680. *Tocopherols. Part V.*[1] *Structural Studies on ε- and ζ-Tocopherol.*

By J. Green, D. McHale, S. Marcinkiewicz, P. Mamalis, and P. R. Watt.

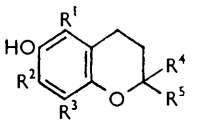
Natural ε-tocopherol has been shown to differ from synthetic 5-methyltocol, with which it had previously been identified. Spectroscopical, chromatographical, synthetical, and degradative evidence is presented confirming the nature of the synthetic compound. ε-Tocopherol would appear to be a 5,8-disubstituted chromanol, related to the tocols, but perhaps with altered substitution at $C_{(2)}$. Natural ζ-tocopherol from wheat bran is not identical with 5,7-dimethyltocol, which structure had been given it, but appears to be the trisubstituted representative in the same series as ε-tocopherol. The ζ-tocopherol that occurs in rice, however, is apparently identical with authentic 5,7-dimethyltocol.

When McHale, Mamalis, Marcinkiewicz, and Green's synthetic 5-methyltocol [1] was converted into a nitroso-derivative by Quaife's method [2] and this compound compared chromatographically with the nitroso-derivative of natural ε-tocopherol [3] the R_f values of the two compounds were different. Since ε-tocopherol has been identified with 5-methyltocol, it was necessary to compare in detail the chemistry of the two compounds.

Eggitt and Ward [4] considered that ε-tocopherol was 5-methyltocol because, on partition chromatography (a system sensitive to molecular-weight differences), it migrated almost identically with 8-methyltocol. This was also the view of Green, Marcinkiewicz, and Watt,[5] who found that the substance migrated in a two-dimensional adsorption-partition chromatographic system to the same position as 7-methyltocol. Eggitt and Norris [6] compared the behaviour of ε-tocopherol with that of other tocopherols and concluded that ε-tocopherol was probably 5-methyltocol, from the failure of the former to enter coupling reactions, its formation of a nitroso-derivative whose ultraviolet and visible spectra were closely similar to those of the nitroso-derivative of 5,8-dimethyltocol, the nearly

[1] Part IV, preceding paper.
[2] Quaife, *J. Biol. Chem.*, 1948, **175**, 605.
[3] Marcinkiewicz and Green, *Analyst*, 1959, **84**, 304.
[4] Eggitt and Ward, *J. Sci. Food Agric.*, 1953, **4**, 569.
[5] Green, Marcinkiewicz, and Watt, *ibid.*, 1955, **6**, 274.
[6] Eggitt and Norris, *ibid.*, p. 689.

quantitative formation of a " tocopheroxide " (now known to be a hemiketal, not an oxide [7]), whose ultraviolet spectrum (with $\lambda_{max.}$ at 265 mμ) was identical with the " tocopheroxide " from 5,8-dimethyltocol, and a number of other oxidation reactions.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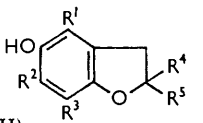
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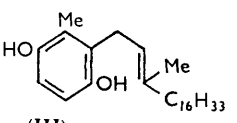
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The identity of the synthetic material with 5-methyltocol (I; $R^1 = R^4 = Me$, $R^2 = R^3 = H$, $R^5 = C_{16}H_{33}$), on the other hand, appeared to rest securely on its method of synthesis, which was parallel to the synthesis of α-tocopherol [8] and of 7-methyltocol,[9] its ultraviolet and infrared spectra, its behaviour towards the *o*-dianisidine and ferric chloride–bipyridyl reagents, and its two-dimensional paper-chromatographic behaviour. The only step in its synthesis with any degree of uncertainty appeared to be the chroman ring closure. If the unsaturated compound (III) is an intermediate in the cyclisation, there is a possibility that, by anti-Markovnikov addition, a coumaran would result. The nature of this type of ring closure and the possibility of coumaran formation was discussed by Karrer, Fritzsche, Ringier, and Salomon [10] and by Smith.[11] The formation of a coumaran (II; $R^1 = Me$, $R^2 = R^3 = R^4 = H$, $R^5 = CHMe{\cdot}C_{16}H_{33}$) would presume that cyclisation did not follow the course shown by Smith and his co-workers [12] to operate with more fully methylated model compounds; nevertheless, this was a possibility that was considered during the earlier structural studies.

ε-Tocopherol was isolated from wheat-bran oil by Ward's method [13] and distilled in a micro-molecular still as a yellow oil. Paper-chromatographic analysis showed that it contained only one reducing substance, corresponding in migration to the material described by Eggitt and Ward. Since the quantity obtained by the laborious procedure was small and its purity was uncertain (although oxidation with ferric chloride indicated that the preparation was of about 90% purity) no combustion analyses were attempted and the available material was used in a comparative study of the chemistry of ε-tocopherol and the synthetic 5-methyltocol of McHale *et al.*, pending further attempts to prepare crystalline derivatives.

Coupling Evidence.—Neither the synthetic material nor natural ε-tocopherol coupled with diazotised *o*-dianisidine, which reaction is given by tocols unsubstituted at $C_{(5)}$. The inactivity at $C_{(7)}$ in these 6-chromanols towards coupling is even more marked than the well-known Mills–Nixon effect in the corresponding 6-tetralols and has been described in some detail.[13a] The position of coupling in 5-coumaranols has not been described and it could not be assumed that, as in the corresponding 5-indanols, coupling would occur only at $C_{(6)}$. Indeed, Arnold and McCool [14] have shown that nitration takes place at $C_{(4)}$ in certain coumarans. Some model compounds were synthesised and tested with the *o*-dianisidine reagent. 4-Methylcoumaran-5-ol (II; $R^1 = Me$, $R^2 = R^3 = R^4 = R^5 = H$) and 2,4,7-trimethylcoumaran-5-ol (II; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$) coupled with the reagent to give a purple dye, whereas 6-methylcoumaran-5-ol (II; $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = Me$) did not. This indication that coumaranols couple at $C_{(6)}$ is strong evidence for the chromanol structure of the synthetic preparation in which the

[7] Martius and Eilingsfeld, *Annalen*, 1957, **607**, 159.
[8] Smith and Miller, *J. Amer. Chem. Soc.*, 1942, **64**, 440.
[9] McHale, Mamalis, Green, and Marcinkiewicz, *J.*, 1958, 1600.
[10] Karrer, Fritzsche, Ringier, and Salomon, *Helv. Chim. Acta*, 1938, **21**, 520.
[11] Smith, *Chem. Rev.*, 1940, **27**, 287.
[12] (*a*) Smith, Ungnade, Hoehn, and Wawzonek, *J. Org. Chem.*, 1929, **4**, 305; (*b*) *idem*, *ibid.*, p. 311; (*c*) Smith, Ungnade, Stevens, and Christman, *J. Amer. Chem. Soc.*, 1939, **61**, 2615.
[13] Ward, *Brit. J. Nutrition*, 1958, **12**, 226.
[13a] Green and Marcinkiewicz, *Analyst*, 1959, **84**, 297.
[14] Arnold and McCool, *J. Amer. Chem. Soc.*, 1942, **64**, 1315.

position of the methyl group is known (leaving the possibility of a $C_{(6)}$-substituted coumaranol structure for natural ε-tocopherol still open).

Spectroscopic Studies.—The ultraviolet spectra of synthetic 5-methyltocol, ε-tocopherol and β-tocopherol were almost identical, all having $\lambda_{max.}$ at 296 mμ and $\lambda_{min.}$ at 258 mμ. The infrared spectra of the three compounds were very similar (Fig. 1). Rosenkrantz,[15] discussing the spectra of α-, γ-, and δ-tocopherol and some related compounds, attributed bands in the 7μ (1430 cm.$^{-1}$) region to the vibration of C–H linkages and methyl groups. In this region, the spectra of 5,8-dimethyltocol and ε-tocopherol each show three bands, respectively, at 1458, 1410, 1373 cm.$^{-1}$, and 1448, 1415, 1377 cm.$^{-1}$. The spectrum of synthetic 5-methyltocol, however, has bands at 1476, 1458, and 1373 cm.$^{-1}$, and resembles more closely the spectra of tocol (bands at 1479, 1455, and 1376 cm.$^{-1}$) and 8-methyltocol (bands at 1463 and 1382 cm.$^{-1}$). The spectra of three model chromanols and four model coumaranols were compared in the 1110—1250 cm.$^{-1}$ region with those of the tocopherols (Table 1). These were 2,5,8-trimethyl-, 2,5,7,8-tetramethyl- and 2,2,5,7,8-pentamethylchroman-6-ol (I; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$; $R^1 = R^2 = R^3 = R^4 = Me$, $R^5 = H$; $R^1 = R^2 = R^3 = R^4 = R^5 = Me$, respectively), 2,4,6,7-tetramethylcoumaran-5-ol (II; $R^1 = R^2 = R^3 = R^4 = Me$, R = H), and 4-methyl-, 6-methyl-, and 2,4,7-trimethyl-coumaran-5-ol. Rosenkrantz has stated that the strong band that appears near 8·6 μ (1162 cm.$^{-1}$) in the spectra of α-, γ-, and δ-tocopherol is characteristic of the tocol type of structure. This band is indeed present in the spectra of all the tocopherols examined by us, of ε-tocopherol, and of the synthetic 5-methyltocol. It is also present in the spectra of the three 2-substituted chromanols, but is absent from those of the two 2-substituted coumaranols. In the spectra of the two coumaranols unsubstituted at $C_{(2)}$, it is apparently replaced by two bands. Rosenkrantz has also discussed the 8 μ (1250 cm.$^{-1}$) band, which probably results from the C–O vibration of the phenolic hydroxyl, and concludes that increased substitution by methyl groups in tocopherols results in a progressive shift, towards shorter wavelengths. When all the spectra are compared, however, this is seen to be a simplification and the position of methyl substitution is more important than the number of methyl groups in determining the wavelength shift. Thus, in the spectrum of 7-methyltocol, the band appears at 1240 cm.$^{-1}$ and is very weak. The band at 1246 cm.$^{-1}$ in the spectrum of synthetic 5-methyltocol may, perhaps, resemble the band in the analogous, *ortho*-substituted 7-methyltocol, whereas the position of the band at 1231 cm.$^{-1}$ in ε-tocopherol seems to place it more nearly in the category of dimethyltocols. The total infrared evidence indicates that ε-tocopherol may be a chromanol, disubstituted in the aromatic nucleus in a similar way to β-tocopherol.

TABLE 1. *Infrared bands in the* 1110—1250 *cm.*$^{-1}$ *region of tocopherols, chromanols, and coumaranols.*

Compound	ν (cm.$^{-1}$)	ν (cm.$^{-1}$)	Compound	ν (cm.$^{-1}$)	ν (cm.$^{-1}$)
Tocol	1218	1152	2,2,5,7,8-Pentamethyl-chroman-6-ol	1222	1166
7-Methyltocol	1240	1178	4-Methylcoumaran-5-ol	1222	1191, 1138
8-Methyltocol	1218	1152	6-Methylcoumaran-5-ol	1265	1187, 1159
5,8-Dimethyltocol	1229	1158	2,4,7-Trimethylcoumaran-5-ol	1208	None
7,8-Dimethyltocol	1221	1155	2,4,6,7-Tetramethylcoumaran-5-ol	1231	None
5,7-Dimethyltocol	1223	1162	Synthetic 5-methyltocol	1246	1155
5,7,8-Trimethyltocol	1266, 1210	1159	ε-Tocopherol	1231	1164
2,5,8-Trimethylchroman-6-ol	1230	1148			
2,5,7,8-Tetramethylchroman-6-ol	1258	1149			

Chromatographic Evidence.—Brown [16] and Eggitt and Ward [4] both remarked that the migration of ε-tocopherol on paper partition chromatograms appeared to be faster than that of 8-methyltocol. The difference is slight and can be overlooked unless the chromatograms are carefully constructed. We therefore compared carefully the R_F values of

[15] Rosenkrantz, *J. Biol. Chem.*, 1948, **173**, 439.
[16] Brown, *Biochem. J.*, 1952, **51**, 237.

synthetic 5-methyltocol, 7-methyltocol, 8-methyltocol, and ε-tocopherol by the descending method, the stationary phase being liquid paraffin and the mobile phase 75% v/v ethanol. After 4 hours' migration, the R_F values were respectively 0·52, 0·49, 0·52, and 0·62. In the same system, tocol had an R_F value of 0·67 and this would indicate that if ε-tocopherol is a chromanol, it probably has a lower molecular weight than the monomethyltocols.

Nitrosation Evidence.—Tocols that are unsubstituted at $C_{(5)}$ or $C_{(7)}$ can be nitrosated under Quaife's conditions.[2] It has been shown by Marcinkiewicz and Green [3] that sub-milligram quantities of almost pure nitrosotocopherols can be obtained by nitrosation at high dilution followed by a two-step paper-chromatographic separation. Attempts to prepare the nitrosotocopherols on a larger scale failed, owing to rapid oxidation of the nitroso-compounds. This kind of difficulty in the preparation of *o*-nitrosophenols may be noted in the work of Cronheim [17] who prepared 52 *o*-nitrosophenols in amounts sufficient for light-absorption studies but was unable to give any analytical results. We have repeated some of Cronheim's preparations and confirmed the minute yields of even the simplest *o*-nitrosophenols under normal preparative conditions. The nitrosotocopherols were therefore characterised by their ultraviolet and infrared spectra.

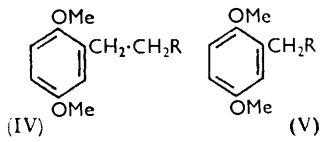
The light-absorption data in the visible and near ultraviolet on the nitroso-derivatives of 5,8-dimethyltocol, 7,8-dimethyltocol, and 8-methyltocol obeyed [2,6] Cronheim's rules for *o*-nitrosophenols. These rules state that (1) the wavelength of minimum absorption increases with the number of substituents in the benzene ring, and (2) the wavelength of maximum absorption depends on the distance between the hydroxy-group and the nearest substituent other than the nitroso-group itself. The nitroso-derivatives of 7-methyltocol and the synthetic 5-methyltocol preparation obeyed Chronheim's rules, both having $\lambda_{max.}$ at 413—415 mμ and $\lambda_{min.}$ at 338—340 mμ. We have, however, confirmed the previous findings [6] that the nitroso-derivative of ε-tocopherol did not have $\lambda_{min.}$ at 340 mμ (as would be expected if ε-tocopherol were a monomethylphenol) but at 358 mμ, identical with $\lambda_{min.}$ of nitroso-5,8-dimethyltocol.

When the nitroso-derivatives of the synthetic 5-methyltocol and ε-tocopherol were compared in the paper-chromatographic system of Marcinkiewicz and Green (93% v/v ethanol against liquid paraffin), the R_F values were quite different, being 0·21 and 0·60, respectively, compared with 0·31 for 7-methyl-5-nitrosotocol. In this system, the R_F values of 7,8-dimethyl-5-nitrosotocol and 5,8-dimethyl-7-nitrosotocol are 0·19 and 0·42, respectively. 8-Methyltocol gave two nitroso-derivatives,[3] with R_F values of 0·79 and 0·48, which, on the basis of their relative proportion formed during nitrosation (*ca.* 1 : 3) and their infrared spectra, are considered to be 8-methyl-7-nitroso- and 5-nitroso-tocol, respectively. The infrared spectra of the 7-nitroso-derivatives of the methylated tocols contained a band of medium strength near 1720 $cm.^{-1}$ (presumably due to carbonyl stretching vibration), which was absent from or very weak in the spectra of the 5-nitroso-derivatives. Comparison of the relative order of the R_F values suggested that the nitroso-derivative from the synthetic 5-methyltocol migrated anomalously. Tocol also gave two nitroso-derivatives which in spite of their lower molecular weights, migrated more slowly than the nitroso-derivatives of 8-methyltocol, having R_F values of 0·59 and 0·27. In view of these anomalous results, and since the constitution of synthetic tocol itself had been based on assumptions derived from the work of Smith *et al.*,[11,12] it seemed desirable at this stage to correlate the structure of tocol and synthetic 5-methyltocol. Tocol was therefore synthesised by a route parallel to that used for 5-methyltocol by McHale *et al.*, and then converted directly into a compound identical with the latter.

New Syntheses of Tocol and 5-Methyltocol.—The intermediate required for the new synthesis of tocol was 2-2′-hydroxyethylquinol dimethyl ether (IV; R = OH). Toluquinol dimethyl ether and *N*-bromosuccinimide readily gave 2-bromomethylquinol dimethyl ether (V; R = Br). Several attempts were made to prepare the Grignard reagent from

[17] Cronheim, *J. Org. Chem.*, 1945, **12**, 20.

this, but the only isolable product was 1,2-bis-(2,5-dimethoxyphenyl)ethane. 2-Chloromethylquinol dimethyl ether (V; R = Cl) had previously been prepared [18] from the hydroxymethyl compound (V; R = OH) by treatment with phosphorus pentachloride. We were able to prepare it in low yield, admixed with a nuclear chlorinated substance,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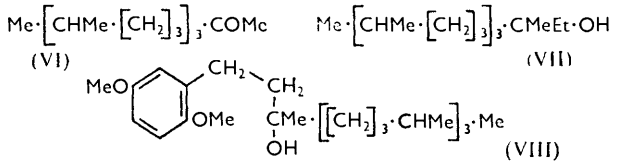
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probably 5-chlorotoluquinol dimethyl ether, by the chlorination of toluquinol dimethyl ether with 1,3-dichloro-5,5-dimethylhydantoin in carbon tetrachloride in the presence of benzoyl peroxide. In view of the poor yield, the chloro-compound was not further investigated. Attempts were then made to replace the bromine atom in the bromomethyl ether by the cyano-group. Although this atom was sufficiently labile to be replaced by methoxyl merely by heating in methanol for a short time, reaction with sodium cyanide in aqueous dioxan was slow. After 5 hr. under reflux, a poor yield of 2-cyanomethylquinol dimethyl ether (V; R = CN) was obtained and this could be smoothly hydrolysed to the known homogentisic acid dimethyl ether [18,19] (V; R = CO_2H). Reduction with lithium aluminium hydride afforded 2-2′-hydroxyethylquinol dimethyl ether, a compound eventually prepared in better yield from 2-bromoquinol.[20] Methylation of the latter gave the dimethyl ether,[21] and reaction of its Grignard reagent with ethylene oxide afforded 2-2′-hydroxyethylquinol dimethyl ether, identical with the product by the first route. Replacement of the hydroxyl group by bromine was best accomplished with phosphorus tribromide in ether containing a trace of pyridine.

The Grignard reagent from 2-2′-bromoethylquinol dimethyl ether, prepared by the entrainment technique with ethyl iodide, and 6,10,14-trimethylpentadecan-2-one (VI) gave two products, a lower-boiling oil, probably 3,7,11,15-tetramethylhexadecan-3-ol (VII), and 1-(2,5-dimethoxyphenyl)-3,7,11,15-tetramethylhexadecan-3-ol (VIII). The latter, when heated with hydrogen bromide in acetic acid, gave an oil, which, after chromatography and molecular distillation, gave pure tocol identical with a specimen prepared by Mamalis *et al.*[22]

The direct introduction of a methyl group at $C_{(5)}$ in tocol is considered difficult.[22,23] Chloromethylation and hydroxymethylation gave unidentified products or unchanged material. This is due to the slow substitution in the unmethylated tocol nucleus compared with the much faster reaction for monosubstituted intermediates. By hydroxymethylation at room temperature with only a trace of alkali, followed by reduction, we were able to prepare, in about 50% yield, a monomethyltocol identical in all respects with the 5-methyltocol of McHale *et al.*

Degradation of Tocol.—The chromanol structure of the synthetic 5-methyltocol was confirmed by oxidative degradations similar to those carried out by Fernholz [24] on α-tocopherol. Tocol itself was used since it was available in larger amounts and its structural relation to the synthetic specimen of McHale *et al.* had been proved. Fernholz used (+)-α-tocopherol and it was thought advisable to compare the degradative products of tocol with those of synthetic (±)-α-tocopherol, in order to avoid possible stereochemical effects. In fact the degradation products of (+)- and (±)-α-tocopherol were identical.

[18] Baumann and Frankel, *Z. physiol. Chem.*, 1895, **20**, 221.
[19] Abbott and Smith, *J. Biol. Chem.*, 1949, 179, 365.
[20] Sarauw, *Annalen*, 1881, **209**, 99.
[21] Noelting and Werner, *Ber.*, 1890, **23**, 3250.
[22] Mamalis, McHale, Green, and Marcinkiewicz, *J.*, 1958, 1850.
[23] Pendse and Karrer, *Helv. Chim. Acta*, 1957, **40**, 1837.
[24] Fernholz, *J. Amer. Chem. Soc.*, 1938, **60**, 700.

Tocol gave the same C_{21}-lactone as α-tocopherol, and the *S*-benzylisothiuronium salts of the parent hydroxy-acids had the same m. p. and mixed m. p. This degradation provided proof of the authentic chromanol structures of tocol and 5-methyltocol.

Chloromethylations and Hydroxymethylations. The Structure of Natural ζ-Tocopherol.—The structure of the synthetic 5-methyltocol was finally confirmed by its conversion into 5,8-dimethyltocol (β-tocopherol) and 5,7,8-trimethyltocol (α-tocopherol). Chloromethylation for 2 hr. at room temperature, followed by reduction,[25] gave 28·5% of β-tocopherol and 26·4% of α-tocopherol. The identity of the β-tocopherol was confirmed by its chromatographic isolation, and conversion into a nitroso-derivative having the same ultraviolet spectrum and R_F value as authentic nitroso-β-tocopherol. The entry of the chloromethyl group *meta* to the hydroxy-group is remarkable and recalls substitutions in 1-substituted 2-naphthols. 5,7-Dimethyltocol could not be detected in the product, indicating either non-formation of a 7-chloromethyl derivative or, alternatively its very rapid conversion into a 7,8-bischloromethyl compound (giving α-tocopherol on reduction). The latter is unlikely since 7-methyltocol under similar conditions gave only 5,7-dimethyltocol, and 8-methyltocol only 5,8-dimethyltocol, showing that the chloromethyl group is strongly deactivating towards a second substitution. In contrast, if 7-methyltocol is chloromethylated in the presence of a reducing agent *in situ* such as stannous chloride, some α-tocopherol is produced.[26] After chloromethylation for 5 hr. and reduction, synthetic 5-methyltocol gave α-tocopherol in 53·5% yield. Isolation on a zinc carbonate–Hyflo Super-cel column, followed by molecular distillation, gave pure α-tocopherol.

Hydroxymethylation of 5-methyltocol and reduction gave only unidentified reducing products. Under slightly different conditions,[27] 7-methyltocol gave 7·8% of 5,7-dimethyltocol (81% unchanged) after 1 hr., and 8-methyltocol gave 47·4% of 5,8-dimethyltocol (40% unchanged) after 30 min. 7,8-Dimethyltocol could not be detected in any of the products. These experiments emphasise the lack of reactivity at $C_{(7)}$ towards the reagent (*meta*-substitution at $C_{(8)}$ is not possible in the Lederer–Manasse reaction).

ε-Tocopherol was chloromethylated under reducing conditions by Eggitt and Norris, who found that the product contained no α-tocopherol but a substance that they identified chromatographically as 5,7-dimethyltocol. It was clear to us that ε-tocopherol could not yield 5,7-dimethyltocol and that the chloromethylation product must have been another substance, chromatographically very similar. Since the naturally-occurring ζ-tocopherol, which is found with ε-tocopherol in the bran fraction of wheat, has previously been identified [5] with 5,7-dimethyltocol, we re-investigated the structures of the two compounds. ζ-Tocopherol was isolated,[5,26] from the lipid fraction of wheat bran, palm oil,* and unpolished rice. ε-Tocopherol was chloromethylated and reduced to give an 18% yield of a material that corresponded to the product described by Eggitt and Norris. This substance, synthetic 5,7-dimethyltocol, and the three specimens of ζ-tocopherol were compared chromatographically by the 4-hr. descending method. The R_F values of the synthetic specimen and of the specimen from rice were identical (0·28). The specimens of ζ-tocopherol from wheat and palm oil had R_F values equal to 0·36, identical with the R_F value of the chloromethylation product from ε-tocopherol. In contrast to the negative result with synthetic 5-methyltocol, hydroxymethylation of ε-tocopherol, followed by reduction, gave a product containing 21% of a substance chromatographically identical with the ζ-tocopherol of wheat. It is clear that neither the ε-tocopherol nor the ζ-tocopherol from wheat is a tocol. The former appears to be a disubstituted (probably dimethyl) chromanol, perhaps with altered substitution at $C_{(2)}$, although the possibility that it has the alternative 6,7-disubstituted coumaranol structure cannot be ruled out. The ζ-tocopherol in wheat is almost certainly the trisubstituted representative in the same series as

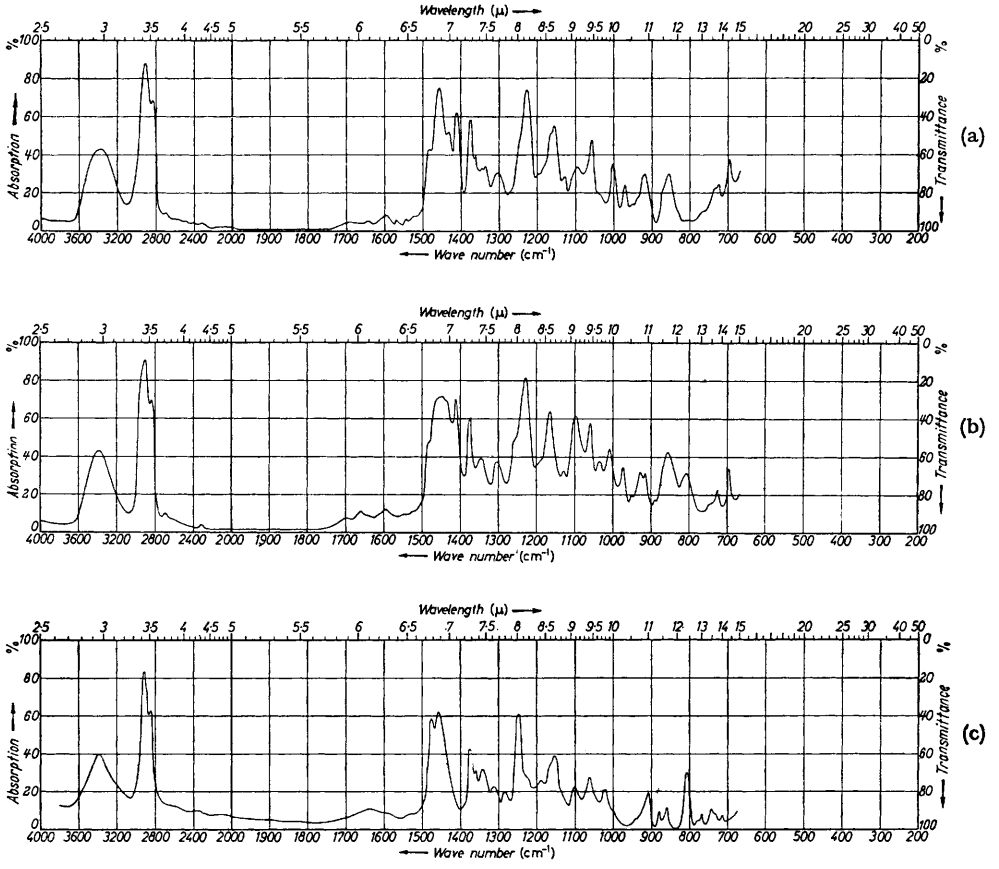
* We are indebted to Dr. R. J. Ward for the palm-oil preparation.

[25] B.P. 629,649, B.P. 639,011.
[26] Green and Marcinkiewicz, *Nature*, 1956, **176**, 86.
[27] B.P. 676,853.

ε-tocopherol. Further evidence for this was obtained by chloromethylating and reducing samples of synthetic 5,7-dimethyltocol and of ζ-tocopherol from wheat bran. The former gave α-tocopherol in 36% yield, whereas the latter gave no α-tocopherol or other identifiable product. The ζ-tocopherol that has been discovered in rice,[26] however, is apparently identical with 5,7-dimethyltocol. Thus, two different naturally-occurring substances, distinguishable from each other only with great difficulty, have both been called ζ-tocopherol. The problem of identity is of some importance from the point of view of animal nutrition.

In the course of this work, the identity of η-tocopherol with 7-methyltocol was confirmed by infrared and nitrosation studies.

Infrared spectra of (a) *β-tocopherol,* (b) *ε-tocopherol, and* (c) *synthetic 5-methyltocol.*



Experimental

Ultraviolet light absorption was measured with a Uvispek spectrophotometer. Infrared spectra were measured with a Grubb-Parsons DB 1/S4 spectrometer fitted with a sodium chloride prism: oils were measured as liquid films, and solids by the potassium bromide disc technique. Pressures cited for molecular distillations are Pirani-gauge measurements. Tocopherol analyses are by the method of Green *et al.*[5]

ε-Tocopherol.—This was obtained by Ward's method.[13] The product from 80 g. of wheat-bran oil when distilled [140° (bath)/5 × 10^{-3} mm.] gave 37 mg. of a pale yellow oil, which was chromatographically pure; $\lambda_{max.}$ 296 mμ, $\lambda_{min.}$ 258 mμ in ethanol; $\nu_{max.}$ 3390m, 2907vs, 1451vs, 1410vs, 1374s, 1229vs, 1164s, 1095s, 1059s, and 857m cm.$^{-1}$.

Chromatography of Tocols and Tocopherols.—The substances (20 μg. of each) were run side

by side on sheets of Whatman No. 1 paper by Eggitt and Ward's descending method.[4] R_F values, measured after 4 hr., for tocol, 5-, 7-, 8-methyltocol, and ε-tocopherol were, respectively, 0·67, 0·52, 0·49, 0·52, and 0·62.

Nitroso-derivatives.—The nitroso-derivatives were prepared by nitrosation of each tocopherol (500 μg.) as previously described [3] and purified chromatographically by separation on sheets of Whatman No. 1 paper impregnated with zinc carbonate. The yellow bands were eluted with ether, and the solutions filtered and evaporated at low temperature to give the nitroso-derivatives as bright yellow oils. Table 2 gives their ultraviolet-absorption maxima, R_F values (93% v/v ethanol as mobile phase), and main infrared absorption bands.

TABLE 2. *Physical data for nitroso-derivatives.*

Derivative	$\lambda_{max.}$ (mμ)	$\lambda_{min.}$ (mμ)	R_F	ν (cm.$^{-1}$)				
5(or 7)-Nitrosotocol *	405—410	326	0·59	2924vs	1732vw	1529m	1460s	1262m
7(or 5)-Nitrosotocol *	404	330—335	0·27	2924vs	—	1529m	1460s	—
5-Nitroso-7-methyltocol	415	358	0·31	2895vs	—	1528m	1456s	1262m
7-Nitroso-5-methyltocol	413	338	0·21	2907vs	1728w	1528m	1458s	1264m
5-Nitroso-8-methyltocol	405	340	0·48	2915vs	—	1519m	1458s	1261m
7-Nitroso-8-methyltocol	405	340	0·79	2907vs	1722w	1522m	1460s	1261m
Nitroso-ε-tocopherol	410	358	0·60	2915vs	1732w	1522m	1456s	1272m
5-Nitroso-7,8-dimethyltocol ...	415	355	0·19	2907vs	—	1522m	1452s	1261m
7-Nitroso-5,8-dimethyltocol ...	410	358	0·42	2907vs	1721w	1522m	1451s	1272m

* Structures uncertain. One is the 5- and the other the 7-nitroso-derivative.

4-Methylcoumaran-5-ol (II; $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = Me$).—3-2′-Hydroxyethyltoluquinol dimethyl ether [1] (0·8 g.), hydrobromic acid (5 g.), and acetic acid (15 ml) were refluxed for 6 hr. The product was diluted with ether, and the extract washed successively with water, saturated aqueous sodium hydrogen carbonate, and water. Evaporation gave a black oil which was refluxed for 3 hr. with anhydrous potassium carbonate (2 g.) and acetone (10 ml.). The product was taken up in ether, washed free from acetone with water, and extracted with N-sodium hydroxide. The alkaline extract was acidified and extracted with ether. Evaporation gave a black oil (0·25 g.) which tended to crystallise. Vacuum sublimation [130° (bath)/0·04 mm.] gave the *coumaranol*, m. p. 111—112°, from water (Found: C, 71·9; H, 6·4. $C_9H_{10}O_2$ requires C, 72·0; H, 6·7%).

5-Hydroxy-2,4,7-trimethylbenzofuran.—To a stirred solution of sodium ethoxide [from sodium (0·56 g.) and ethanol (30 ml.)] containing ethyl acetoacetate (3·6 g.) at room temperature was added 2,5-dimethylbenzoquinone (3·3 g.) in ethanol (100 ml.) during 1·5 hr. The solution was kept overnight and poured on ice (250 g.) and concentrated hydrochloric acid (10 ml.), giving a tar from which the product was isolated by steam-distillation. The *benzofuran* separated as long white needles (0·25 g.), m. p. 133—134°, from aqueous ethanol (Found: C, 74·7; H, 6·8. $C_{11}H_{12}O_2$ requires C, 75·0; H, 6·9%).

2,4,7-Trimethylcoumaran-5-ol (II; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$).—The benzofuran (0·5 g.) in ethanol (15 ml.) was shaken with hydrogen and palladised charcoal (10%) until uptake ceased (*ca.* 5 hr.; 65 ml. absorbed). Removal of catalyst and concentration gave white needles (0·33 g.), m. p. 85—87°, and a second crop (0·10 g.), m. p. 83—86°. Two recrystallisations from light petroleum (b. p. 40—60°) yielded the *coumaranol*, m. p. 97—99° (Found: C, 74·4; H, 8·0. $C_{11}H_{14}O_2$ requires C, 74·2; H, 8·0%).

5-2′-Bromoethyltoluquinol.—5-2′-Hydroxyethyltoluquinol dimethyl ether [9] (4·5 g.), hydrobromic acid (25 g.), and acetic acid (75 ml.) were refluxed for 6 hr. The product was diluted with ether, and the ethereal solution washed successively with water, saturated aqueous sodium hydrogen carbonate, and water. Evaporation and crystallisation from benzene gave the *quinol* as needles (2·1 g.), m. p. 156—157° (Found: C, 47·5; H, 5·1; Br, 33·8. $C_9H_{11}O_2Br$ requires C, 46·8; H, 4·8; Br, 34·6%).

6-Methylcoumaran-5-ol (II; $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = Me$).—5-2′-Bromoethyltoluquinol (0·8 g.), anhydrous potassium carbonate (3·5 g.), and acetone (25 ml.) were refluxed for 1 hr. An ethereal solution of the product was washed with water and dried. Evaporation and crystallisation from benzene–light petroleum (b. p. 40—60°) gave the *coumaranol* (0·25 g.), m. p. 135—136° (Found: C, 71·5; H, 6·8. $C_9H_{10}O_2$ requires C, 72·0; H, 6·7%).

6-Hydroxy-2,5,8-trimethylchroman-4-one.—2,5-Dimethylquinol (14·0 g.), ethyl acetoacetate (18·0 g.), and ethanol (10 ml.) were stirred at 0—5° while phosphoric oxide (30 g.) was added in

small portions. The temperature was then slowly raised to 140° and the mixture kept thereat for 2 hr. The mixture was cooled, crushed ice added, and the product extracted with ether. The dark ethereal extracts were shaken with aqueous 2N-sodium hydroxide (3 × 20 ml.), and the alkaline washings acidified. The *chromanone* (1·9 g.) crystallised from ethanol as needles, m. p. 280—282° (Found: C, 70·1; H, 5·8. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·9%).

2,5,8-*Trimethylchroman*-6-*ol* (I; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$).—The chromanone (1·0 g.) in ethanol (100 ml.) was hydrogenated, a palladised charcoal (10%) catalyst being used. The *chromanol* was obtained as white prismatic needles, m. p. 126—128° (from ethanol) (Found: C, 75·0; H, 8·4. $C_{12}H_{16}O_2$ requires C, 75·0; H, 8·4%).

2,5,7,8-*Tetramethylchroman*-6-*ol* (I; $R^1 = R^2 = R^3 = R^4 = Me$, $R^5 = H$).—This chromanol was prepared by hydrogenation of 6-hydroxy-2,5,7,8-tetramethylchroman-4-one [28] and formed white needles from light petroleum (b. p. 40—60°), m. p. 144—146°. John, Günther, and Schmeil [29] give m. p. 145°.

2,2,5,7,8-*Pentamethylchroman*-6-*ol* (I; $R^1 = R^2 = R^3 = R^4 = R^5 = Me$).—The pentamethyl compound was made according to Smith *et al.*,[12b] m. p. 93—94° (aqueous ethanol) (lit., m. p. 94—94·5°).

2,4,6,7-*Tetramethylcoumaran*-5-*ol* (II; $R^1 = R^2 = R^3 = R^4 = Me$, $R^5 = H$).—This coumaranol was obtained by reduction (hydrogen–palladised charcoal) of 5-hydroxy-2,4,6,7-tetramethylbenzofuran [30] and formed white needles, m. p. 133—134°, from aqueous ethanol. The literature records m. p.s for this compound varying from 123° to 131·5°; cf. Smith *et al.*[12a].

2-*Bromomethylquinol Dimethyl Ether* (V; R = Br).—Toluquinol dimethyl ether (8·0 g.), *N*-bromosuccinimide (9·8 g.), carbon tetrachloride (100 ml.), and benzoyl peroxide (100 mg.) were heated under reflux for 2 hr. After cooling, the mixture was filtered, the solid washed with carbon tetrachloride, and the combined filtrates evaporated. Crystallisation of the solid from light petroleum (b. p. 60—80°) afforded the *bromomethyl derivative* as needles (9·0 g.), m. p. 75—76° (Found: C, 47·2; H, 5·0; Br, 34·1. $C_9H_{11}O_2Br$ requires C, 46·8; H, 4·8; Br, 34·6%). When this was heated with methanol, rapid reaction yielded an oil, b. p. 68°/0·07 mm., n_D^{21} 1·5250, which from the analytical figures appeared to be 2-*methoxymethylquinol dimethyl ether* (Found: C, 65·9; H, 7·6. $C_{10}H_{14}O_3$ requires C, 66·0; H, 7·8%).

1,2-*Bis*-(2,5-*dimethoxyphenyl*)*ethane*.—2-Bromomethylquinol dimethyl ether (8·5 g.) in dry ether (40 ml.) was slowly added to magnesium shavings (0·9 g.) and a crystal of iodine. Reaction proceeded readily and was completed under reflux for 1 hr. The mixture was cooled to room temperature and stirred while a stream of formaldehyde (from 2·2 g. of paraform-formaldehyde) in nitrogen was blown in. After treatment with sulphuric acid (10%; 45 ml.) the ether layer was separated, filtered, and distilled in steam. The residual oil, after isolation with ether, slowly crystallised. The solid (3·3 g.) when crystallised from aqueous ethanol yielded the *diphenylethane*, m. p. 64—66° (Found: C, 71·2; H, 7·4. $C_{18}H_{22}O_4$ requires C, 71·5; H, 7·3%). In subsequent experiments, the oil which separated from the initial reaction was shown to be the diphenylethane; no variation in the experimental conditions was found that would prevent the formation of this product.

5-*Chlorotoluquinol Dimethyl Ether*.—Toluquinol dimethyl ether (9·0 g.), 1,3-dichloro-5,5-dimethylhydantoin [31] (11·8 g.; m. p. 125—132°), benzoyl peroxide (100 mg.), and carbon tetrachloride (80 ml.) were heated under reflux for 30 min. After cooling, the solid was collected, washed with carbon tetrachloride and rejected (9·0 g.; m. p. 120—130°). The combined filtrates were evaporated and the oil taken up in hot light petroleum (30 ml.; b. p. 60—80°). The filtered solution was cooled, large white prisms then separating (5·6 g.), m. p. 86—90°, and a second crop being obtained on concentration of the mother liquors (1·75 g.), m. p. 90—92°. Recrystallisation yielded prisms of 5-*chlorotoluquinol dimethyl ether*, m. p. 92—93° (Found: C, 57·7; H, 5·5; Cl, 19·5. $C_9H_{11}O_2Cl$ requires C, 57·8; H, 5·9; Cl, 19·0%). The product did not give a precipitate with silver nitrate–nitric acid; its infrared absorption corresponded closely with that of 5-bromotoluquinol dimethyl ether.

Chlorination of Toluquinol Dimethyl Ether with Purified 1,3-*Dichloro*-5,5-*dimethylhydantoin*.—Toluquinol dimethyl ether (9·0 g.), 1,3-dichloro-5,5-dimethylhydantoin (11·8 g., m. p. 131—132°), benzoyl peroxide (50 mg.), and carbon tetrachloride (80 ml.) were heated under reflux for

[28] Werder and Jung, *Ber.*, 1938, **71**, 2650.
[29] John, Günther, and Schmeil, *Ber.*, 1938, **71**, 2637.
[30] Smith and MacMullen, *J. Amer. Chem. Soc.*, 1936, **58**, 629.
[31] B.P. 707,990.

2 hr. The product was obtained in light petroleum, as in the previous experiment, and fractionally crystallised from it, giving as a less-soluble fraction (3·4 g.), 5-chlorotoluquinol dimethyl ether, m. p. 92—93°, and as more-soluble fraction, 2-chloromethylquinol dimethyl ether (2·9 g.), softening at 62—65° and melting at 72° (lit.[20] m. p. 72—73°). Its infrared spectrum differed from that of the nuclear chlorinated product in the longer wavelength region and closely resembled that of 2-bromomethylquinol dimethyl ether. A precipitate was obtained with silver nitrate–nitric acid.

2-Cyanomethylquinol Dimethyl Ether (V; R = CN).—Sodium cyanide (5·1 g.) and water (6 ml.) were stirred on the steam-bath while 2-bromomethylquinol dimethyl ether (10·2 g.) in dioxan (100 ml.) was slowly added (30 min.). After a further 5 hr. the mixture was cooled, water added, the product extracted with ether, and the extracts washed with water, dried, and evaporated. Distillation afforded a pale yellow liquid (2·9 g.), b. p. 100—102°/0·05 mm., which solidified, and a viscous yellow oil, b. p. 103—140°/0·05 mm., which was not further investigated. The solid fraction when crystallised from aqueous ethanol furnished the *nitrile* as prisms, m. p. 52—54° (Found: C, 67·3; H, 6·5; N, 7·7. $C_{10}H_{11}O_2N$ requires C, 67·8; H, 6·3; N, 7·9%).

Homogentisic Acid Dimethyl Ether (V; R = CO_2H).—The nitrile (4·0 g.), sodium hydroxide (1·6 g.), water (2·5 ml.), and ethanol (20 ml.) were heated under reflux for 24 hr. On cooling, the sodium salt separated; water (30 ml.) was added, the ethanol removed under reduced pressure, and the clear solution acidified, a white solid then separating. The acid was collected (4·0 g.); it crystallised from water containing a little ethanol as white needles, m. p. 122—123° (lit.,[20] m. p. 124·5°).

Reduction of Homogentisic Acid Dimethyl Ether.—To a stirred mixture of lithium aluminium hydride (0·6 g.) and dry ether (10 ml.) a solution of the foregoing acid (1·4 g.) in dry ether (50 ml.) was slowly added. The mixture was stirred for 1 hr., cooled, and treated successively with moist ether (15 ml.) and water (10 ml.). The ether layer was separated and dried, the solvent removed, and the residual oil (1·08 g.) distilled in a short-path still to give 2-hydroxyethylquinol dimethyl ether (0·80 g.) [100° (bath)/0·3 mm.], n_D^{23} 1·5378 (Found: C, 65·3; H, 8·0. Calc. for $C_{10}H_{14}O_3$: C, 66·0; H, 7·8%), and an impure higher-boiling fraction (0·13 g.) [110° (bath)/0·3 mm.], the infrared absorption of the ether was identical with that of the product described earlier in this section.

2-2′-Hydroxyethylquinol Dimethyl Ether (IV; R = OH).—To magnesium shavings (3·1 g.) were added a few ml. of a solution of 2-bromoquinol dimethyl ether [19] (26·4 g.) in dry ether (100 ml.), and the reaction initiated by the addition of ethyl iodide (1 drop). The remaining ethereal solution was slowly added with stirring so as to maintain reflux, and the reaction completed by heating for 1·5 hr. The solution, after being cooled to 0°, was slowly treated with ethylene oxide (17·0 g.) in dry ether (35 ml.), and the mixture refluxed for 1·5 hr. and decomposed with dilute sulphuric acid (10%). After separation of the ethereal layer the aqueous layer was extracted with ether and the combined ethereal solutions were washed with water and concentrated to a pale yellow oil. Steam-distillation afforded a white solid that crystallised from light petroleum (b. p. 40—60°) as large plates (2·1 g.), m. p. 55° not depressed on admixture with an authentic specimen of quinol dimethyl ether. The oil non-volatile in steam was isolated with ether and distilled, the required *product* being obtained as a pale yellow oil (10·0 g.). On redistillation this had b. p. 108°/0·2 mm., n_D^{25} 1·5370 (Found: C, 65·3; H, 7·9. $C_{10}H_{14}O_3$ requires C, 66·0; H, 7·8%). The *3,5-dinitrophenylurethane* separated from aqueous acetone as orange-yellow needles, m. p. 166—167° (Found: C, 52·1; H, 4·6; N, 10·8. $C_{17}H_{17}O_8N_3$ requires C, 52·2; H, 4·4; N, 10·7%).

2-2′-Bromoethylquinol Dimethyl Ether (IV; R = Br).—A stirred mixture of 2-2′-hydroxyethylquinol dimethyl ether (6·0 g.) in dry ether (30 ml.) containing pyridine (6 drops) was heated under reflux, and phosphorus tribromide (2·3 ml.) in light petroleum (5·0 ml.; b. p. 80—100°) added during 20 min. After a further 1·5 hr. it was cooled and treated with water (10 ml.), the ether layer separated, and the aqueous layer extracted with ether. The combined ethereal extracts were washed successively with saturated aqueous sodium hydrogen carbonate (3 × 15 ml.), N-sodium hydroxide (2 × 10 ml.), and water. Acidification of the sodium hydroxide washings afforded a trace of oil that was rejected. Similar treatment of the sodium hydrogen carbonate solution yielded, after isolation with ether, a yellow oil (2·3 g.), probably the crude dihydrogen phosphite of the starting material, since a clear solution in N-sodium hydroxide (25 ml.), when warmed on the steam-bath for 30 min., precipitated an oil (1·05 g.)

identified as the unchanged hydroxyethyl derivative. The original ethanol extract was evaporated to give a pale yellow mobile oil (4·15 g.), distillation of which afforded the *bromoethyl product*, b. p. 93—94°/0·1 mm., n_D^{23} 1·5572 (Found: C, 49·4; H, 5·5; Br, 31·8. $C_{10}H_{13}O_2Br$ requires C, 49·0; H, 5·3; Br, 32·7%). The *isothiuronium bromide* separated from acetone as prisms, m. p. 115—116° (Found: C, 41·2; H, 5·5; N, 8·6. $C_{11}H_{17}O_2N_2SBr$ requires C, 41·1; H, 5·3; N, 8·7%). The derived *picrate* formed yellow needles, m. p. 215—216°, from ethanol (Found: C, 43·6; H, 3·9; N, 15·1. $C_{17}H_{19}O_9N_5S$ requires C, 43·4; H, 4·1; N, 14·9%).

1-(2,5-*Dimethoxyphenyl*)-3,7,11,15-*tetramethylhexadecan*-3-*ol*.—To magnesium shavings (0·85 g.) and a crystal of iodine were added a few ml. of a solution of 2-2′-bromoethylquinol dimethyl ether (3·98 g.) and ethyl iodide (2·55 g.) in dry ether (20 ml.). Stirring was begun and the remaining solution added at such a rate as to maintain gentle reflux. The mixture was heated for a further 1·5 hr., and then cooled, and a solution of 6,10,14-trimethylpentadecan-2-one (8·7 g.) in dry ether (15 ml.) slowly added. The mixture was then refluxed for 1·5 hr. and cooled, and the complex decomposed by addition of 2N-hydrochloric acid (30 ml.). Ethereal extracts were washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated to give a red oil (11·3 g.). Lower-boiling material was distilled off: (*a*) pale yellow oil (1·07 g.), b. p. 68—70°/0·05 mm., n_D^{19} 1·5026; (*b*) pale yellow oil (3·82 g.), b. p. 114—120°/0·05 mm., n_D^{19} 1·4593, probably crude 3,7,11,15-tetramethylhexadecan-3-ol. The remaining oil was distilled at 5×10^{-3} mm. to give the following fractions: (*a*) pale yellow oil (1·37 g.) [140—150° (bath)], (*b*) yellow oil (0·80 g.) [150—170° (bath)], (*c*) yellow oil (2·67 g.) [170—180° (bath)]. Fraction (*c*) was used for the preparation of tocol and from it the analytical sample of the *hexadecanol* was obtained as a yellow oil by a further distillation [170° (bath)/5×10^{-3} mm.], n_D^{20} 1·4788 (Found: C, 78·4; H, 11·6. $C_{28}H_{50}O_3$ requires C, 77·7; H, 11·7%).

Tocol.—The hexadecanol (300 mg.) was heated under reflux with hydrogen bromide in acetic acid (6·0 ml.; 25%) for 8 hr., the dark red solution evaporated, and the residual oil taken up in ether and washed with aqueous sodium hydrogen carbonate and water. The ethereal solution was evaporated and the brown oil (270 mg.; assay 22%) chromatographed in light petroleum (10 ml.; b. p. 40—60°) on alumina (Peter Spence type " O "; 10 × 1 cm.). The chromatogram was developed with benzene–light petroleum (b. p. 40—60°) (150 ml.; 1 : 1); the eluate contained practically no tocol and was rejected, the tocol being recovered by elution with ether (180 ml.). The brown oil (48 mg.) was distilled, affording a yellow oil [160° (bath)/5×10^{-3} mm.] (assay 98%), which on two-dimensional paper-chromatography behaved identically with tocol previously prepared,[22] and had the same infrared spectrum.

Methylation of Tocol.—Tocol (1 g.) was dissolved in a mixture of ethanol (7·8 ml.) and formaldehyde solution (40%; 2·2 ml.), and saturated potassium hydroxide (0·22 ml.) added. The solution was kept at room temperature for 72 hr. Isopropyl ether (free from peroxides; 30 ml.) and concentrated hydrochloric acid (10 ml.) were added and the mixture was heated under reflux for 2 hr. while zinc powder (2 g.) was added in small portions. The ether solution was washed, dried, and evaporated to give a yellow oil.

The oil was chromatographed in light petroleum (b. p. 40—60°) on alumina (Peter Spence, type " O "; 100 g.). The column was rendered fluorescent before use as follows: alumina (5 g.) was mixed with a 0·1% solution of sodium fluorescein in methanol (25 ml.), filtered off, and washed with methanol. It was dried at 200° for 1 hr. and then intimately mixed with the remaining 95 g. of alumina. The column was developed with benzene, then benzene containing 1% v/v of ethanol. The column was extracted under ultraviolet light, and the dark tocopherol band cut out and extracted with ethanol. Evaporation gave a yellow oil (300 mg.; 97% purity by analysis) which was distilled [170° (bath)/5×10^{-3} mm.] to give 5-methyltocol, identical with that prepared by McHale *et al.*[1] (paper chromatography, ultraviolet absorption, and infrared spectrum) (Found: C, 80·1; H, 11·5. Calc. for $C_{27}H_{46}O_2$: C, 80·5; H, 11·5%).

Oxidation of (±)-α-*Tocopherol*.—(±)-α-Tocopherol (1·2 g.) was oxidised by Fernholz's method.[24] Distillation [140° (bath)/5×10^{-3} mm.] of the neutral fraction (0·56 g.) gave 4-hydroxy-4,8,12,16-tetramethylheptadecanoic lactone, n_D^{21} 1·4600, identical in refractive index and infrared spectrum with the lactone obtained from (+)-α-tocopherol. The *S*-benzylisothiuronium salts prepared from both lactones had m. p. and mixed m. p. 117—118°.

Oxidation of Tocol.—Tocol (3 g.) was oxidised in the same manner. Distillation [140° (bath)/5×10^{-3} mm.] of the neutral fraction (1 g.) gave a lactone, n_D^{20} 1·4590, from which an *S*-benzylisothiuronium salt, m. p. 117—118°, was prepared. The lactone had the same infrared spectrum as 4-hydroxy-4,8,12,16-tetramethylheptadecanoic lactone.

Chloromethylations.—(*a*) Synthetic 5-methyltocol (80 mg.) and paraformaldehyde (50 mg.) were dissolved in dry ether saturated with hydrogen chloride (5 ml.), and kept for 2 hr. More ether and concentrated hydrochloric acid (5 ml.) were added, followed by an excess of zinc dust (*ca.* 0·5 g.) added during 30 min. The ethereal solution was separated, washed twice with water, and evaporated to give a pale yellow oil (71 mg.) containing 28·5% of β-tocopherol and 26·4% of α-tocopherol, identified by two-dimensional paper-chromatography. The product (2 mg.) was chromatographed on paper impregnated with zinc carbonate, and the β-tocopherol band removed (*ca.* 500 μg.) and separately nitrosated. The nitroso-derivative behaved identically, on two-dimensional chromatography, with authentic 7-nitroso-β-tocopherol. The remainder of the product was chloromethylated for a further 3 hr. The product (65 mg.) then contained 53·5% of α-tocopherol and no β-tocopherol. It was chromatographed on a column (2·5 cm. × 20 cm.) of zinc carbonate–Hyflo Super-cel (1 : 1; rendered fluorescent by mixing the column packing with a 0·02% w/v solution of sodium fluorescein in methanol, washing it with methanol, and then drying it at 150° for 1 hr.) in light petroleum (60—80°), and the column developed with benzene–light petroleum (1 : 2). The column was extracted under ultraviolet light, and the dark band due to the tocopherol cut out and eluted with ether. Evaporation and distillation [160° (bath)/5 × 10^{-3} mm.] gave α-tocopherol (infrared spectrum identical with that of an authentic sample).

(*b*) 8-Methyltocol (20 mg.) and paraformaldehyde (50 mg.) were dissolved in dry ether saturated with hydrogen chloride (5 ml.) and kept for 5 min. Reduction with zinc dust and hydrochloric acid and working up gave a product containing 22% of β-tocopherol, 27·7% of an unknown reducing substance, and some starting material. In another reaction, after 30 min. the product contained 47·6% of β-tocopherol. No 7,8-dimethyltocol could be detected (*o*-dianisidine coupling test).

(*c*) 7-Methyltocol (1 mg.) and paraformaldehyde (0·4 mg.) were dissolved in dry ether saturated with hydrogen chloride (5 ml.) and kept for 30 min. After reduction and working up, the product contained 34% of 5,7-dimethyltocol and 48% of unchanged 7-methyltocol.

(*d*) 5,7-Dimethyltocol (1 mg.) was treated as in (*c*) above; the product contained 36% of α-tocopherol.

(*e*) ε-Tocopherol (1 mg.) was treated as in (*c*) above. The product contained 18% of a substance, chromatographically identical with a specimen of natural ζ-tocopherol from wheat bran (R_F = 0·36). Continuing the reaction for 4 hr. did not produce α-tocopherol.

Hydroxymethylations.—(*a*) 5-Methyltocol (1 mg.) was dissolved in a mixture of ethanol (0·75 ml.) and formaldehyde solution (40%; 0·2 ml.), and saturated potassium hydroxide solution (0·02 ml.) was added. After 3 days, isopropyl ether (10 ml.), concentrated hydrochloric acid (5 ml.), and zinc dust (0·5 g.) were added, and the mixture was heated under reflux at 60° for 2 hr. The ethereal solution was separated, washed three times with water, and evaporated to give a product which contained no identifiable products.

(*b*) ε-Tocopherol (1 mg.) was treated as in (*a*) above. The product, on chromatographic separation, contained 21% of a substance identical with ζ-tocopherol from wheat bran (R_F = 0·36).

(*c*) 7-Methyltocol (50 mg.) was dissolved in a mixture of ethanol (3 ml.), formaldehyde solution (40%; 0·3 ml.), and potassium hydroxide (0·1 g.). The solution was heated in a sealed ampoule at 65° for 1 hr. After reduction and working up the product contained 7·8% of 5,7-dimethyltocol (R_F = 0·28, identical with that of an authentic specimen), and 81% of unchanged material.

(*d*) 8-Methyltocol (50 mg.) was treated as in (*c*) above for 30 min. After reduction and working up, the product contained 47·4% of 5,8-dimethyltocol and 40% of unchanged material.

Chromatography of 5,7-Dimethyltocol and ζ-Tocopherols.—Specimens of ζ-tocopherol were obtained from wheat bran, palm oil, and unpolished rice as previously described.[5,26] These substances (20 μg.), together with 20 μg. of synthetic 5,7-dimethyltocol, were run side by side for 4 hr. by the descending method, Whatman No. 4 papers being used. The ζ-tocopherol from wheat bran and palm oil had an R_F value of 0·36. The ζ-tocopherol from rice and 5,7-dimethyltocol had an R_F value of 0·28.

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