

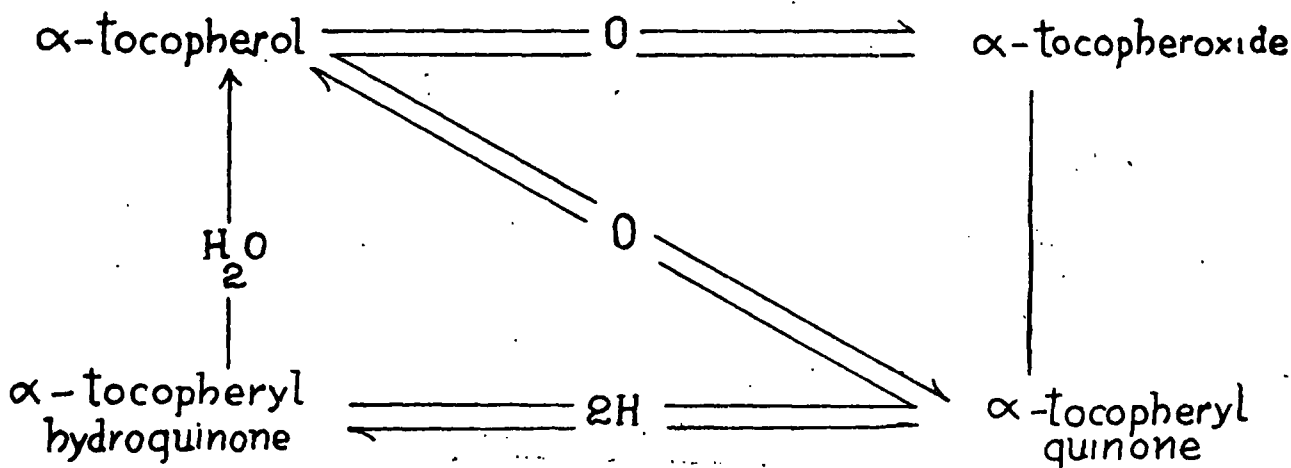
STUDIES ON THE TOCOPHEROLS OF COCONUT OIL

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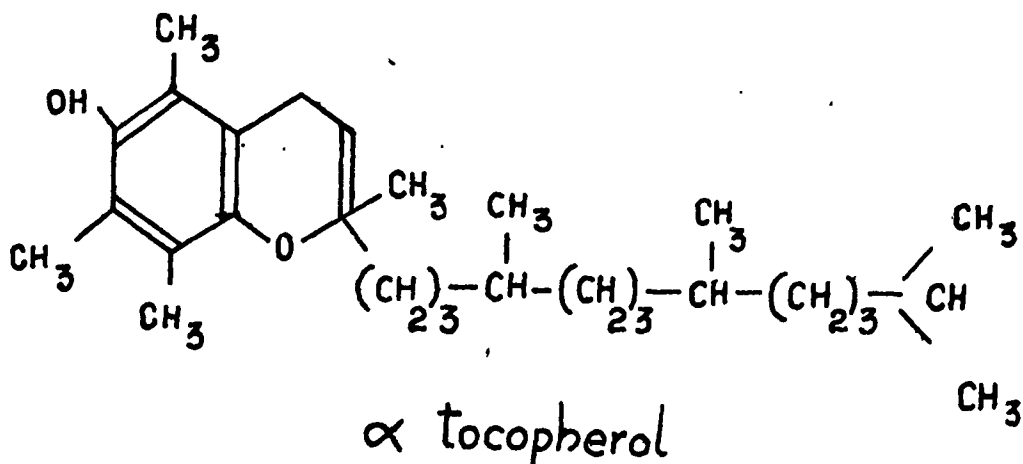
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INTRODUCTION

The tocopherols discovered by Evans and Co-workers were demonstrated by Olcott and Emmerson to be effective antioxidants. Like other antioxidants tocopherols are themselves readily oxidizable. Mild oxidation causes a cleavage in the heterocyclic ring and tocoquinones are formed which are not antioxidants. Fruton and Simmonds (1960) have postulated a scheme for inter-conversions of α -tocopherol.



Though much work on tocopherols in coconut oil has not been done by earlier workers, four different tocopherols have so far been identified in other vegetable oils (eg. cotton seed, soya bean etc.) and these are designated as α —, β —, γ —, and δ — tocopherols. Out of these α — tocopherol (α — T) is commonly known as Vitamin E. β — T and γ — T differ from α — T in having one less methyl group substituted in the benzene ring. In β — T, the methyl groups are at 5 and 8 positions while in γ — T at 7 and 8 positions. δ — T has one such group in the 8 position.



Also previous workers have shown the natural occurrence of tocotrienols related to tocopherols but having three unsaturated isoprenoid groups in the side chains and the esters of the tocopherols. On saponification these esters yield free tocopherols.

EXPERIMENTAL

Column Chromatography

A column (25 cm in length and 2.5 cm in diameter) was prepared with the acid washed alumina suspended in pet-ether (40-60°C). Purified coconut oil (10 ml) from B.C.C. Ltd., Colombo, Ceylon, was chromatographed on this column of alumina. The compounds were eluted from the column with pet-ether (40-60°C) which contained increasing amounts of acetone (up to 10%). Fractions of 10 ml each were collected in a LKB RadiRac Fraction Collector. Those fractions which gave a positive Emmerie-Engel⁶ reaction for tocopherols were combined into 3 portions, evaporated and redissolved in 5 ml of absolute ethanol.

Spectrophotometric Estimation

A standard curve, Fig. (1) (Absorbance Vs Concentration) was prepared from samples of known α -tocopherol content using Beckmann Spectrophotometer at 520 $m\mu$. One ml of solution from each of the above 3 portions was placed in an opaque glass stoppered bottle separately. To this, 0.5 ml of 0.2% solution of $FeCl_3 \cdot 6H_2O$ in ethanol and 0.5 ml of 0.5% solution of α - α dipyridyl reagent in ethanol were added in this order followed by 11 ml of purified absolute ethanol. Exactly 15 minutes after addition of the reagents, the absorbance of the resulting mixture was read in the Beckmann Spectrophotometer at 520 $m\mu$. The tocopherol content in all 3 portions corresponding to the absorbances was calculated with the help of the standard curve.

RESULTS

Fractions collected with acetone in pet-ether (1-2%) and (4-6%) gave the intense colour reaction with Emmerie-Engel reagent for tocopherols. Fractions (3%) gave very weak colour while other fractions (7-10%) indicated the absence of tocopherols.

Qualitative Data

Percentage of acetone in the combined fractions	Total volume in ml	Absorbance per ml as measured by the Beckmann Spectrophotometer	Equivalent Tocopherol content from std. curve $\mu\text{g/ml}$	Total Tocopherol content (μg)
1—2%	10	80	30	300
3%	50	82	36	1800
4—6%	50	85.2	48	2400

It was observed that the total tocopherol content in 10 ml of processed coconut oil (B.C.C.) was 4500 μg (i.e. 450 μg per ml).

Thin Layer Chromatography

The thickness of the silica gel (Merck, Darmstadt, Germany) layer on the glass plate was 0.25 μ and the plate was activated for 30 minutes at 110°C before use. Two dimensional TLC was carried out with the fractions which gave positive Emmerie-Engel reaction. The solvents used were (1) Chloroform (2) 20% Diethyl ether in light-petroleum. The spots were located by spraying with the Emmerie-Engel reagent.

Method of breaking down the esters to free tocopherols (Saponification)

Combined fractions were evaporated and were dissolved in 4 ml of ethanol. Pyrogallol (200 mg) was added and the resulting solution was shaken for 15 minutes in machine with 1 ml of saturated KOH.

RESULTS AND DISCUSSION

One dimensional thin layer chromatography of the unsaponified extract revealed that in nature, the tocopherols occurred mainly in an esterified form. Free tocopherols were also detected in this extract. On a saponification of the extract, α — tocopherol was detected in very high concentration with trace amounts of β and δ tocopherols. No attempt was made to identify the fatty acid moieties of the esters and although the tocopherol ester fraction gave one spot on the chromatoplate, it is possible that this spot consisted of more than one tocopherol esters.

Combined fractions containing increasing amounts of acetone	Rf value	Probable identity
1, 2 and 3%	0.73	Tocopherol esters
4, 5 and 6%	0.82	α — tocopherol
1, 2 and 3% saponified	0.82	α — tocopherol

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REFERENCES

- Evans, H. M., Emmerson, O. H. and Ammerson, G. A. (1936). *J. biol. Chem.* 113, 319-332.
- Fرتون, J. S. and Simmonds, S. (1960) '*General Biochemistry*' New York, John Wiley and Sons Inc. 777 pp.
- Guillermina, C., Manalac, G. C. and Harder-Soliven, A. (1967). *Philipp. J. Sci.* 94, 3.
- Morton, R. A., *Wissenschaftliche Veroffentlichungen der Deutschen Gesellschaft fur Ernahrung* (1967) 16, 1-13.
- Olcott, H. S. and Ammerson, O. H. (1937). *J. Am. Chem. Soc.* 59, 1008.
- Stahl, E. (1965) Ed. '*Thin layer chromatography*,' Berlin, Springer-Verlag, 553 pp.
- Sturin, P. A., Parkhurst, R. M. and Skinner, W. A. (1966) *Analyt. Chem.* 38., 1244.
- Tirimanna, A. S. L. and Wickremasinghe, R. L. (1960). *Tea Q.* 37, 229-231.