A REASSESSMENT OF TOCOPHEROL CHEMISTRY

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This paper describes the presence in Nature of four methylated tocols (the 8-monomethyl, 5,8- and 7,8-dimethyl and 5,7,8-trimethyl derivatives) and the four related tocotrienols (for nomenclature see Bunyan, McHale, Green and Marcinkiewicz, 1961).

A new 'tocopherol' from human bone marrow

A survey of the unsaponifiable lipids of human bone marrow (Kasimuddin, Morton, Hemming and Pennock, 1964) revealed, in certain cases, a compound which reduced $FeCl_3$, and showed $\lambda_{max.}$ 295 and 302 mμ (cyclohexane, $E^{1\%}_{1cm.}$ for both maxima near 80). Thin layer chromatography (TLC) on silica gel (benzene/ $CHCl_3$, 1/1) showed the unknown had the same R_f as β- and γ-tocopherol but on using a solvent system capable of separating these two tocopherols (Stowe, 1963) the unknown had a lower R_f than either. Besides staining with Emmerie-Engel reagent, the unknown gave a colour with diazotised o-dianisidine showing the presence of a free 5-position in a tocol system (Green & Marcinkiewicz, 1959), An infrared spectrum of the unknown showed that it was more like δ-tocopherol than any other tocopherol. Hydrogenation (PtO as catalyst) gave a product which could not be separated from δ-tocopherol by TLC. The nitroso derivatives (Marcinkiewicz and Green, 1959) of β-, γ-tocopherol, the unknown and hydrogenated unknown were prepared and gave R_f values on TLC (silica gel, CCl_4) of 0.43, 0.62, 0.53 and 0.61 respectively.

Since tocotrienols related to β- and α-tocopherols have been found (Green, Mamalis, Marcinkiewicz and McHale, 1960) it seemed plausible that the unknown might be the tocotrienol related to γ-tocopherol. Accordingly 7,8-dimethyl tocotrienol was synthesised (Coop and Pennock, 1964) by a

method used by Schudel, Mayer, Metzger, Rüegg and Isler (1963) for the synthesis of other tocotrienols. The product was identical with the tocopherol from bone marrow with respect to ultraviolet and infrared spectra, R_f values on TLC, R_f values of nitroso-derivatives and colours produced with dianisidine and phosphomolybdic acid. The two compounds were also indistinguishable by two-dimensional TLC.

The identity of η -tocopherol.

Since β - and γ-tocopherols are not separable by two dimensional paper chromatography (Analytical Methods Committee, 1959) it seemed possible that the tocotrienols related to these tocopherols might run together. In fact η -tocopherol runs with ϵ -tocopherol (5,8-dimethyl tocotrienol) and so we decided to check the identity of η -tocopherol. Green and Marcinkiewicz (1956) identified η -tocopherol from rice as 7-methyl tocol but the evidence presented did not preclude our considering the possibility of its being 7,8-dimethyl tocotrienol. η -Tocopherol has also been described in palm oil (Ward, 1958) and so the nature of η -tocopherol from these two sources was reinvestigated.

Unpolished rice (purchased locally) was ground finely and extracted with ether/light petroleum (1/1) in a Soxhlet and the oil so obtained was saponified by the recommended method (Analytical Methods Committee, 1959). Samples of crude palm oil (kindly given by Merseyside Food Products) were saponified similarly. Aliquots of the unsaponifiable lipid (0.3 - 0.5 mg.) containing 15-30 µg. total tocopherol were spotted on 20 x 20 cm. plates with silica gel as adsorbent and the chromatogram was developed in one direction with $CHCl_3$ and in the second with 20% isopropyl ether/light petroleum. In the $CHCl_3$ tank (lined with paper) good separations of δ -, from β - + γ - and from α -tocopherols were obtained while 20% isopropyl ether/light petroleum (freshly made up and tank unlined) gave good separations of β - and γ -tocopherols (see Stowe, 1963) and also separated the methylated tocols from their related tocotrienols (see fig.1).

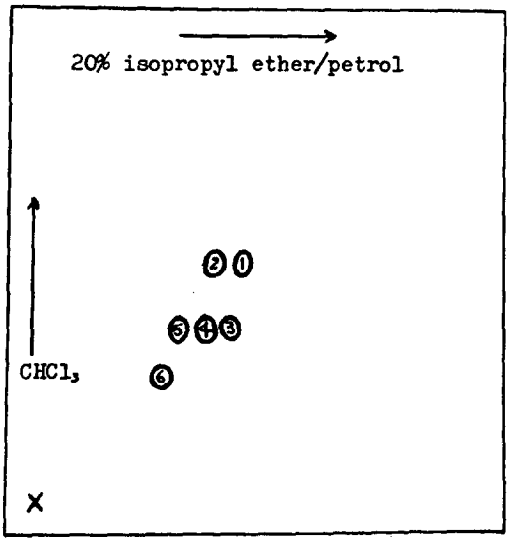

Figure 1

Two dimensional TLC of a tocopherol mixture

1	=	α -tocopherol	(5,7,8-trimethyl tocol)
2	=	ζ -tocopherol	(5,7,8-trimethyl tocotrienol)
3	=	β -tocopherol	(5,8-dimethyl tocol)
4	=	ϵ -tocopherol + γ -tocopherol	(5,8-dimethyl tocotrienol) + (7,8-dimethyl tocol)
5	=	———	(7,8-dimethyl tocotrienol)
6	=	δ -tocopherol	(8-methyl tocol)

As can be seen in figure 1, spot 4 represents ϵ - plus γ-tocopherol. γ-Tocopherol gives a stain with dianisidine whereas ϵ -tocopherol does not. To show the presence of ϵ -tocopherol if γ -tocopherol is also present the two tocopherols have to be separated. This can be achieved by reversed-phase chromatography or by chromatography of the nitroso derivatives.

Rice showed three main spots which were clearly α -, ζ - and η -tocopherols. Two smaller spots were present which were identified by position and by reason of not staining with dianisidine as β- and ϵ -tocopherols. The η-tocopherol ran in the same position as 7,8-dimethyl tocotrienol and this was confirmed by adding a sample of the latter before chromatography. When the rice unsaponifiable lipid was hydrogenated (PtO catalyst) the spot in the η -position disappeared and a large spot appeared in the γ -tocopherol position which stained with

dianisidine and was clearly γ-tocopherol When synthetic 7-methyl tocol was added to rice unsaponifiable lipid and the mixture chromatographed the 7-methyl tocol appeared as a distinct spot being slightly more polar in both solvent systems than η-tocopherol. Thus η-tocopherol from rice is not 7-methyl tocol but is 7,8-dimethyl tocotrienol.

Palm oil similarly gave five distinct reducing spots on TLC. These appeared to be α-, ζ_1-, η-, ϵ-tocopherols and a δ-tocopherol-like material. In a manner identical as for rice it was shown that the η-tocopherol from palm oil was 7,8-dimethyl tocotrienol and not 7-methyl tocol. Thus there is no evidence at present that 7-methyl tocol appears naturally.

The identity of ζ-tocopherol in rice.

Two ζ-tocopherols are believed to occur in Nature. ζ-Tocopherol was originally found in wheat, barley and rye by Green, Marcinkiewicz and Watt (1955) and later by Green and Marcinkiewicz (1956) in rice. The ζ-tocopherol was identified as 5,7-dimethyl tocol. More recently Green, McHale, Marcinkiewicz, Mamalis and Watt (1959) claimed that the ζ-tocopherol in wheat and palm oil was 5,7,8-trimethyl tocotrienol but the rice ζ-tocopherol was different and was 5,7-dimethyl tocol as originally suggested. Bunyan et al. (1961) refer to these two compounds as ζ_1-(5,7,8-trimethyl tocotrienol) and ζ_2-tocopherol (5,7-dimethyl tocol).

When examining the tocopherols from rice the ζ-tocopherol ran in the position of ζ_1-tocopherol and on hydrogenation no spot appeared there but the α-tocopherol spot had increased in size. When synthetic 5,7-dimethyl tocol was chromatographed together with rice unsaponifiable lipid a spot distinct from any of the rice tocopherols was observed. 5,7-Dimethyl tocol was a little more polar than ζ-tocopherol of rice in both systems. The ζ-tocopherol of rice behaved in exactly the same manner as ζ-tocopherol from palm oil and the only ζ-tocopherol so far found in Nature is 5,7,8-trimethyl tocotrienol, ζ_1-tocopherol. There is no evidence at the moment for the existence of 5,7-dimethyl tocol.

A new 'tocopherol' from palm oil.

It was mentioned earlier that palm oil contained a δ -tocopherol-like compound. When synthetic δ -tocopherol was chromatographed with the unsaponifiable lipid from palm oil it appeared as a distinct spot and the unknown was in a position which could be assigned to 8-methyl tocotrienol if such a compound existed. The new 'tocopherol' could be separated from tocol by two dimensional TLC and gave a slate-coloured stain with dianisidine, similar to that of δ -tocopherol but different from the brown colour given by tocol. On hydrogenation the new 'tocopherol' was converted into δ-tocopherol. This new 'tocopherol' can be identified as 8-methyl tocotrienol.

A revised picture of tocopherol chemistry.

We have offered evidence for the presence in Nature of four tocols and four tocotrienols. The 8-methyl group is present in all these compounds which are 8-methyl; 5,8-dimethyl; 7,8-dimethyl and 5,7,8-trimethyl derivatives of tocol and tocotrienol. Table 1 shows the revised position of tocopherol chemistry compared with the position hitherto.

Table 1

A Revision of Tocopherol Chemistry

Previous position	Present position
7-methyl tocol (η)	8-methyl tocol (δ)
8-methyl tocol (δ)	5,8-dimethyl tocol (β)
5,7-dimethyl tocol (ζ_2)	7,8-dimethyl tocol (γ)
5,8-dimethyl tocol (β)	5,7,8-trimethyl tocol (α)
7,8-dimethyl tocol (γ)	8-methyl tocotrienol
5,7,8-trimethyl tocol (α)	5,8-dimethyl tocotrienol (ϵ)
5,8-dimethyl tocotrienol (ϵ)	7,8-dimethyl tocotrienol (η)
5,7,8-trimethyl tocotrienol (ζ_1)	5,7,8-trimethyl tocotrienol(ζ)

The authors suggest that the Greek letters for the tocotrienol series (ζ_1, ϵ and η) be dropped and these compounds be referred to as α-, β-, γ-, and δ-tocotrienols related to α-, β-, γ- and δ-tocopherols. We have found it convenient to use the abbreviations α_3, β_3, γ_3 and δ_3 to denote the presence of three double bonds in the side chain of these tocotrienols (see Table 2).

Table 2

A suggested nomenclature for Tocopherols

Substitution	Tocopherol	Tocotrienol
8-methyl	δ-tocopherol (δT)	δ-tocotrienol (δ_3T)
5,8-dimethyl	β-tocopherol (βT)	β-tocotrienol (β_3T)
7,8-dimethyl	γ-tocopherol (γT)	γ-tocotrienol (γ_3T)
5,7,8-trimethyl	α-tocopherol (αT)	α-tocotrienol (α_3T)

Metabolic Relations of the Tocopherols

An interesting picture of tocopherols is presented by palm oil which contains all four tocotrienols and α-tocopherol. The possibility arises that the four tocotrienols are intermediates in the biosynthesis of α-tocopherol. 8-Methyl tocotrienol is made first and is then methylated to form 5,8- and 7,8-dimethyl tocotrienols (the latter predominating) and these two compounds are further methylated to 5,7,8-trimethyl tocotrienol which is then saturated to form 5,7,8-trimethyl tocol, α-tocopherol. It would appear that methylation occurs before saturation of the side chain. If a plant has a tocopherol other than α-tocopherol then saturation occurs at the required step.

It is interesting that Baszyn ski (1961) found that α-, γ- and δ-tocopherols occurred during the first days of germination of the pea but δ-tocopherol disappeared by the time a height of 3 cm. was reached. He found that α-tocopherol increased with a simultaneous decrease in γ-tocopherol and that methionine and ATP stimulated the synthesis of α-tocopherol.

It remains to be seen whether tocotrienol itself will be found in Nature or whether 8-methyl tocotrienol is the first product. If the latter is the case it is possible that a related quinone, 3-methyl-5-geranyl-geranyl-1:4-benzoquinone occurs in plants.

It is hoped to publish fuller details of the properties of the two new tocotrienols later.

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