

PALM OIL TOCOTRIENOLS: ASPECTS OF THEIR CHEMISTRY AND BIOCHEMISTRY

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ABSTRACT

Palm oil is a unique vegetable oil because of its high content of tocotrienols which are natural vitamin E compounds but are not found in most of the other vegetable oils. Various chemical and biochemical aspects of the palm-oil tocotrienols studied in this research include their oxidation and electron transfer reactions, their antioxidant activities, their distribution in organs of animals consuming them and their effects in the inhibition of atherosclerosis and tumour promotion.

The oxidation of tocotrienols and tocopherols by alkaline potassium ferricyanide gave rise to chromanoxyl radicals, the spectra of which were recorded by electron spin resonance spectroscopy. Oxidation of γ -tocotrienol and γ -tocopherol produced radical-coupling products, viz. ether dimers and diastereomeric pairs of bichromanil dimers whose structures and absolute configurations have been determined by nuclear magnetic resonance spectroscopy. Further oxidation of the ether dimers derived from γ -tocotrienol and γ -tocopherol also gave stable radicals which dimerize to new tetramers. Oxidation of the bichromanil dimers of both γ -tocotrienol and γ -tocopherol also gave rise to stable radicals which are detectable by ESR and the resulting products were diastereomeric dienone dimers. Oxidation of α -tocotrienol gave rise to various dimers and trimers. The present studies demonstrate that tocotrienol compounds, like tocopherols, are good natural antioxidants and their activity can be regenerated by reducing agents.

α -Tocotrienol and α -tocopherol were found to be oxidized by 4-nitrobenzyl chloride under basic conditions. The major product was a nitrobenzyl ether cross-product but diastereomeric carbon-carbon cross-products were also obtained. The major products from the reactions of γ -tocotrienol and γ -tocopherol with 4-nitrobenzyl chloride were nitrobenzyl ether cross-products; ether dimers derived from the radicals of γ -vitamers were also formed and these dimers further reacted with 4-nitrobenzyl chloride to give nitrobenzyl ether cross-products. Formation of products arising from radical intermediates indicates single electron transfer reactions are involved. Preliminary kinetic results showed

that the α -vitamers are relatively more reactive than the γ -vitamers in the reactions with 4-nitrobenzyl chloride. The reaction of α -tocopherol with triphenylmethyl chloride was also carried out. Analysis of the products indicated that hydrolysis of the triphenylmethyl chloride is dominant, but a competing electron transfer reaction was also observed. Such a result is expected from a mechanism involving ionization followed by hydroxide ion capture or a competing electron transfer from tocopheroxyl anion.

The effects of dietary soyabean, coconut and palm oils on the oxidative susceptibility of plasma low density lipoproteins were studied on rabbits fed with semi-synthetic diets (for 12 weeks) supplemented with dietary fats (15% by weight) and cholesterol (0.5% by weight). Dietary palm oil is better than soyabean and coconut oils in eliciting greater oxidative resistance to the low density lipoproteins because of a combination of low levels of polyunsaturated fatty acids and relatively high levels of vitamin E compounds. Beneficial effects of various natural antioxidants (tocopherols, tocotrienols and vitamin C) supplemented in the semi-synthetic diets containing refined-bleached-deodorized palm olein were demonstrated. Dietary supplementation of vitamin E as well as its combination with vitamin C conferred excellent oxidative resistance to the low density lipoproteins. Experiments to determine the extent of the formation of atherosclerotic plaques in rabbits fed with soybean, coconut and palm oils supplemented with cholesterol showed that there was no statistically significant difference among these oils. Supplementation of γ -tocotrienol (209 ppm) or δ -tocopherol (863 ppm) in the atherogenic diets reduced the severity of atherosclerosis in the rabbits.

The distribution and bioavailability of tocotrienols have been studied in guinea pigs and rabbits. When palm-oil vitamin E (1000 mg per kg body weight per day) containing 57.3% of tocotrienols was fed to guinea pigs for two and a half months, the relative concentrations of α -tocopherol in the liver, plasma and kidney of the treated guinea pigs were remarkably increased, whereas most of the ingested tocotrienols apparently were not accumulated. Similarly, when rabbits fed with 0.2 g of palm-oil vitamin E (containing

69.4% tocotrienols and 30.6% tocopherols) per kg body weight per day for one month, the tocotrienols levels were surprisingly low in their plasma and other organs; however, there was an accumulation of α -tocopherol in their plasma and livers. In another experiment, rabbits were fed with semi-synthetic diets containing refined-bleached-deodorized palm olein low in α -tocopherol but enhanced with γ -tocotrienol or δ -tocopherol. Although most of the γ -tocotrienol and δ -tocopherol apparently were not accumulated, the relative concentrations of α -tocopherol in their plasma and livers were significantly elevated. These results suggest that a bioconversion of the tocotrienols to α -tocopherol possibly occurred in the animals. However, preliminary *in vitro* experiments using radiolabelled *S*-adenosyl-¹⁴C-methyl-methionine and δ -tocopherol incubated with the rabbit liver microsomal fraction did not show biomethylation of the δ -tocopherol to α -tocopherol.

Various vitamin E compounds have been examined for their anti-tumour promoting effect using an *in vitro* assay based on the detection of Epstein-Barr virus early antigen. Results showed that γ -tocotrienol and δ -tocotrienol (at concentration 20 μ g/mL cell culture and above) effectively inhibited the tumour promotion induced by 12-*O*-tetradecanoylphorbol-13-acetate; α -tocotrienol and δ -tocopherol exhibited the anti-tumour promoting effect only at higher concentrations (i.e. > 80 μ g/mL cell culture), whereas α -tocopherol, γ -tocopherol and various dimeric derivatives of tocopherols and tocotrienols did not show any activity at the experimental concentrations (i.e. 10 - 320 μ g/mL cell culture). Two-stage skin carcinogenesis experiments have also been carried out on mice, but the preliminary *in vivo* results were ambiguous. The latency for tumour appearance in the mice treated with γ -tocotrienol and δ -tocotrienol was slightly shorter than that for the mice without application of the vitamin E compounds; however, over a longer period the control mice had more tumours than the mice treated with tocotrienols.

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a	ESR hyperfine coupling constant
$^{\circ}\text{C}$	degree Celsius
^{13}C	carbon-13
cm	centimetre
CDCl_3	deuterated chloroform
COSY	correlated spectroscopy
δ_{C}	carbon chemical shift
δ_{H}	proton chemical shift
DMBA	7,12-dimethylbenz[a]anthracene
EBV EA	Epstein Barr virus early antigen
EI	electron impact
ESR	electron spin resonance
Fig.	figure
FT	Fourier transformed
g	gram
G	gauss (for ESR)
G	gravitational force (for centrifugation)
GHz	gigahertz
^1H	proton
HDL	high density lipoproteins
HETCOR	heteronuclear correlated spectroscopy
HPLC	high performance liquid chromatography
Hz	hertz
i.d.	internal diameter
IDL	intermediate density lipoproteins
IR	infrared

K	Kelvin
$K_3Fe(CN)_6$	potassium ferricyanide
kcal	kilocalories
KOH	potassium hydroxide
kHz	kilohertz
LDL	low density lipoproteins
M	molarity (mol/dm ³)
mg	milligram
MHz	megahertz
min	minute
mL	millilitre
mm	millimetre
mm ²	millimeter square
MS	mass spectrum/spectra
mV	millivolt
m.w.	molecular weight
m/z	mass/charge
nm	nanometre
N ₂	nitrogen gas
N.A.	not available
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
OFR	off-resonance
<i>p</i> -DNB	<i>para</i> -dinitrobenzene
PFAD	palm fatty acid distillate

Ph_3CH	triphenylmethane
$\text{Ph}_3\text{C}^\bullet$	triphenylmethyl radical
ppm	parts per million
RBD	refined, bleached and deodorized
rpm	revolutions per minute
r.t.	retention time
s	second
SET	single electron transfer
S_N	nucleophilic substitution
$\text{S}_\text{N}2$	bimolecular nucleophilic substitution
$\text{S}_\text{RN}1$	unimolecular nucleophilic radical substitution
T	tocopherol
T^\bullet	tocopheroxyl radical
$\gamma\text{-T}_3\text{DED}$	γ -tocotrienol dichromanyl ether dimer
T_3	tocotrienol
T_3^\bullet	tocotrienoxyl radical
$\gamma\text{-TDED}$	γ -tocopherol dichromanyl ether dimer
TLC	thin layer chromatography
TMS	tetramethylsilane
TPA	12- <i>O</i> -tetradecanoylphorbol-13-acetate
UV	ultra-violet
v	volume
VLDL	very low density lipoproteins
w	weight
μg	microgram
μl	microlitre
μm	micrometre