, N-sodium hydroxide, and water. Evaporation of the organic layer gave a brown oil (1·05 g.) from which a phenylazobenzoate (0·2 g.) (Found: C, 78·3; H, 7·0; N, 5·4. Calc. for $C_{36}H_{42}N_2O_3$: C, 78·5; H, 7·7; N, 5·1%) was obtained. Saponification and short-path distillation [120°(bath)/5 × 10^{-3} mm.] gave a yellow oil (0·05 g.) (Found: C, 80·3; H, 9·5. Calc. for $C_{23}H_{34}O_2$: C, 80·7; H, 10·0%) that showed only one reducing band, R_F 0·56 (60% ethanol). A hydrogenated sample separated into three bands (R_F 0·56, 0·46, 0·36).

Cyclisation of 3-farnesyl-2,6-dimethylquinol gave a product, R_F 0·56, which after hydrogenation, separated into two bands (R_F 0·45, 0·36). 6-Farnesyl-2,3,5-trimethylquinol behaved similarly.

Cyclisation of 3-Farnesyl-2,5-dimethylquinol with Hydrogen Fluoride.—Similarly treated, the condensation product from 2,5-dimethylquinol (0·5 g.) and nerolidol (0·8 g.) gave a *phenylazobenzoate* (0·23 g.) (Found: C, 78·2; H, 7·8. $C_{36}H_{42}O_3N_2$ requires C, 78·5; H, 7·7%), from which 2-(4,8-*dimethylnona*-3,7-*dienyl*)-2,5,8-*trimethylchroman*-6-ol (0·05 g.) was obtained as a yellow oil [140°(bath)/7 × 10^{-3} mm.] (Found: C, 80·3; H, 9·8. $C_{23}H_{34}O_2$ requires C, 80·7; H, 10·0%), R_F 0·56 (60% ethanol). The oil (23·5 mg.) took up 2·3 mol. of hydrogen and the hydrogenated sample had R_F 0·35.

Condensation of 2,5-Dimethylquinol with Nerolidol in the Presence of Hydrogen Fluoride.—2,5-Dimethylquinol (0·3 g.) and nerolidol (0·5 g.) in dry ether (5 ml.) were treated with hydrogen fluoride (0·1 g.) in dry ether (5 ml.) and left for 30 hr. The product was worked up in the usual way and then examined in the partition system (65% ethanol): only one band was present on the chromatogram and this on the origin line, the position 2-(4,8-dimethylnona-3,7-dienyl)-7-farnesyl-2,5,8-trimethylchroman-6-ol would occupy. Similar results were obtained with 2,3-dimethylquinol.

Attempted Preparation of 2,5,8-Trimethyl-2-(4,8,12-trimethyltrideca-3,7,11-*trienyl)chroman*-6-*ol* (II; R′ = H, R = R″ = Me, *n* = 3).—All *trans*-geranyl-linaloöl (2·0 g.) in dry ether (10 ml.) was added, dropwise, at room temperature to a solution of 2,5-dimethylquinol (0·9 g.) and freshly distilled boron trifluoride–ether (1·0 ml.) in dry ether (10 ml.) in a Polythene vessel and left for 48 hr. Hydrogen fluoride (0·6 g.) in ether (15 ml.) was added and the mixture left for a further 72 hr. and then washed with water, N-sodium hydroxide, and water. Evaporation of the solvent gave an oil (2·35 g.) that was dissolved in light petroleum and adsorbed on alumina (50 g.). Elution with light petroleum gave a non-reducing oil (0·75 g.) and continued elution with benzene gave an oil (0·26 g.). 5% v/v Ethanol–benzene eluted an oil (1·1 g.) that contained 41% of ε-tocopherol (by assay) but showed two reducing bands on a partition chromatogram (65% ethanol). The main band migrated to the same position as ε-tocopherol and the second band, which remained on the origin line, was almost certainly 7-geranylgeranyl-2,5,8-trimethyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol (II; R = R″ = Me, R′ = geranylgeranyl, *n* = 3). After hydrogenation the main band migrated to the same position as 5,8-dimethyltocol. The oil was converted into the phenylazobenzoate (1·2 g.) and chromatographed on alumina (50 g.). Benzene eluted a red oil (0·65 g.) that failed to crystallise. A portion of the *phenylazobenzoate* was purified by short-path distillation [230°(bath)/7 × 10^{-3} mm.] (Found: C, 80·0; H, 7·8; N, 3·9. $C_{41}H_{50}N_2O_3$ requires C, 79·6; H, 8·1; N, 4·5%); it had $\nu_{max.}$ 2880vs, 1720vs, 1595m, 1465s, 1440s, 1405s, 1370m, 1260vs, 1230vs, 1165m, 1140m, 1095vs, 1065s, 1010m, 917w, 883w, 862m, 773s, 692s cm.$^{-1}$.

Saponification of the remainder of the phenylazobenzoate (0·4 g.) gave an oily *substance* (0·18 g.), which was purified by short-path distillation [150°(bath)/7 × 10^{-3} mm.] (Found: C, 81·5; H, 10·1. $C_{28}H_{42}O_2$ requires C, 81·9; H, 10·3%), then having $\lambda_{max.}$ 295·5 mμ ($E^{1\%}_{1cm.}$ 76),

$\lambda_{min.}$ 255 mμ, $\nu_{max.}$ 3365m, 2905vs, 1455s, 1410s, 1375m, 1340w, 1305w, 1230s, 1165m, 1100m, 1060m, 1005w, 976w, 926w, 855w, 773w, 721w, 697w cm.$^{-1}$, nuclear magnetic resonance (of italicised protons) 9·12 [CH_3·C], 8·77 [CH_3·C·O], 8·44 [CH_3·C:], 7·99 [aromatic CH_3], 7·95 [:C·CH_2·CH_2·C:]7·42, [·O·C(CH_3)·CH_2·CH_2·], 4·95 [·CH:], 3·7 [O*H*] p.p.m. The oil had R_F 0·66 (70% ethanol) and gave a nitroso-derivative, R_F 0·60 (93% ethanol), which migrated to the same position as nitroso-ε-tocopherol. The oil (47·6 mg.) in acetic acid (3 ml.) absorbed 2·4 mol. of hydrogen over pre-reduced platinum oxide (7 mg.). The product was diluted with light petroleum and filtered, and the filtrate washed free from acetic acid and evaporated. Short-path distillation [150°(bath)/7 × 10^{-3} mm.] gave a yellow oil (Found: C, 80·6; H, 11·0. Calc. for $C_{28}H_{48}O_2$: C, 80·7; H, 11·6%) almost identical in infrared spectrum with 5,8-dimethyltocol and having nuclear magnetic resonance 9·11 [$(CH_3)_2$CH and CH_3·CH·], 8·82 [·CH_2 and CH_3·C·O], 8·04 [aromatic CH_3], 7·46 [·O·C(CH_3)·CH_2·CH_2·], 5·07 [·CH:(impurity)], 3·7 [O*H*] p.p.m.

The migration (70% ethanol) was identical with that of 5,8-dimethyltocol, R_F 0·33, although on heavy loading two other bands (R_F 0·53, 0·44) were detected. The oil gave a nitroso-derivative, R_F 0·42 (93% ethanol), which migrated to the same position as 5,8-dimethyl-7-nitrosotocol.

Attempted Preparation of 2,5,7,8-*Tetramethyl*-2-(4,8,12-*trimethyltrideca*-3,7,11-*trienyl*)-*chroman*-6-*ol* (II; R = R′ = R″ = Me, *n* = 3).—Under the same conditions as above geranyl-linaloöl (2·0 g.) and trimethylquinol (1·0 g.) gave an oil (2·9 g.), from which a phenylazobenzoate (0·12 g.) was obtained. Saponification gave an oil (60 mg.) which was shown by gas-chromatography to contain ξ_1-tocopherol and at least three other substances.

Isolation of Fast-running Reducing Material from Bran Oil.—Fractions 2 and 3 which preceded the ε-tocopherol fractions were combined and rechromatographed on alumina (500 g.). The benzene eluate (4·1 g.) in 77% ethanol (previously equilibrated by shaking with liquid paraffin) was chromatographed on a partition column prepared from hydrophobic " Hyflo " (1 kg.) and liquid paraffin (700 g.), with 77% ethanol (equilibrated) as mobile phase. The " Hyflo " was made hydrophobic by treatment with dichlorodimethylsilane in light petroleum. The first eluate (3 l.) was rejected; the second eluate (3 l.) contained reducing material and was evaporated, diluted with water, and extracted with light petroleum. Evaporation of the extract gave a pale oil (2·23 g.), which was freed from liquid paraffin by adsorption on alumina and elution with light petroleum. Subsequent elution with 5% v/v ethanol–benzene gave a reducing oil (1·2 g.), which was converted into a phenylazobenzoate and purified in the usual way on alumina. Benzene eluted the phenylazobenzoate (0·7 g.) as an oil that on treatment with propan-2-ol gave a solid (0·2 g.). The soluble portion was diluted with light petroleum, washed with water, and recovered. Saponification of the resulting orange oil (0·5 g.) and short-path distillation gave a non-reducing oil (137 mg.) [100°(bath)/0·2 mm.], n_D^{20} 1·4662, and a reducing oil (47 mg.) [140°(bath)/10^{-3} mm.], which after redistillation had $\lambda_{max.}$ 295 mμ ($E^{1\%}_{1cm.}$ 38·4), $\lambda_{min.}$ 265 mμ $\nu_{max.}$ 3310m, 2900vs, 1665w, 1440s, 1400m, 1370m, 1305w, 1230m, 1165w, 1100w, 1060w, 1020w, 1005w, 833w cm.$^{-1}$, R_F 0·33 (50% ethanol) and R_F 0·16 after hydrogenation. A sample chromatographed by the descending method in a system having olive oil as stationary phase and 70% ethanol as mobile phase had R_F 0·24 [calculated R_F in this system for compound (II; R = R′ = R″ = Me, *n* = 2) is 0·23].[19]

Samples (25 g.) of English, Manitoba, Up-river Plate, and Australian bran were extracted with light petroleum (400 ml.). The extracts were evaporated and the oils examined as described in the Analytical Methods Committee Report.[20] No fast-running material was observed on the chromatograms.

The solid phenylazobenzoate obtained as above had m. p. 68—69° (from propan-2-ol) (Found: C, 77·1; H, 9·5; N, 6·2%), $\lambda_{max.}$ 323 mμ ($E^{1\%}_{1cm.}$ 520), which corresponds to a molecular weight of 515. Hydrolysis and short-path distillation [120°(bath)/0·4 mm.] gave a colourless oil (Found: C, 80·35; H, 13·2%), n_D^{22} 1·4623, $\nu_{max.}$ 3300m, 2900vs, 1660w, 1460m, 1370w, 1005w cm.$^{-1}$.

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[19] Marcinkiewicz, Green, and McHale, unpublished work.
[20] Analytical Methods Committee, *Analyst*, 1959, **84**, 356.