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The Isolation and Properties of δ-Tocotrienol from *Hevea* Latex

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1. δ-Tocotrienol (8-methyltocotrienol) was isolated from the latex of *Hevea brasiliensis*. This new member of the tocopherol family is a pale-yellow oil at room temperature. 2. The properties of δ-tocotrienol are very similar to those of δ-tocopherol and the small differences can be explained by the change in side chain. 3. The ultraviolet and infrared spectra of δ-tocotrienol were determined and a conversion factor for use with the Emmerie–Engel reaction was worked out. Details are given for the chromatography of δ-tocotrienol on thin layers (adsorption and partition) and reversed-phase paper, and the nitroso derivatives were formed. 4. An ethyl carbonate ester of δ-tocotrienol was prepared and compared with a similar ester of δ-tocopherol. 5. Hydroxymethylation of δ-tocotrienol followed by reduction gave β-tocotrienol as a major product.

Stern, Robeson, Weisler & Baxter (1947) described a derivative of tocol, δ-tocopherol, which was related to the already well-known α-, β- and γ-tocopherol. Thus α-, β- and γ-tocopherol were the 5,7,8-trimethyl, 5,8-dimethyl and 7,8-dimethyl derivatives of tocol respectively, and Stern *et al.* (1947) showed δ-tocopherol to be the 8-methyl derivative (see Fig. 1).

δ-Tocopherol, found originally in soya-bean oil at a level of 30% of the total tocopherols present, was soon found to be present in peanut and cottonseed oils (Weisler, Robeson & Baxter, 1947). Since that time δ-tocopherol has been described in castor, neem, safflower, sesame, mustard and corn oils (Herting & Drury, 1963; Rao, Rao & Achaya, 1965), in raspberry and blackberry fruits (Booth & Bradford, 1963), in plant leaves (Booth, 1962) and in some seaweeds (Brown, 1953).

It was shown (Green, Mamalis, Marcinkiewicz & McHale, 1960; McHale, Green, Marcinkiewicz, Feeney & Sutcliffe, 1963) that two tocopherols, designated ζ_1 and ϵ, were related to α- and β-tocopherol respectively, the side chain at position 2 having three double bonds and being truly isoprenoid (see Fig. 1). Pennock, Hemming & Kerr (1964) realized that the η-tocopherol of rice and palm oil was not 7-methyltocol, as had seemed likely previously (Green & Marcinkiewicz, 1956), but was the unsaturated derivative related to γ-tocopherol. The name 'tocotrienol' was suggested as a trivial name for those members of the tocopherol family with true isoprenoid side chains by Bunyan, McHale, Green & Marcinkiewicz (1961). We can therefore call ζ_1-, ϵ- and η-tocopherol by the names α-, β- and γ-tocotrienol respectively. When examining palm oil, Pennock *et al.* (1964) located another tocopherol, this time related to δ-tocopherol and shown to be δ-tocotrienol.

It was hoped to isolate sufficient δ-tocotrienol from palm oil to allow physical and chemical properties of this compound to be determined. However, the amount in palm oil was relatively small (about 70 μg./g. of oil) and purification was made difficult by the large amounts of carotenoid present. It was therefore very fortunate that δ-tocotrienol was found in quite large amounts in latex from *Hevea brasiliensis*, which contained only very small quantities of carotenoids. Dunphy, Whittle, Pennock & Morton (1965) reported on tocotrienols in commercial latex from *Hevea*, finding a total of about 46 μg. of δ-tocotrienol/g. of latex of which 25 μg./g. was free and 21 μg./g. was esterified. The commercial latex could be extracted to yield an oil containing a total of 4200 μg. of δ-tocotrienol/g., and accordingly it was decided to isolate δ-tocotrienol from this source and investigate its properties.

MATERIALS AND METHODS

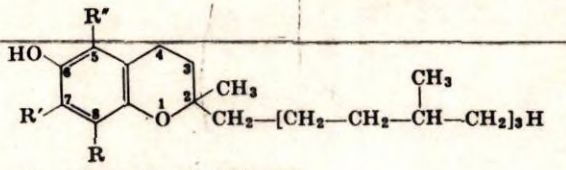
Latex. Commercial latex from Malaya was used. This material had been concentrated to 60% rubber on the plantation and contained ammonia as a stabilizer.

Alumina. This was acid-washed and deactivated with water to Brockmann grade 3 (Laidman, Morton, Paterson & Pennock, 1960).

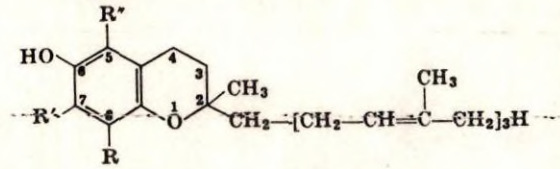
Diethyl ether. This was dried over sodium wire and distilled over reduced iron.

Light petroleum. This was dried over sodium wire and distilled (b.p. 40–60°).

Di-isopropyl ether. This was technical grade and contained quinol (0·01%) to prevent peroxide formation.



α-Tocopherol: R=R′=R″=CH_3
β-Tocopherol: R=R″=CH_3, R′=H
γ-Tocopherol: R=R′=CH_3, R″=H
δ-Tocopherol: R=CH_3, R′=R″=H



α-Tocotrienol (ζ1-tocopherol): R=R′=R″=CH_3
β-Tocotrienol (ε-tocopherol): R=R″=CH_3, R′=H
γ-Tocotrienol (η-tocopherol): R=R′=CH_3, R″=H
δ-Tocotrienol: R=CH_3, R′=R″=H

Fig. 1. Naturally occurring tocopherols and tocotrienols.

Chloroform. This was a general analytical reagent containing ethanol (1%) as a stabilizer.

Paraffin. Liquid paraffin was obtained from A. Gallenkamp and Co., Widnes, Lancs.

Extraction of the latex. (i) The first method was based on the Folch method for lipid extraction (Folch, Ascoli, Lees, Meath & Le Baron, 1951). Samples of latex (50ml.) were homogenized in an Ultra-Turrax macerator with 750ml. of chloroform–methanol (2:1, v/v) for 2–3min. During this process the rubber coagulated and was filtered off with a sintered-glass funnel. The chloroform–methanol solution was mixed with water and the chloroform was allowed to settle out overnight. The supernatant was removed by suction and the chloroform reduced in volume by distillation under vacuum. At this stage diethyl ether was added, the ethereal solution was washed with water and dried over anhydrous Na_2SO_4, and after removal of the latter by filtration the solvent was removed by vacuum distillation, yielding the latex lipid.

(ii) The second method, which was found to be much easier from a practical point of view, consisted in pouring 50ml. of latex into 500ml. of chloroform with constant stirring and then homogenizing in the Kenwood Chef homogenizer at half speed for 1min. Methanol (250ml.) was added to the emulsion that had formed and the mixture was stirred until the rubber coagulated. The mixture was homogenized for a few seconds at high speed and then for 1½min. at a lower speed, after which the rubber was removed by filtering with glass wool and the filtrate treated as in the first method. The two methods of extraction were used to about the same extent.

Column chromatography. The total lipid was chromatographed on 250g. columns of Brockmann grade-3 alumina, the following solvents being used for developing the column: light petroleum, 2% E/P,* 4% E/P, 6% E/P, 8% E/P, 15% E/P, 30% E/P and diethyl ether. In general about 2l. of each solvent was used and approx. 5·5g. of lipid was put on each column. The 2%-E/P fraction contained tocotrienyl esters, and δ-tocotrienol was eluted by 8% and 15% E/P with a trace in the 30%-E/P fraction. The main weight of the later fractions was a sterol, probably β-sitosterol, from which it was inseparable on thin-layer chromatography.

Saponification of the tocotrienyl esters. The material eluted by 2% E/P, i.e. the fraction containing tocotrienyl esters, was saponified to yield the free tocotrienols by a method that was essentially that of Idler & Baumann (1952). The fraction was dissolved in 1l. of boiling benzene, 100g. of pyrogallol was added and then 1l. of 15% (w/v) KOH in 85% (v/v) ethanol was added cautiously. The mixture was refluxed for 20min., cooled, diluted with water and extracted four times with diethyl ether. The extract yielded an orange–yellow solid that was shown by thin-layer chromatography to contain mainly γ-tocotrienol together with α- and δ-tocotrienol.

The material was chromatographed on a 300g. column of Brockmann grade-3 alumina and fractions eluted by 2% E/P, 4% E/P, 5% E/P, 6% E/P, 7% E/P, 9% E/P and 12% E/P were collected. δ-Tocotrienol was present in the 7%-E/P, 9%-E/P and 12%-E/P fractions.

Removal of sterol. (i) By crystallization. δ-Tocotrienol was found in the 8–30%-E/P fractions in the initial chromatography and in the 7–12%-E/P fractions from the chromatography of the hydrolysed product, and these fractions were combined, dissolved in methanol and left overnight at −20°. The white crystalline material was centrifuged down, the supernatant reduced in volume and more sterol removed by a second crystallization at −20°. The final methanol supernatant was diluted with water and extracted with light petroleum to yield a red oily solid.

(ii) By precipitation with digitonin. The material obtained from the mother liquors above was dissolved in 100ml. of 95% (v/v) ethanol and heated to boiling. A solution of digitonin (4·4g.) in 100ml. of 90% (v/v) ethanol (hot) was added and almost immediately a precipitation of sterol digitonide occurred. The mixture was stirred for 15min. at room temperature and was then left overnight at 0°. The precipitate was centrifuged down and, after removal of the supernatant, the sterol digitonide was washed with ice-cold 95% (v/v) ethanol and recentrifuged. The combined supernatants were diluted with water and extracted with diethyl ether to yield an orange oil.

Preparative thin-layer chromatography. The oil was chromatographed on thin layers (500μ) of silica gel G with chloroform as solvent in tanks lined with filter paper saturated with solvent. Before use the silica-gel plates were developed with diethyl ether as solvent to remove any lipid impurities in the adsorbent to the solvent front. The ether was allowed to evaporate off and the silica-gel layers were reactivated before use. About 30mg. of tocotrienol concentrate was chromatographed on each plate and after development the chromatograms were sprayed with 0·002% (w/v) fluorescein in ethanol. The bands of silica gel corresponding to γ- and δ-tocotrienol showed up pink and were scraped off and extracted exhaustively with diethyl ether.

The δ-tocotrienol fraction was chromatographed once again on thin layers with chloroform as solvent and then on thin layers with 20% (v/v) di-isopropyl ether in light

* Abbreviation: E/P, solution of diethyl ether in light petroleum.

petroleum as solvent. In the latter case the chromatography tanks were not lined with filter paper. The final product was a very pale-yellow oil that remained an oil even at −5° and gave only one spot on thin-layer chromatography with four separate systems. This material was used for the experimental work.

Analytical thin-layer chromatography. (i) Adsorption chromatography was carried out on thin layers (250 μ) of silica gel G with four different solvent systems. With chloroform and 1% (v/v) methanol in benzene as solvents the chromatography tanks were lined with filter paper to help to saturate the atmosphere, but with 20% (v/v) di-isopropyl ether in light petroleum or 20% (v/v) di-isopropyl ether in benzene as solvents the tanks were unlined. The two-dimensional system of Pennock *et al.* (1964) was also used.

(ii) Partition chromatography was performed on kieselguhr G thin layers (250 μ thick) coated with paraffin. The kieselguhr plates were dipped into a 5% (v/v) solution of paraffin in light petroleum and the solvent was allowed to evaporate. The solvents (saturated with liquid paraffin before use) were ethanol–water and acetone–water systems.

Paper chromatography. Whatman no. 1 paper was dipped into 5% (v/v) paraffin in light petroleum and the solvent evaporated. Dimethylformamide–water mixtures (saturated with paraffin) were used as solvents.

Ultraviolet spectra. These were examined in the Unicam SP.500 spectrophotometer and the SP.800 recording spectrophotometer. Spectra were usually recorded in cyclohexane and sometimes in ethanol. Unless stated otherwise the solvent used was cyclohexane.

Infrared spectra. These were examined as oily smears on NaCl disks in the Perkin–Elmer 137 Infracord instrument.

Reaction with Emmerie–Engel reagent. The method used was that described by the Analytical Methods Committee (1959); to 3 ml. of the tocotrienol solution in ethanol 0·5 ml. of 0·5% (w/v) $\alpha\alpha'$-bipyridyl in ethanol was added followed by 0·5 ml. of 0·2% (w/v) $FeCl_3$ in ethanol. The reaction was studied by following colour production over a period of time and also by studying the colour produced at specified times by different amounts of tocotrienol.

Ethyl carbonate ester. The ethyl carbonate esters of δ-tocopherol and δ-tocotrienol were made by a method used by J. Glover & M. A. Malik (personal communication) of this Department. A twofold excess of ethyl chloroformate was added to 10 mg. of δ-tocopherol or δ-tocotrienol in 2 ml. of acetone and the mixture was shaken vigorously and then cooled in ice. Another portion of ethyl chloroformate was added and the mixture shaken once more, and then 0·1 ml. of N-NaOH was added and the ester extracted with light petroleum. The yield was essentially quantitative.

RESULTS

Commercial latex (4 kg.) was extracted to yield 33·5 g. of pale-yellow lipid, i.e. 0·84% of the latex. This lipid was chromatographed on six 250 g. columns of Brockmann grade-3 alumina and Table 1 shows the results from one such column. The fraction eluted by light petroleum contained a

Table 1. *Chromatography of latex lipid on alumina*

Latex lipid (5·6 g.) was chromatographed on 250 g. of Brockmann grade-3 alumina. The fractions were examined by thin-layer chromatography on silica gel G with 5% (v/v) diethyl ether in light petroleum as solvent for the light-petroleum and 2%-E/P fractions and 25% (v/v) di-isopropyl ether in light petroleum for the others.

Eluent	Volume (l.)	Material eluted (mg.)	Absorption spectra λ (mμ)		$E^{1\%}_{1cm.}$	Details from thin-layer chromatography
Light petroleum	1·5	84·1	255	(infl.)	38·3	Squalene and saturated hydrocarbons
			428	(infl.)		
			452	(max.)	2·6	
			478	(max.)	2·1	
2% E/P	2·0	606·4	280·5	(max.)	25·8	Squalene, tocotrienyl ester and a trace of α-tocopherol
			286	(max.)	27·5	
			290	(max.)	28·6	
4% E/P	2·0	231·8	292	(max.)	26·9	α-Tocotrienol
			298	(max.)	27·2	
6% E/P	2·0	654·6	294·5	(max.)	11·4	α- and γ-Tocotrienol
			301·5	(max.)	11·6	
8% E/P	2·0	237·6	295	(max.)	33·7	α- (trace), γ- and δ- (trace) Tocotrienol and sterol
			301	(max.)	33·7	
15% E/P	2·0	543·3	294·5	(max.)	5·6	γ- and δ-Tocotrienol and sterol
			301	(infl.)	5·4	
30% E/P	1·5	144·7	291	(max.)	6·3	γ- and δ-Tocotrienol and sterol
			301	(infl.)	5·7	
			270	(infl.)	4·8	
Diethyl ether	1·0	179·4	291	(max.)	6·7	δ-Tocotrienol (trace) and sterol
			301	(infl.)	6·1	

rather small amount of β-carotene, as shown by the absorption spectrum, and squalene was detected by thin-layer chromatography. Tocotrienyl ester was found in the 2%-E/P fraction and the ultraviolet spectrum of this fraction (see Fig. 2) was qualitatively identical with that of γ-tocotrienyl palmitate, which showed $\lambda_{max.}$ at 280, 285·5 and 289·5mμ ($E^{1\%}_{1cm.}$ 40·4). The 4%-E/P fraction showed the characteristic spectrum (Fig. 2) of α-tocotrienol (or α-tocopherol) and the presence of this compound was confirmed. The 8%-E/P fraction, also shown in Fig. 2, shows the spectrum of a 7,8-dimethylchromanol, γ-tocotrienol or γ-tocopherol, and the first-named was shown to be present in this fraction. The δ-tocotrienol that appeared in the 8%-E/P, 15%-E/P and 30%-E/P fractions was present with γ-tocotrienol and sterol. The recovery from the column was 48% and the material remaining on the column was most likely to be phospholipid.

The 2%-E/P fractions were combined to yield 5·6g. of oil and of this 4·7g. was saponified to give an orange–yellow solid (3·1g.) showing $\lambda_{max.}$ at 295mμ ($E^{1\%}_{1cm.}$ 85·1) and 300·5mμ. Chromatography of this material on alumina yielded 0·37g. of a δ-tocotrienol-containing fraction that was added to the δ-tocotrienol fractions (5·39g.) from the original chromatography. From this, sterol was removed by crystallization from methanol (3·77g.) and by precipitation with digitonin (another 0·83g.), leaving 1·16g. of orange oil containing γ- and δ-tocotrienol.

Preparative thin-layer chromatography (64 plates) yielded concentrates of γ-tocotrienol (0·4g.) and δ-tocotrienol (0·27g.), and the latter was chromatographed once more (15 plates) to give 0·17g. of pale-yellow oil with $\lambda_{max.}$ at 294·5mμ ($E^{1\%}_{1cm.}$ 53) with inflexions at 300 and 303mμ. With chloroform as solvent the fraction showed only one spot on thin layers but with 20% di-isopropyl ether in light petroleum several impurities could be detected. Chromatography on nine thin-layer plates with the di-isopropyl ether–light petroleum solvent yielded 0·084g. of material that was chromatographed through a small alumina column and gave a pale-yellow oil (0·06g.). The tocotrienol was clearly unstable and further purifications were not attempted so as to conserve the small amount of material remaining.

Table 2 shows the results of thin-layer chromatography (both adsorption and partition) of δ-tocopherol and δ-tocotrienol. With adsorption chromatography a separation of the two 8-methylchromanol derivatives could be achieved only with 20% (v/v) di-isopropyl ether in light petroleum. This separation is the basis of the two-dimensional system used (Pennock *et al.* 1964). The tocopherol and tocotrienol were detected on the thin layers by spraying with the Emmerie–Engel reagent. Phosphomolybdic acid (10%, v/v) was also used, both tocotrienol and tocopherol giving a blue colour without heating the plate. δ-Tocopherol gives a positive reaction with diazotized *o*-dianisidine (Analytical Methods Committee, 1959) and it was found that δ-tocotrienol too gave a mauve colour (as does δ-tocopherol) with this reagent.

Excellent separations of δ-tocopherol and δ-tocotrienol could be achieved on the reversed-phase systems with paraffin-coated kieselguhr plates.

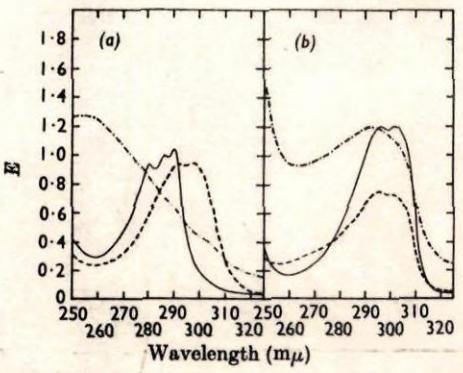


Fig. 2. Ultraviolet spectra of some fractions from chromatography of latex lipid on alumina. (*a*) –·–·, Light-petroleum fraction (0·042%); ——, 2%-E/P fraction (0·036%); – – –, 4%-E/P fraction (0·035%). (*b*) ——, 8%-E/P fraction (0·036%); – – –, 15%-E/P fraction (0·136%); –·–·, diethyl ether fraction (0·180%).

Table 2. *Thin-layer chromatography of δ-tocopherol and δ-tocotrienol*

All solvents in section (*B*) were saturated with paraffin.

Solvent	R_F δ-Tocopherol	R_F δ-Tocotrienol
(*A*) Adsorption chromatography with silica gel G		
20% Di-isopropyl ether in light petroleum	0·31	0·27
20% Di-isopropyl ether in benzene	0·47	0·46
Chloroform	0·37	0·37
1% Methanol in benzene	0·30	0·29
(*B*) Reversed-phase chromatography on kieselguhr coated with paraffin		
70% Acetone in water	0·14	0·50
80% Acetone in water	0·41	0·72
90% Acetone in water	0·79	0·90
60% Ethanol in water	0·35	0·61
80% Ethanol in water	0·66	0·87

Chromatography with aqueous ethanol as solvent took 2¼ hr. as compared with only 70 min. for the acetone solvents and so the latter system is to be preferred. Reversed-phase paper chromatography (Table 3) gave separations similar to those on reversed-phase thin-layer chromatography but here the time taken for development was 3–3½ hr.

The ultraviolet spectra of δ-tocopherol and δ-tocotrienol were examined in ethanol and cyclohexane (see Fig. 3). Both compounds gave clean peaks in ethanol but in cyclohexane fine structure was apparent. With cyclohexane as solvent all the tocopherols show fine structure that is characteristic for the different methylated chromanols and for this reason we prefer it to ethanol as a spectroscopic solvent. As shown in Table 4 the two 8-methylchromanol derivatives have qualitatively identical spectra, δ-tocopherol marginally having the higher $E^{1\%}_{1cm.}$ values. One might have expected the tocotrienol to have had slightly the higher $E^{1\%}_{1cm.}$ value, having a lower molecular weight (397 for δ-tocotrienol, 403 for δ-tocopherol). The δ-tocotrienol may have 2–3% of impurity in it, which would account for the discrepancy. The spectra of δ-tocotrienol and δ-tocopherol in cyclohexane show rather more fine structure than the more methylated chromanol derivatives and this is consistent with the 8-methyl derivatives being closer structurally to the parent phenol molecule. The spectrum of tocol itself resembles phenol even more closely, showing $\lambda_{max.}$ at 297·5 and 307·5 mμ with inflexions at 302 and 292 mμ. With cyclohexane as solvent one gets a slightly more intense spectrum than with ethanol. δ-Tocotrienol in cyclohexane has $E^{1\%}_{1cm.}$ 92·8 at $\lambda_{max.}$ and in ethanol the value is 88·1.

The infrared spectra of δ-tocotrienol and δ-tocopherol as oily smears were examined in a Perkin–Elmer Infracord and as shown in Fig. 4 the two spectra are very similar. The spectrum of δ-tocotrienol is contaminated by a small carbonyl impurity at 5·9 μ and we have observed that δ-tocopherol develops a similar band in time. This may suggest that the carbonyl band is due to a decomposition product. The tocotrienol shows a band for O–H stretching at 3·01 μ, a strong band at 8·21 μ, which may be due to the aryl ether absorption, a band at 8·74 μ for the alkyl ether, and aromatic absorption at 6·27 μ (C=C skeletal in-plane vibrations) and 11·7 μ (isolated free hydrogens in an aromatic ring). The tocotrienol differs from the tocopherol in having a weak band at 6·0 μ for unconjugated double bonds and a band of increased intensity at 9·1 μ. One would expect the tocotrienol to show absorption near 12 μ for trisubstituted ethylene groups but there is not a specific band in that region. The tocotrienol does,

Table 3. *Reversed-phase paper chromatography of δ-tocotrienol*

Whatman no. 1 paper coated with paraffin was used, and the solvents were saturated with paraffin before use.

Solvent	R_F δ-Tocotrienol	R_F δ-Tocopherol
5% Dimethylformamide in water	0·88	0·78
15% Dimethylformamide in water	0·79	0·51
25% Dimethylformamide in water	0·51	0·11

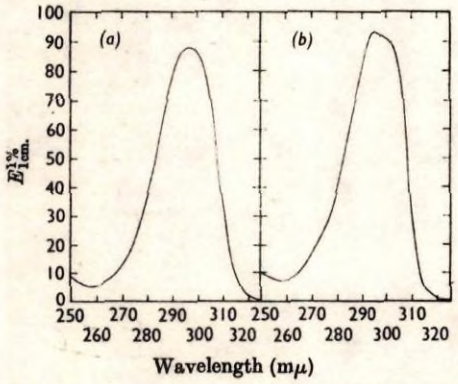


Fig. 3. Ultraviolet spectra of δ-tocotrienol: (*a*) in ethanol; (*b*) in cyclohexane.

Table 4. *Ultraviolet spectra of δ-tocopherol and δ-tocotrienol*

Solvent	δ-Tocopherol λ (mμ)	δ-Tocopherol $E^{1\%}_{1cm.}$	δ-Tocotrienol λ (mμ)	δ-Tocotrienol $E^{1\%}_{1cm.}$
Ethanol	257 (min.)	4·1	257 (min.)	4·8
	297 (max.)	90·3	297 (max.)	88·1
Cyclohexane	256·5 (min.)	4·4	256·5 (min.)	6·9
	295 (max.)	93·8	295 (max.)	92·8
	300 (infl.)	91·6	300 (infl.)	90·2
	304 (infl.)	84·2	304 (infl.)	81·9

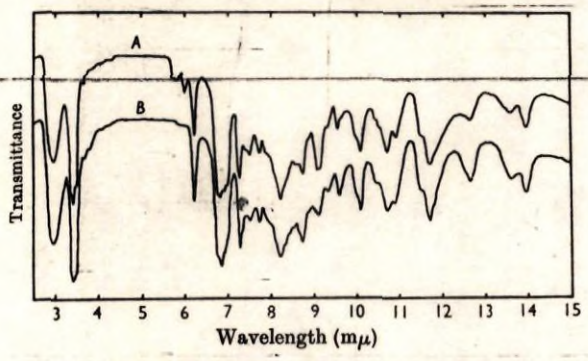


Fig. 4. Infrared spectra of δ-tocotrienol (*A*) and δ-tocopherol (*B*). Both compounds were examined as oily films.

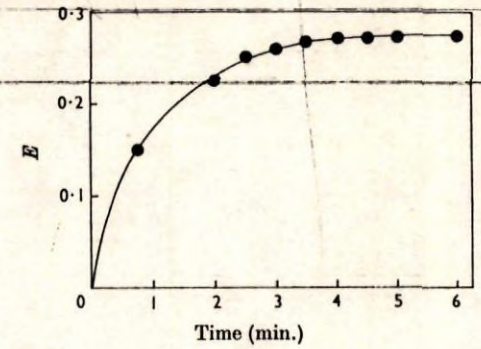


Fig. 6. Rate of colour production for δ-tocotrienol with the Emmerie–Engel reagent. The δ-tocotrienol concentration (in the reaction mixture) was 0·000493%.

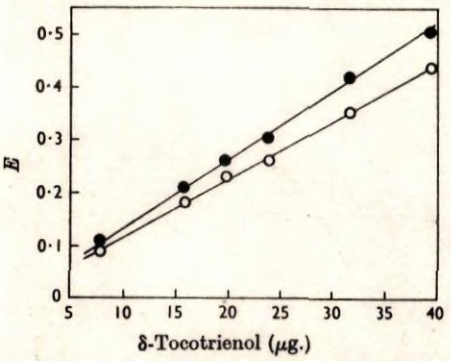


Fig. 5. Standardization graph for reaction between δ-tocotrienol and the Emmerie–Engel reagent. ○, Readings 2 min. after adding $FeCl_3$; ●, readings 3 min. after adding $FeCl_3$.

however, have a broader band at 11·7 μ than the tocopherol and there is no real minimum between that band and the adjacent band at 12·7 μ as there is in δ-tocopherol. It would appear therefore that there is absorption near 12 μ in the tocotrienol spectrum.

The reaction of δ-tocotrienol with the Emmerie–Engel reagent (ferric chloride–αα′-bipyridyl) was studied to find the necessary conversion factor for estimation of δ-tocotrienol with this reagent. Fig. 5 shows that the colour production is directly proportional to the amount of tocotrienol present. Fig. 5 also shows that colour development is not complete after 2 min. and so the reaction of δ-tocotrienol with ferric chloride–αα′-bipyridyl over a period of time was studied. Fig. 6 shows that colour production is not complete even after 3 min. and this is in contrast with α-, β- and γ-tocopherol, which have reacted completely by this time. Several determinations were carried out on δ-tocotrienol and conversion factors for 2 min. and 3 min. colour development were found to be 84·3 and 75·2 respectively. The data of Fig. 6 can be replotted as 1/time against 1/E value and a straight line is obtained, showing that although colour development is far from complete at 2 min. it is still justifiable to use a conversion factor for this time.

A similar series of experiments were carried out with δ-tocopherol and the results were similar to those obtained with the tocotrienol. The Emmerie–Engel conversion factors determined for δ-tocopherol were 87·3 after a 2 min. colour development and 76·2 after 3 min. The Analytical Methods Committee (1959) recommended a conversion factor of 75 for δ-tocopherol for a 2 min. colour development but this is in our experience only reasonable for a 3 min. reading. Many other workers have noted that periods longer than 2 min. are required with a conversion factor of 75 (Booth, 1963; Tsen, 1961; Green, Marcinkiewicz & Watt, 1955), and Stern & Baxter (1947) showed that colour development of δ-tocopherol with the Emmerie–Engel reagent was much slower than was the reaction of other tocopherols with this reagent.

δ-Tocotrienol was hydrogenated in a Towers Microhydrogenation Apparatus with Adams catalyst (platinum oxide) in acetic acid–ethanol–cyclohexane (1:1:1, by vol.). The hydrogen uptake was complete in 6 min. and the uptake was 0·78 mole of hydrogen/100 g. of tocotrienol, which is equivalent to 3·1 moles of hydrogen/mol. wt. 397, in agreement with the proposed structure of the compound. The hydrogenated material was extracted from the reaction mixture and was chromatographed on thin layers of silica gel G with 20% (v/v) di-isopropyl ether in light petroleum as solvent, and in both cases the hydrogenated material was identical with δ-tocopherol and separated from δ-tocotrienol.

The ethyl carbonate esters of δ-tocotrienol and

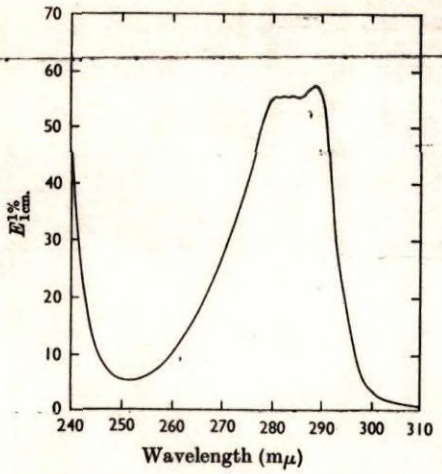


Fig. 7. Ultraviolet spectrum of δ-tocotrienyl ethyl carbonate in cyclohexane.

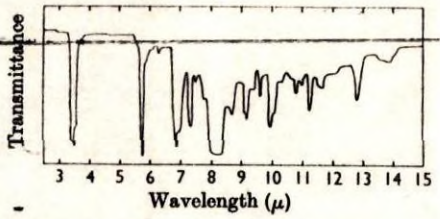


Fig. 8. Infrared spectrum of δ-tocotrienyl ethyl carbonate. The sample was examined as an oily film.

δ-tocopherol gave the same ultraviolet spectra, δ-tocotrienol ethyl carbonate showing $\lambda_{max.}$ at 288·5mμ ($E^{1\%}_{1cm.}$ 57·5) with a broad maximum between 280 and 284mμ of slightly lower intensity (see Fig. 7). The spectrum of the δ-tocopherol derivative gave $E^{1\%}_{1cm.}$ 54·1 at 288·5mμ. With benzene as solvent and on silica gel G the ethyl carbonate esters of δ-tocotrienol and δ-tocopherol could be separated, R_F values respectively being 0·51 and 0·55. The infrared spectra of the esters were very similar, showing carbonyl absorption at 5·71μ and C–O stretching at 7·98 and 8·3μ. The two bands for C–O stretching were probably due to the ethyl carbonate and tocotrienyl carbonate parts of the molecule respectively. Fig. 8 shows the infrared spectrum of the ethyl carbonate ester of δ-tocotrienol and it shows some unexpected differences in intensity in the 11–15μ and 6·27μ regions compared with the spectrum of δ-tocotrienol.

The nitroso derivatives of δ-tocopherol and δ-tocotrienol were prepared according to the method of Marcinkiewicz & Green (1959). The yellow products were chromatographed on thin layer by using both adsorption and reversed-phase methods. Both δ-tocopherol and δ-tocotrienol nitroso derivatives were resolved into two products that were yellow and stained pink with the Emmerie–Engel reagent. Marcinkiewicz & Green (1959) found that δ-tocopherol gave two nitroso derivatives, the 5- and 7-nitroso compounds. They found that the 5-nitroso derivative was the major component (70–80%) and had the higher R_F value in adsorption chromatography and the lower in reversed-phase chromatography. Table 5 shows the values obtained with δ-tocopherol and δ-tocotrienol. The 5-nitroso derivatives were the major components with both the tocopherol and the tocotrienol, amounting to 70–80% of the mixture, as found by Marcinkiewicz & Green (1959). The ultraviolet spectra of the nitroso compounds were determined in cyclohexane. The 5-nitroso derivatives of δ-tocopherol and δ-tocotrienol showed similar spectra, with $\lambda_{max.}$ at 258·5, 291 and 408mμ and $\lambda_{min.}$ at 246·5, 275 and 340mμ. Likewise the two 7-nitroso derivatives showed similar spectra, with $\lambda_{max.}$ 263, 292·5 and 407mμ and $\lambda_{min.}$ at 247, 279 and 340mμ. As only very small amounts of the nitroso compounds were made it was not possible to determine $E^{1\%}_{1cm.}$ values.

Table 5. *Thin-layer chromatography of the nitroso derivatives of δ-tocopherol and δ-tocotrienol*

For adsorption chromatography, silica gel G was used as adsorbent with benzene as solvent. For reversed-phase chromatography, kieselguhr impregnated with paraffin was used with 75% acetone in water as solvent.

	R_F	
Nitroso derivative	Adsorption chromatography	Reversed-phase chromatography
5-Nitroso-δ-tocopherol	0·52	0·16
7-Nitroso-δ-tocopherol	0·33	0·49
5-Nitroso-δ-tocotrienol	0·48	0·47
7-Nitroso-δ-tocotrienol	0·29	0·76

A small sample of δ-tocotrienol was hydroxymethylated according to the method of Green, McHale, Marcinkiewicz, Mamalis & Watt (1959). The method involved warming the tocotrienol with potassium hydroxide and 40% (v/v) formaldehyde in ethanol in a sealed capsule for 1hr. at 65° and then reducing the products with zinc and hydrochloric acid. The products contained β-tocotrienol and α-tocotrienol as well as unchanged δ-tocotrienol and some unidentified material. The β-tocotrienol was isolated by preparative thin-layer chromatography and its identity was checked by colour reactions and chromatography. Green

et al. (1959) found that δ-tocopherol could be hydroxymethylated and reduced to yield β-tocopherol.

DISCUSSION

δ-Tocotrienol has been isolated from a preparation of latex from *Hevea brasiliensis* and chemical and physical investigations have confirmed its structure. The ultraviolet spectrum of δ-tocotrienol is as expected almost identical with that of δ-tocopherol, and the Emmerie–Engel conversion factors for the two are virtually the same. The infrared spectrum of the tocotrienol differs from that of δ-tocopherol only in those bands attributable to the unsaturated nature of the former. With most solvent systems on thin-layer chromatograms δ-tocotrienol and δ-tocopherol are inseparable, but the two can be distinguished by using 20% (v/v) di-isopropyl ether in light petroleum as solvent. All reversed-phase systems tried either on paper or thin layers gave good separations of the tocopherol and tocotrienol. Hydrogenation of δ-tocotrienol gave a product that was indistinguishable from δ-tocopherol. In all other tests the results were predictable if one took into account that the unsaturation of the side chain is the only difference between this tocotrienol and δ-tocopherol.

The distribution of δ-tocotrienol as we know it so far is limited to two sources, palm oil and *Hevea brasiliensis* latex. As such it is unlikely to be of much interest nutritionally, especially since only the palm oil might find its way into the diet. δ-Tocotrienol has been found (F. W. Hemming, J. D. Kerr & J. F. Pennock, unpublished work) in a variety of margarines, which presumably contain hydrogenated palm oil. It is noteworthy that even though the carotenoids have been hydrogenated in the palm oil some tocotrienol survives. It is possible that this member of the tocopherol family has been overlooked in previous studies on tocopherols or even mistaken for δ-tocopherol. In the two places it has been found so far it has been accompanied by the more methylated tocotrienols, α-, β- and γ- in palm oil and α- and γ- in *Hevea* latex. Therefore one might suspect that if other sources are to be found they will be in tissues containing other tocotrienols, e.g. the seeds of wheat, barley or rice.

What is the function of δ-tocotrienol? The most likely suggestion would seem to be that it is an intermediate in the biosynthesis of α-tocopherol and the other tocopherols (see Pennock *et al.* 1964). δ-Tocotrienol could be hydrogenated to yield δ-tocopherol or methylated to β-, γ- and α-tocotrienol. Both palm oil and *Hevea* latex suggest, in their tocotrienol patterns, a biosynthetic pathway to α-tocopherol through δ-, γ- and α-tocotrienol. Palm oil contains some β-tocotrienol, which suggests that an alternative pathway may lie through this compound, but the presence of a small quantity of β-tocopherol might indicate that the β-tocotrienol is there as an intermediate in the biosynthesis of β-tocopherol. It is not clear why some tissues possess tocotrienols and others tocopherols, or why some contain α-tocopherol whereas β-, γ- or δ-tocopherol predominate in other cases.

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