CHAPTER 1

OXIDATION OF TOCOTRIENOLS AND TOCOPHEROLS

1.1 INTRODUCTION

1.1.1 Vitamin E Components

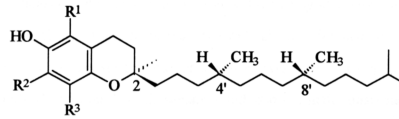
Vitamin E was first discovered by Evans and Bishop [165-167], and it has also been described by Mattill & Stone [404] and Sure [615] in the 1920's. Vitamin E was found as a factor required in animal nutrition and was thought to be a dietary constituent essential for normal reproduction. Naturally occurring vitamin E comprises of eight types of compounds which are classified into two homologous series, i.e. α-, β-, γ- and δ-tocopherols (T) and tocotrienols (T_3) (Table 1.1). Each pair of the two series of vitamin E components (T and T_3) have similar chromanyl structures but differ in their side chains; tocopherols have a saturated phytyl side chain whereas the corresponding tocotrienols have a tri-unsaturated isoprenoid chain. The correct structure of α-tocopherol (α-T) was first proposed by Fernholz in 1938 [170]. Natural tocopherols have all *R*-configuration at carbons 2, 4' and 8', and the 2*R*,4'*R*,8'*R*-α-tocopherol has been shown to be the most bioactive stereoisomer [673]. Tocotrienols have an *R*-configuration at carbon-2 and all-*trans* (*E*,*E*)-configuration for the double bonds [407,561].

The generic name "tocopherol" was derived from the Greek words "tokos" means offspring, "pherein" means to bear and "ol" to indicate the phenolic hydroxy group [168]. The trivial name "tocotrienols" was introduced by Bunyan *et al.* to represent the vitamin E homologues with isoprenoid side chain [80]. Since the discovery of vitamin E compounds, α-T has been widely misunderstood as the only vitamin E component, this is because of its widespread distribution in plants and there are many reports suggesting that α-T is the biologically most active vitamin E compound. In previous years, the synthetic *dl*-α-tocopheryl acetate has been commonly consumed as the vitamin E for pharmaceutical uses and food supplements despite the fact that it is a mixture consisting of eight stereoisomers with varying degrees of activity. Since the last two decades, a tremendous improvement in the technology for extraction and purification of vitamin E has been achieved and a lower production cost has made it more economical to obtain the α-T from natural sources such

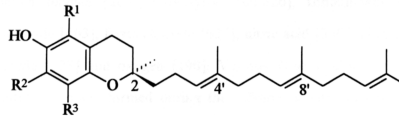
Table 1.1 Structures of naturally occurring vitamin E components

Substituent	Tocopherols (2*R*,4'*R*,8'*R*-)				Tocotrienols (2*R*-all *trans* (*E*,*E*)-)			
	α-T	β-T	γ-T	δ-T	α-T_3	β-T_3	γ-T_3	δ-T_3
R^1	CH_3	CH_3	H	H	CH_3	CH_3	H	H
R^2	CH_3	H	CH_3	H	CH_3	H	CH_3	H
R^3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3

Tocopherols



Tocotrienols



as deodorization distillates of various vegetable oils. Apart from that, there is an increasing preference for naturally occurring vitamin E in pharmaceuticals and many healthcare products.

1.1.2 Oxidation of Vitamin E

To date, there are thousands of reports on the chemical, biological and medical aspects of vitamin E encompassing a large variety of its functions. In most cases, the basic feature of this well-known lipid-soluble antioxidant is that the vitamin E exhibits fundamental roles in preventing the primary peroxidation of unsaturated lipids and protecting biomolecules from radical damage. However, while functioning as an antioxidant, the vitamin E itself is also being oxidized to form chromanoxyl radicals which are relatively stable and can be detected by electron spin resonance (ESR). These chromanoxyl radicals can be reduced to non-radical forms by coupling with other radicals such as peroxyl radicals. Apart from that, the radicals can dimerize or undergo further oxidation to other products such as quinones and epoxides. Because of its high reactivity towards free radicals, vitamin E is air-sensitive and also thermally-unstable. A diversity of oxidation products of vitamin E can be obtained by numerous types of oxidizing agents such as potassium ferricyanide [448,468,580], benzoyl peroxide [581], *p*-benzoquinone [466,467], singlet oxygen [223], photolysis [108,226], trimethylamine oxide [294], potassium superoxide [403], ferric chloride [627], nitric acid [350], nitrogen oxide [566], hydrogen peroxide [223] and oxygen [190]. Various dimers of tocopherols have been found as oxidative products formed during the refining of seed oils [448,573], dimeric metabolites of α-T have also been reported to be present in animal liver extracts [121,122].

1.1.3 Oxidation of α-Tocopherol

α-T, the most reactive of the vitamin E components, prevents the oxidation of lipids by scavenging peroxyl radicals in chain propagating reactions while itself being

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1.1.3 Oxidation of α-Tocopherol

α-T, the most reactive of the vitamin E components, prevents the oxidation of lipids by scavenging peroxyl radicals in chain propagating reactions while itself being

oxidized to the α-tocopheroxyl radical. α-Tocotrienol (α-T_3) has a similar chromanyl structure as α-T and therefore can be oxidized to produce a similar type of chromanoxyl radical as indicated by their almost identical ESR spectra shown in Fig. 1.1 [219]. The α-tocopheroxyl and α-tocotrienoxyl radicals are stabilized by resonance structures (Fig. 1.2) but are unstable to air. *o*-Quinone methide was also thought to be another reactive intermediate formed during the oxidation of α-T. Several types of oxidation products including quinones and dienones (Fig. 1.3), dimers (Fig. 1.4) and trimers (Fig. 1.5) can be derived from the various intermediates depending on the method of oxidation. For example, in the photooxidation of α-T by singlet oxygen, a quinone and its epoxide and a hydroperoxy dienone were found to be the major products [225,696]. When alkaline potassium ferricyanide was used as the oxidizing agent, the main oxidation products of α-T were found to be a spirodienone dimer (also called ketoether dimer or spirodimer) [448,449], a trimer [579], a dihydroxy dimer and a quinone have also been detected as minor products; the major products derived from α-T oxidized in the autoxidation of methyl linoleate were also found to be a spirodienone dimer and a trimer [123]. However, when α-T was oxidized by benzoquinone, a dihydroxy dimer was the main product [413]. A quinone dimer has been reported in the oxidation of α-T by *t*-butyl hydroperoxide; it was expected to be a Diels-Alder product of *o*-quinone methide with α-tocopheryl-*p*-quinone [608]. Following administration of α-T-^{14}C-5-methyl to animals, two labelled metabolites isolated from their livers were α-tocopheryl-*p*-quinone [119], and a dimeric product [120,124] which was suggested to be di-α-tocopherone [121] and its *cis-trans* isomers were claimed to be synthesized by oxidation of α-T using alkaline potassium ferricyanide [121,122]. However, the structure of this dimeric product was demonstrated to be identical to the spirodienone dimer [413], a reinvestigation of the structure using near infrared spectroscopy, silylation and deuterium labelling technique suggested that it is a dienone dimer which has a 9-membered chelate ring (Fig. 1.4) [124].

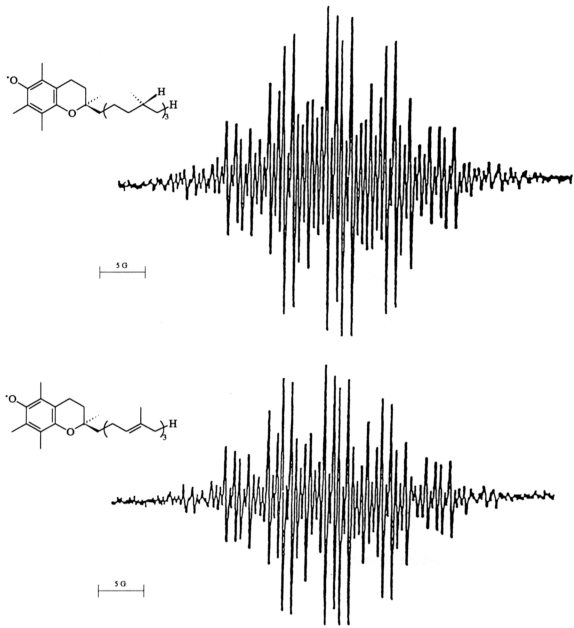
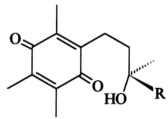
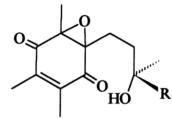


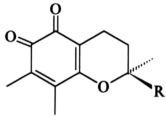
Fig. 1.1 ESR spectra of α-tocopheroxyl and α-tocotrienoxyl radicals.



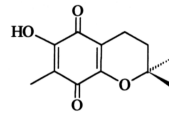
α-tocopherol quinone
(α-tocopheryl-*p*-quinone)



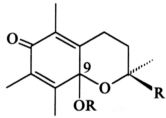
α-tocoquinone-2,3-oxide
(α-tocopheryl quinone epoxide)



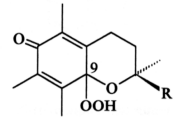
α-tocored



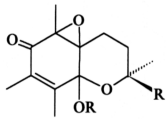
α-tocopurple



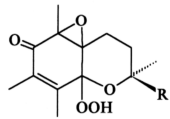
9*RS*-α-tocopheroxide
(9*RS*-alkoxy-α-tocopherone)



9*RS*-hydroperoxy-α-tocopheryl dienone
(9*RS*-hydroperoxy-α-tocopherone)

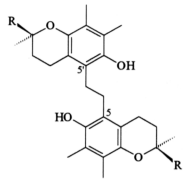


epoxy-9*RS*-alkoxy-α-tocopherone

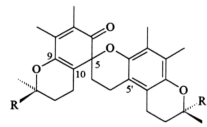


epoxy-9*RS*-hydroperoxy-α-tocopherone

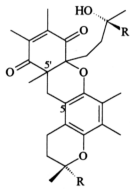
Fig. 1.3 Some oxidation products of α-tocopherol.



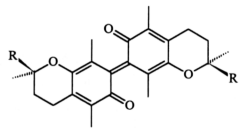
5,5'-α-T dihydroxy dimer



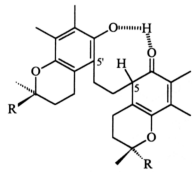
5,5'-α-T spirodienone dimer



α-T quinone dimer

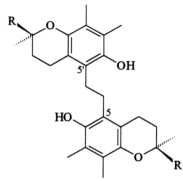


di-α-tocopherone dimer

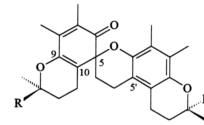


α-T dienone dimer

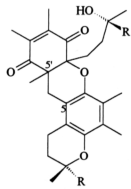
Fig. 1.4 Several possible structures of α-tocopherol dimers.



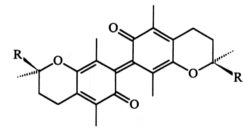
5,5'-α-T dihydroxy dimer



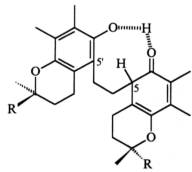
5,5'-α-T spirodienone dimer



α-T quinone dimer

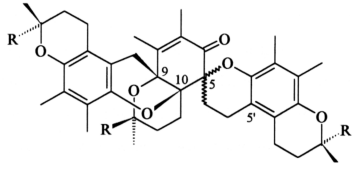


di-α-tocopherone dimer

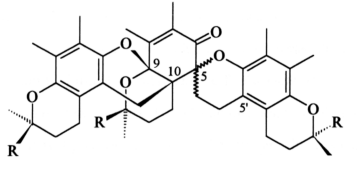


α-T dienone dimer

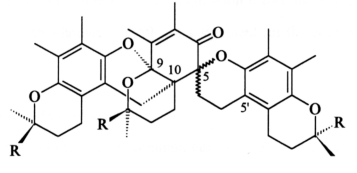
Fig. 1.4 Several possible structures of α-tocopherol dimers.



spiroether trimer



spiroketal trimer A



spiroketal trimer B

Fig. 1.5 Possible structures of α-tocopherol trimers.

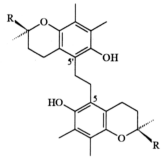
Stereoisomers of the α-T spirodienone dimers and trimers

The 5,5'-α-T dihydroxy dimer (Fig. 1.6) is the major dimeric product formed in the oxidation of α-T by $K_3Fe(CN)_6$ and further oxidation gives the 5,5'-α-T spirodienone dimer *via* a ring closure process [466]. Three other isomeric structures of the spirodienone dimers derived from different dihydroxy dimers having C5-C7', C7-C5' and C7-C7' linkages are also possible but these are always found to be minor oxidation products of α-T [579]. All of the four structurally isomeric spirodienone dimers derived from natural α-T (i.e. 2*R*,4'*R*,8'*R*-α-T) in fact can exist in diastereomeric forms as shown in Fig. 1.6; however, carbon assignments by ^{13}C NMR and the configuration of these diastereomers are still not available [448,466].

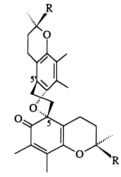
The reported structures of α-T trimers are shown in Fig. 1.5. An α-T *o*-quinone methide (Fig. 1.2) has been reported to be an intermediate formed during the oxidation of α-T [464], a trimer can be formed possibly *via* the Diels-Alder addition of the *o*-quinone methide to a spirodienone dimer at the C-9, C-10 double bond [466]. Two possible modes of the addition process will give rise to a spiroether trimer which is expected to be acid resistant [580], and a spiroketal trimer which is acid labile owing to the presence of a ketal group [699]. In fact, various possible diastereomers of the α-T trimer can be derived from 5*RS*,5'-α-T spirodienone dimers as shown in Fig. 1.7 but only two diastereomeric structures of the spiroketal trimers derived from natural α-T have been obtained during the autoxidation of methyl linoleate [699] and assignment for the configuration at C-5 has not been made.

1.1.4 Oxidation of γ-Tocopherol

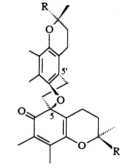
γ-Tocopherol (γ-T) is another common vitamin E component which can also easily dimerize when subjected to oxidation. The major oxidation products are γ-tocopherol dichromanyl ether dimer (γ-TDED) and γ-tocopherol bichromanyl dimer (γ-TBD) which have also been obtained during the fractionation of corn oil [465]. Both the ether dimer [413] and bichromanyl dimer [466] have been successfully synthesized by oxidation of γ-T



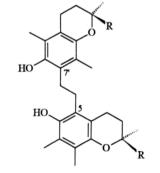
5,5'-α-T dihydroxy dimer



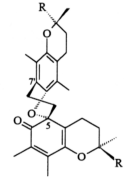
5*R*,5'-α-T spirodienone dimer



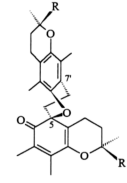
5*S*,5'-α-T spirodienone dimer



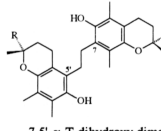
5,7'-α-T dihydroxy dimer



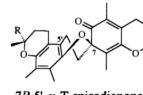
5*R*,7'-α-T spirodienone dimer



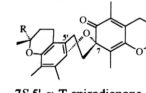
5*S*,7'-α-T spirodienone dimer



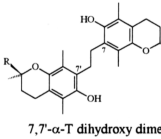
7,5'-α-T dihydroxy dimer



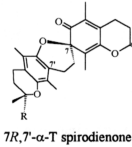
7*R*,5'-α-T spirodienone dimer



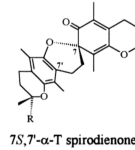
7*S*,5'-α-T spirodienone dimer



7,7'-α-T dihydroxy dimer



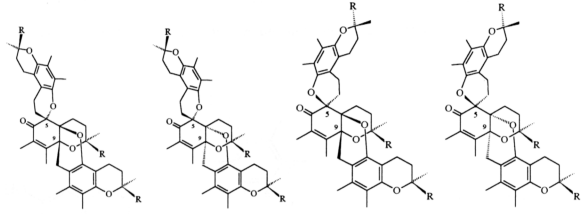
7*R*,7'-α-T spirodienone dimer



7*S*,7'-α-T spirodienone dimer

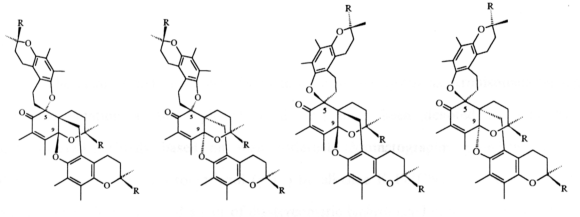
Fig. 1.6 Possible structures of various α-T dihydroxy dimers and spirodienone diastereomers.

spiroether trimers



5*R*,9*R* 5*R*,9*S* 5*S*,9*R* 5*S*,9*S*

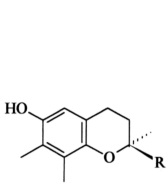
spiroketal trimers



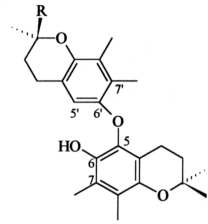
5*R*,9*S* 5*R*,9*R* 5*S*,9*S* 5*S*,9*R*

Fig. 1.7 Possible diastereomeric structures of α-T trimers.

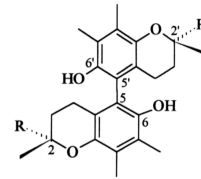
using *p*-benzoquinone as the oxidizing agent. These dimers were also formed when γ-T was oxidized in an autoxidizing oil [295,296] or in liquid paraffin at 180°C in the presence of trimethylamine oxide. The yields and ratio of these dimers were dependent on the presence of the synergist, oxidizing medium and concentration of γ-T [295,296].



γ-T



γ-TDED



RS-γ-TBD

The bichromanyl dimer was found to exist in two forms as atropisomers having restricted rotation at the biphenyl bond, and have been denoted as γ-TBD(H) and γ-TBD(L) chiefly based on their different chromatographic properties [297]. Previously, oxidation of γ-tocotrienol (γ-T_3) by alkaline $K_3Fe(CN)_6$ has been found to produce one ether dimer and a pair of diastereomeric bichromanyl dimers, their structures were resolved by ^{13}C NMR spectral assignments, but absolute configurations of the bichromanyl dimers have not been made. The dimers can be formed *via* coupling of the γ-tocotrienoxyl radical according to its resonance structures. The γ-tocotrienoxyl radical has been recorded by ESR as shown in Fig. 1.8. The γ-tocotrienol diphenyl ether dimer (γ-T_3DED) is also a good antioxidant due to the presence of a phenolic group, when it was subjected to oxidation by alkaline $K_3Fe(CN)_6$, a chromanoxyl radical can be generated and its ESR spectrum is shown in Fig. 1.8 [219].

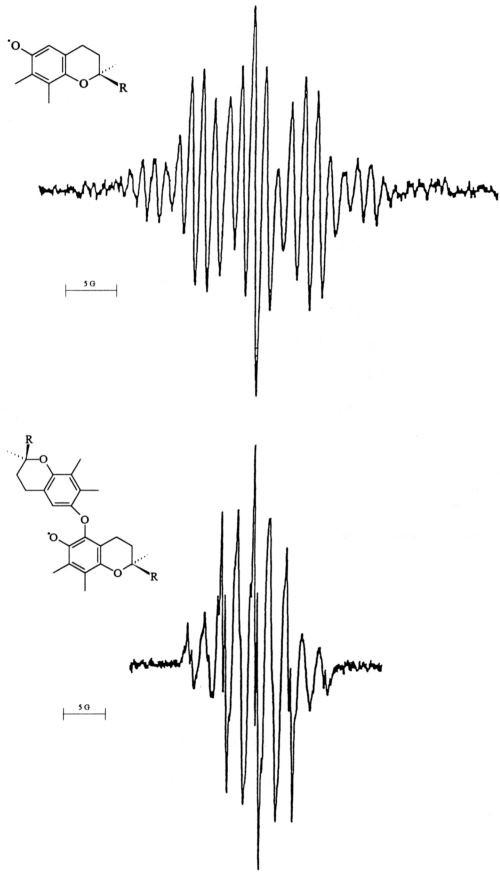


Fig. 1.8 ESR spectra of γ-tocotrienoxyl and γ-tocotrienol dichromanyl ether dimer radicals.

1.1.5 Inhibition of Lipid Peroxidation by Vitamin E

Autoxidation of unsaturated lipids is known to proceed by a free radical chain process [179,515] as exemplified by equations 1.1-1.5. The oxidation of lipid biomolecules possessing unsaturated bonds has long been considered related to degenerative diseases such as atherosclerosis, ageing and carcinogenesis. Initiation of the oxidation (Equation 1.1) *in vivo* is generally triggered by exogenous radical-generating agents (such as peroxides from foodstuffs) or through variable metabolic pathways involving electron transfer or oxidation-reduction systems. The lipid radicals are susceptible to attack by oxygen and will give rise to peroxyl radicals (Equation 1.2) which in turn will abstract a hydrogen atom from another lipid molecule or other biomolecules to produce hydroperoxide as well as regenerate another radical (Equation 1.3). Antioxidants are compounds which play an important role to scavenge the active radicals. Examples of the natural antioxidants are carotenoids, vitamins E and C. It is generally recognized that the antioxidant property of vitamin E is due to its ability to donate a phenolic hydrogen to a radical or active oxygen species (Equation 1.4) [89]; this process gives rise to vitamin E chromanoxyl radicals which can be regenerated by a sparing or secondary antioxidant such as vitamin C [495].

$$LH \longrightarrow L^{\bullet} \quad (1.1)$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \quad (1.2)$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet} \quad (1.3)$$

$$\alpha\text{-TOH} + LOO^{\bullet} \longrightarrow \alpha\text{-TO}^{\bullet} + LOOH \quad (1.4)$$

$$\alpha\text{-TO}^{\bullet} + LOO^{\bullet} \longrightarrow \text{non-radical products} \quad (1.5)$$

LH = unsaturated lipids, $L^{\bullet}$ = lipid radical, $LOO^{\bullet}$ = lipid peroxyl radical, LOOH = hydroperoxide, α-TOH = α-tocopherol, $\alpha\text{-TO}^{\bullet}$ = α-tocopheroxyl radical

Another pathway of the antioxidant action by vitamin E is that its chromanoxyl radical can scavenge other free radicals (peroxy, hydroperoxy or carbon-centred radicals) by forming non-radical products (Equation 1.5); supporting evidence for this is the isolation of non-radical products derived from α-tocopheroxyl radical and alkylperoxyl radicals [687,698] or methyl linoleate-peroxyl radicals [700].

1.1.6 Antioxidant Reactivity of Vitamin E Components

Effect of the isoprenoid side chain on the antioxidant activity of vitamin E

The phytyl side chain of tocopherols has a more remarkable effect on the reactivity of tocopherols in micellar dispersion [82,192,388,436,523] than in homogeneous solution [437]; it may facilitate the incorporation and retention of vitamin E in biomembranes [391,461]. Isoprenoid side chains of vitamin E have been suggested to be able to enhance the lipophilicity [85] of the vitamin E in biological systems such as biomembranes with a possible phytyl tail-to-phytyl tail arrangement in the bilayer [89], and it has been shown that tocopherols possess the optimum length of side chain in assisting 6-hydroxy-tetramethylchromans to penetrate monolayers of phospholipid molecules [394]. More importantly, certain biological activities of tocotrienols have been related to the unsaturation of their side chains [528].

Effect of the number of methyl substituents in the chromanyl ring

The antioxidant reactivity order of various tocopherols has been predicted and explained by the number and position of methyl groups substituted at the aromatic ring [466,562]. Recently, Mukai *et al.* [437] demonstrated that increasing total electron-donating capacity of the alkyl substituent on the aromatic ring of tocopheroxyl radicals significantly increased their reactivity being an electron acceptor in the reaction of abstracting a hydrogen from hydroperoxy methyl linoleates. The excellent antioxidant activity of the tocopherols may also be explained by the stabilization of tocopheroxyl radicals [82] which is related to the substantial orbital overlapping (stereoelectronic effect)

between the 2*p* type lone pair on the *para*-oxygen atom and the aromatic π electron system, and the electron donating or withdrawing character (inductive effect) of the substituents bonded at C-2. Another group of researchers, however, claimed that the relative effectiveness of tocopherols is dependent on the experimental conditions [323,376] such as the type of substrate, temperature [114,284] and the presence of synergists [294]. Remarkably however, instead of being an antioxidant, α-T present at high concentrations may exhibit prooxidant activity [319].

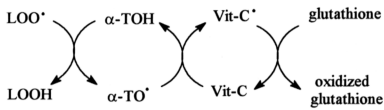
Reactivity order of tocopherols

There are numerous discrepancies concerning the antioxidant reactivity of the various tocopherol homologues in preventing *in vitro* lipid peroxidation. A number of reports have shown that the reactivity of tocopherols [87,434,462] as well as the stability of their tocopheroxyl radicals [432,433] is generally in the decreasing order of α > β > γ > δ [323,370] which is parallel to the order of certain biological activities [79,426,660] such as the rat fetal resorption bioassay [97]. However, the antioxidant efficiency of tocopherols has also been adequately demonstrated to be in the general order as δ > γ > β > α [426,486,499] as well as γ > β > α [284,485]. Although α-T has been widely known to be a better biological antioxidant and has better biological activity than γ-T [1,102,320], the γ-T has been reported to be more effective than α-T in preventing lipid peroxidation [115,294,318]. In contradiction to the reactivity order δ > γ > β > α determined by the disappearance (stability) of tocopheroxyl radicals, the initial rate of formation of the tocopheroxyl radicals by peroxyl radicals has shown the reverse order α > β > γ > δ [371]. Moreover, γ-T and δ-T have also been proven as better antioxidants than α-T in preventing the autoxidation of linoleic acid in aqueous media [105]. Generally, *in vitro* evidence supporting the antioxidant reactivity in the decreasing order α > β > γ > δ is merely based on the decay of radical initiators or oxygen uptake [82,84,87], consumption of the tocopherols [224,462], formation or decay of the tocopheroxyl radicals [436]. On the other hand, the reactivity of tocopherols in the reverse order α < β < γ < δ can be

easily realized as most of these experiments were based on the gross evaluation of both antioxidant and prooxidant effects of the tocopherols and also the influence from their oxidation products.

1.1.7 Regeneration of Vitamin E

There is a large body of evidence to support the hypothesis that vitamin E interacts synergetically with other antioxidants in a variety of *in vitro* model systems, and the synergism is commonly attributed to the regeneration of the vitamin E through reduction of its chromanoxyl radical by various compounds such as vitamin C [385,463]. However, contradictory observations from *in vivo* studies indicated that when vitamin C and radiolabelled vitamin E were fed to guinea pigs for eight weeks, the vitamin C does not spare vitamin E in various tissues [90]. Generally, *in vitro* results suggest that the regeneration of vitamin E radical by vitamin C [474] and glutathione [460] occurs by hydrogen transfer processes as shown below:



1.1.8 Objectives of the Present Research

Since research on tocotrienols is very much less than those for tocopherols, the present study was undertaken to look into some fundamental features of the antioxidant activity of tocotrienols. Oxidations of tocotrienols and tocopherols by alkaline potassium ferricyanide were carried out, reaction products derived from the oxidation of tocotrienols were characterized and compared with those of tocopherols. 2D-NMR spectroscopy was used to characterize new products and to determine the relative configuration of bichromanyl dimers of γ-T_3 and γ-T. The antioxidant behaviour of tocotrienols and their

dimers was further investigated by ESR spectroscopy, and experiments on the regeneration of certain vitamin E radicals by other types of vitamin E compounds are also presented.

1.2 EXPERIMENTAL

1.2.1 Materials

All reagents and solvents of analytical grade were used in the oxidation of vitamin E. Redistilled solvents were used for column chromatography. Silica gel (40-63 μm) for column chromatography and thin layer chromatography (TLC) silica gel $60F_{254}$ plates (5 cm x 10 cm) were purchased from Merck. For high performance liquid chromatography (HPLC), solvents of HPLC grade were filtered through a 0.5 μm membrane filter, degassed with an ultrasonicator and then equilibrated to room temperature prior to use.

Vitamin E components

α-T, α-T_3 and γ-T_3 were isolated from a vitamin E concentrate derived from palm fatty acid distillate (PFAD), a byproduct obtained during the refining of palm oil [218]. The free fatty acids were crystallized and removed by filtration, the residual fatty acids were converted to methyl esters in methanol. Most of the fatty acid methyl esters were distilled off at less than 200°C at a pressure of less than 2 mmHg. The residue contained a mixture of vitamin E compounds, *viz.* α-T, α-T_3, γ-T_3 and δ-T_3; individual vitamin E components were isolated by silica gel column chromatography using 2-10% of ethyl acetate in hexanes as the eluent. γ-T was isolated from a tocopherol mixture by silica gel column chromatography using hexanes-ethyl acetate (gradient 2-10%) as the mobile phase. Purities of the vitamin E components used for reactions were more than 98%.

1.2.2 General Procedures for the Oxidation of Vitamin E

A quantity (1 - 4 g) of each vitamin E component (α-T, α-T_3, γ-T or γ-T_3) or their derivatives (e.g. γ-TDED or γ-T_3DED) was dissolved in 100 mL *n*-hexane in a two-neck round bottom flask capped with rubber septum and flushed with nitrogen gas. For the oxidation of α-T_3 and α-T, toluene was used as the organic solvent. A degassed solution of 100 mL 0.2 M $K_3Fe(CN)_6$ was added into the vitamin E solution, followed by 100 mL 0.2 M NaOH. The mixture was vigorously stirred under nitrogen at room temperature for

less than half an hour until most of the vitamin E compounds reacted; however, for the oxidation of γ-tocotrienol dichromanyl ether dimer (γ-T_3DED) and γ-tocopherol dichromanyl ether dimer (γ-TDED), extensive oxidation was avoided because this would give rise to decomposed and polar compounds. The duration for oxidation of various vitamin E compounds was monitored by TLC using 1 - 3% of ethyl acetate in *n*-hexane as developing solvents, and oxidation products were visualized by short-wave ultra-violet light and iodine staining. After removal of solvents, the crude products were column chromatographed on silica gel using hexanes-ethyl acetate (gradient 0.2 - 20%) as the eluent. The oxidation products of γ-T_3 and γ-T were also separated by HPLC equipped with a 4.6 x 250 mm silica column, the detection was by a Waters 470 fluorescence detector using wavelengths for excitation at 295 nm and emission at 335 nm, the solvent mixture was hexane-tetrahydrofuran-isopropanol (984:15:1) eluted at 0.5 mL/min.

1.2.3 Characterization of Vitamin E Oxidation Products

^1H and ^{13}C NMR spectra for the pure products dissolved in $CDCl_3$ were recorded on a JEOL JNM-GSX270 FT NMR spectrometer. Proton chemical shifts are reported as δ_H ppm downfield from tetramethylsilane as internal standard, whereas δ_C 77.00 ppm is used as the reference peak of chloroform in the ^{13}C NMR data. Electron impact (EI) mass spectra of pure samples were recorded with a Fisons ProSpec spectrometer. Fast atom bombardment-mass spectra for the dimers and larger molecules were also recorded with the same spectrometer. Infrared spectra were recorded with a Perkin-Elmer, model 1650 FT-IR spectrophotometer. Assignments for the *R*- and *S*-configurations of the γ-T bichromanyl dimers and γ-T_3 bichromanyl dimers were done by ^1H-NOE difference experiments using a Varian VXR 300 NMR spectrometer, the irradiation time was 0.5 s, 16 scans were collected and the spectra were Fourier transformed with line broadening of 3 Hz; molecular modelling for the diastereomeric bichromanyl dimers was also analyzed using a simulation programme MM2, CAChe-Tektronix System.

Elemental analysis for γ-TDED dimer found: C, 80.98; H, 11.63. $C_{56}H_{94}O_4$ requires C, 80.96, H, 11.40%. Elemental analysis for the (*R*)-5,5'-bi-γ-T found: C, 80.83, H, 11.30. $C_{56}H_{94}O_4$ requires C, 80.96, H 11.40%.

1.2.4 ESR Studies on Vitamin E Compounds

Generation of vitamin E radicals

Prior to oxidation, 5 mg of α-T, α-T_3 or γ-T_3 in 2 mL of toluene in a 10 mL sample vial was repeatedly vacuum-degassed and flushed with nitrogen several times. The oxidation of vitamin E was initiated by vigorous mixing with 2 mL 0.1 M $K_3Fe(CN)_6$ and 2 mL 0.1 M NaOH degassed aqueous solutions. For the oxidation of vitamin E dimers, 10 mg of the compounds in 2 mL of hexane were used. Immediately after centrifugation at 2000 rpm for 1 min, 0.2 mL of the organic aliquot was transferred (by an air-tight glass syringe) into an ESR quartz tube (i.d. 3 mm) and kept under nitrogen atmosphere. First derivative ESR spectra of the vitamin E free radicals were recorded at room temperature (300 K) using a Bruker B-R70 ESR spectrometer with 100 kHz magnetic field modulation, frequency 9.35 GHz, microwave power 20 - 30 dB, time constant 0.5 second, sensitivity 50 mV/cm, and scan range of 200 G per 60 cm, modulation range (0.01 - 1 Gpp) was kept low but varied with signal gain ($1x10^4$ - $2x10^6$). For simple kinetic studies and determination of the half-live of various chromanoxyl radicals, ESR spectra were recorded periodically and intensities of their central peak were measured.

Regeneration of vitamin E radicals

2 mg of δ-tocotrienol dichromanyl ether dimer (δ-T_3DED) were subjected to ferricyanide oxidation to yield relatively stable chromanoxyl radicals which were detected by ESR; 2 mg of γ-T_3 in 0.2 mL toluene were then added into the ESR quartz tube, and changes in the spectra were recorded. In another experiment, 2 mg of γ-T_3DED was oxidized by alkaline ferricyanide to produce the chromanoxyl radical, 2 mg of α-T_3 in 0.2

mL toluene was then introduced into the radical solution and the ESR spectrum was recorded every 15 minutes until all of the radicals decayed.

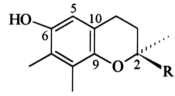
1.3 RESULTS AND DISCUSSION

1.3.1 Oxidation Products of γ-Tocotrienol and γ-Tocopherol

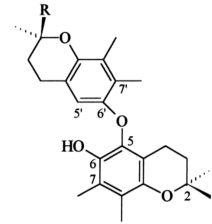
Oxidation of γ-T_3 by alkaline $K_3Fe(CN)_6$ gave rise to a dichromanyl ether dimer (γ-T_3DED, yield 55%) and two bichromanyl diastereomers, i.e. (*R*)-5,5'-bi-γ-T_3 (20%) and (*S*)-5,5'-bi-γ-T_3 (18%). Three major oxidation products obtained from the reaction of γ-T by alkaline $K_3Fe(CN)_6$ are γ-TDED (yield 49%), (*R*)-5,5'-bi-γ-T (22%) and (*S*)-5,5'-bi-γ-T (20%). Structures of the dimers are shown in Figure 1.9, and their characteristic data are given in Table 1.2. Analyses of the oxidation products by TLC and HPLC gave satisfactory separation of the γ-T_3DED, (*S*)-5,5'-bi-γ-T_3 and (*R*)-5,5'-bi-γ-T_3 by using 2% ethyl acetate in *n*-hexane; the γ-T dimers have comparatively higher R_f values than the γ-T_3 dimers. Mass spectroscopic data ($M^{+\cdot}$) for all of the dimers obtained from the oxidation of γ-vitamers (γ-T and γ-T_3) are in agreement with the calculated values based on their molecular formula. ^1H and ^{13}C NMR spectral data of the oxidation products are given in Tables 1.3 and 1.4, respectively.

γ-Tocotrienol dichromanyl ether dimer and γ-tocopherol dichromanyl ether dimer

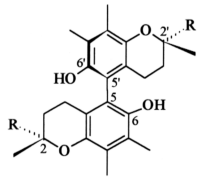
Dimers γ-T_3DED and γ-TDED were found to be the major oxidation products of γ-T_3 and γ-T, respectively (Fig. 1.9). ^1H and ^{13}C NMR data are given in Tables 1.3 and 1.4, respectively. The dimer γ-T_3DED shows a broad peak at δ_H 5.15 ppm assigned to the vinyl protons from two isoprenoid side chains which are not present in the γ-TDED. Assignments of the two oxygenated aromatic carbons in γ-T are based on the chemical shift data induced by shift reagents [190], the phenolic carbon C-6 (δ_C 146.24 ppm) resonates at lower field as compared to the ethereal carbon C-9 (δ_C 145.69 ppm). The carbon assignments for γ-T_3DED have been made previously [219]. The present ^{13}C NMR spectral data of γ-TDED indicate that carbon chemical shifts for its two phytyl side chains are similar with those in γ-T, but different resonance peaks for the two chromanyl rings of γ-TDED were observed (Table 1.4). Four resonance peaks in the upfield region are assigned to aromatic methyl carbons C-7a and C-8a from the γ-tocopheryl



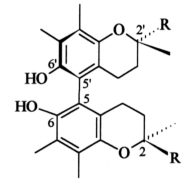
γ-T or γ-T_3



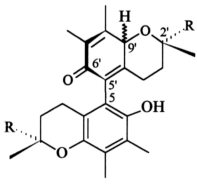
γ-TDED or γ-T_3DED



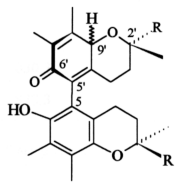
(*R*)-bi-5,5'-γ-T or (*R*)-bi-5,5'-γ-T_3



(*S*)-bi-5,5'-γ-T or (*S*)-bi-5,5'-γ-T_3



(*R*)-5,5'-bi-γ-T dienone dimer



(*S*)-5,5'-bi-γ-T dienone dimer

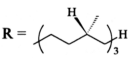
T_3 = Tocotrienol, R =  T = Tocopherol, R = 

Fig. 1.9 Structures of the dimers of γ-T_3 and γ-T.

Table 1.2 Characteristics of the oxidation products* of γ-T_3 and γ-T

Product	TLC[a] R_f	m/z calculated	m/z recorded	HPLC r.t.[b] (min)	Yield (%)
Oxidation of γ-T_3					
γ-T_3DED	0.28	818.62	818.4	2.7	55
(*S*)-5,5'-bi-γ-T_3	0.19	818.62	818.4	3.1	18
(*R*)-5,5'-bi-γ-T_3	0.10	818.62	818.4	4.2	20
γ-T_3 (unreacted)	0.03	410.31	410.0	18.2	5
Oxidation of γ-T					
γ-TDED	0.29	830.71	830.5	2.3	49
(*S*)-5,5'-bi-γ-T	0.22	830.71	830.5	2.5	20
(*R*)-5,5'-bi-γ-T	0.12	830.71	830.0	3.5	22
γ-T (unreacted)	0.04	416.36	416.0	14.8	6
Oxidation of γ-TDED					
γ-T tetramer#	0.35	1659.41	1659.6	-	20
Oxidation of (R)-5,5'-bi-γ-T					
(*S*)-5,5'-bi-γ-T dienone dimer	0.27	830.71	830.3	-	19
(*R*)-5,5'-bi-γ-T dienone dimer	0.15	830.71	830.3	-	21

* Structures of the oxidation products of γ-T_3 and γ-T are given in Fig. 1.9.

Structure is shown in p. 38.

[a] R_f values for TLC developed by 2% of ethyl acetate in *n*-hexane.

[b] HPLC r.t. is the retention time for compounds separated by HPLC using silica column (4.6 x 250 mm) and *n*-hexane-tetrahydrofuran-isopropanol (984:15:1) eluting at 0.5 mL/min.

Table 1.3 ^{1}**H NMR spectral data of γ-tocotrienol, γ-tocopherol and their dimers***

Proton	Tocotrienol				Tocopherol					
	γ-T_3	γ-T_3DED	(*S*)-5,5'-bi-γ-T_3	(*R*)-5,5'-bi-γ-T_3	γ-T	γ-TDED	(*S*)-5,5'-bi-γ-T	(*R*)-5,5'-bi-γ-T	dienone dimer (*S*)-	dienone dimer (*R*)-
5-H	6.39	6.11	-	-	6.38	6.13	-	-	-	-
6,6'-OH	4.90	5.04	4.42	4.46	4.84	5.07	4.47	4.45	4.40	4.40
4,4'-CH_2-	2.72	2.59	2.6	2.6	2.69	2.59	2.0-2.4	2.0-2.4	2.0-2.4	2.0-2.4
7a-CH_3	2.20	2.35	2.20	2.21	2.18	2.37	2.22	2.22	2.17	2.17
7a'-CH_3	-	2.20	2.20	2.21	-	2.22	2.22	2.22	1.59	1.58
8a-CH_3	2.20	2.24	2.19	2.20	2.17	2.26	2.20	2.20	2.47	2.49
8a'-CH_3	-	2.18	2.19	2.20	-	2.19	2.20	2.20	1.48	1.47
3,3'-CH_2-	1.8-2.2	1.7-2.2	1.9-2.2	1.9-2.2	1.6-1.8	1.6-1.8	1.6-1.8	1.6-1.8	1.6-1.8	1.6-1.8
2a,2a'-CH_3	1.34	1.28	1.24	1.27	1.30	1.32	1.29	1.29	1.43	1.43

* Structures of the compounds are shown in Fig. 1.9. Protons from the tocotrienoxy (in γ-T_3DED) or one of the tocotrienyl (in 5,5'-bi-γ-T_3) fragments are prime-numbered. Only the data for chromanyl moieties are given.

Table 1.4 ^{13}C NMR spectral data of γ-tocotrienol, γ-tocopherol and their dimers*

Carbon	γ-T_3	γ-T_3DED	(*S*)-5,5'-bi-γ-T_3	(*R*)-5,5'-bi-γ-T_3	γ-T	γ-TDED	(*S*)-5,5'-bi-γ-T	(*R*)-5,5'-bi-γ-T	dienone dimer (*S*)-	dienone dimer (*R*)-
5	112.30	140.17	115.19	115.18	112.27	140.11	115.19	115.20	129.61	129.40
5'	-	109.84	115.19	115.18	-	109.82	115.19	115.20	151.57	151.45
6	146.26	146.65	145.90	145.94	146.24	146.68	145.95	145.92	147.93	147.84
6'	-	148.13	145.90	145.94	-	148.09	145.95	145.92	182.04	181.92
7	118.14	118.14	117.42	117.29	118.23	118.17	117.46	117.37	118.75	118.41
7'	-	111.91	117.42	117.29	-	111.96	117.46	117.37	133.46	133.23
8	121.85	121.89	122.23	122.23	121.80	121.87	122.19	122.22	124.95	124.74
8'	-	121.65	122.23	122.23	-	121.62	122.19	122.22	130.07	130.12
9	145.63	145.05	144.57	144.65	145.69	145.08	144.53	144.64	146.44	146.47
9'	-	137.13	144.57	144.65	-	137.08	144.53	144.64	98.03	98.00
10	125.71	126.18	126.91	126.90	125.76	126.18	126.86	126.89	125.58	125.69
10'	-	123.56	126.91	126.90	-	123.55	126.86	126.89	147.29	147.11

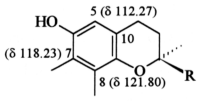
* Structures of the compounds are shown in Fig. 1.9.

Table 1.4 (continued)

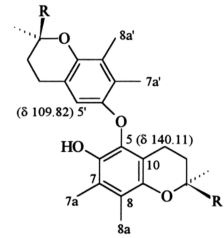
Carbon	γ-T_3	γ-T_3DED	(*S*)-5,5'-bi-γ-T_3	(*R*)-5,5'-bi-γ-T_3	γ-T	γ-TDED	(*S*)-5,5'-bi-γ-T	(*R*)-5,5'-bi-γ-T	dienone dimer (*S*)-	dienone dimer (*R*)-
2	75.22	75.41	74.97	74.97	75.46	75.64	75.17	75.20	76.44	76.58
2'	-	75.02	74.97	74.97	-	75.23	75.17	75.20	75.54	75.41
2a	23.95	24.03	23.67	23.80	24.08	24.90	23.74	23.98	24.49	24.29
2a'	-	23.79	23.67	23.80	-	24.90	23.74	23.98	26.95	27.41
3	31.47	31.36	31.31	31.21	31.42	31.29	31.21	31.24	31.64	31.64
3'	-	30.81	31.31	31.21	-	30.75	31.21	31.24	32.69	32.71
4	22.23	22.43	20.56	20.58	22.32	22.79	20.59	20.66	22.76	22.75
4'	-	22.43	20.56	20.58	-	22.79	20.59	20.66	23.44	23.07
7a	11.86	12.10	12.31	12.30	11.91	12.14	12.32	12.32	12.42	12.40
7a'	-	11.98	12.31	12.30	-	11.99	12.32	12.32	15.54	15.67
8a	11.86	11.98	12.02	12.03	11.91	12.02	12.02	12.03	11.38	11.29
8a'	-	11.63	12.02	12.03	-	11.64	12.02	12.03	14.43	14.49

* Structures of the compounds are shown in Fig. 1.9.

and γ-tocopheroxy moieties in the γ-TDED. The protonated C-5 in γ-T shows a doublet signal in an off resonance (OFR) decoupled spectrum, it resonates at higher field (δ_C 112.27 ppm) as compared to those substituted aromatic carbons C-7, C-8 and C-10 in the γ-T [604]. As a result of an ethereal bonding formed in the γ-TDED, the chemical shift for the protonated C-5' in the tocopheroxy moiety is shifted to higher field (δ_C 109.82 ppm) whereas the substituted C-5 is shifted to lower field (δ_C 140.11 ppm).



γ-T

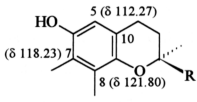


γ-TDED

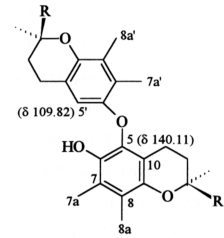
5,5'-Bi-γ-tocotrienyl dimers and 5,5'-bi-γ-tocopheryl dimers

Two diastereomeric bichromanyl dimers obtained from the oxidation of γ-T_3 and γ-T are (*R*)- & (*S*)-5,5'-bi-γ-T_3 and (*R*)- & (*S*)-5,5'-bi-γ-T, respectively (Fig. 1.9). These diastereomers possess different chromatographic behaviours and have been separated by silica gel column chromatography, TLC and HPLC. These diastereomeric dimers are expected to be the result from restricted rotation at the C5-C5' bichromanyl linkage, they are thermally interconvertible and can be isomerized by oxidation using alkaline $K_3Fe(CN)_6$. Isomerization of the pure compounds can be slowed down by keeping at low temperature (0°C), and acetylation of the hydroxyl groups also satisfactorily inhibited the isomerization. Normal ^1H NMR spectra for both of the

and γ-tocopheroxy moieties in the γ-TDED. The protonated C-5 in γ-T shows a doublet signal in an off resonance (OFR) decoupled spectrum, it resonates at higher field (δ_C 112.27 ppm) as compared to those substituted aromatic carbons C-7, C-8 and C-10 in the γ-T [604]. As a result of an ethereal bonding formed in the γ-TDED, the chemical shift for the protonated C-5' in the tocopheroxy moiety is shifted to higher field (δ_C 109.82 ppm) whereas the substituted C-5 is shifted to lower field (δ_C 140.11 ppm).



γ-T

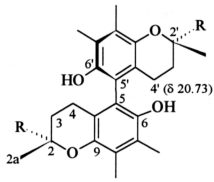


γ-TDED

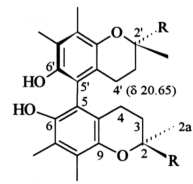
5,5'-Bi-γ-tocotrienyl dimers and 5,5'-bi-γ-tocopheryl dimers

Two diastereomeric bichromanyl dimers obtained from the oxidation of γ-T_3 and γ-T are (*R*)- & (*S*)-5,5'-bi-γ-T_3 and (*R*)- & (*S*)-5,5'-bi-γ-T, respectively (Fig. 1.9). These diastereomers possess different chromatographic behaviours and have been separated by silica gel column chromatography, TLC and HPLC. These diastereomeric dimers are expected to be the result from restricted rotation at the C5-C5' bichromanyl linkage, they are thermally interconvertible and can be isomerized by oxidation using alkaline $K_3Fe(CN)_6$. Isomerization of the pure compounds can be slowed down by keeping at low temperature (0°C), and acetylation of the hydroxyl groups also satisfactorily inhibited the isomerization. Normal ^1H NMR spectra for both of the

diastereomeric bichromanyl dimers are almost identical (Table 1.3); HETCOR and COSY data have been used to assign the proton signals. Carbons from the two chromanyl moieties of the respective diastereomers have similar chemical shifts as given in Table 1.4. The chemical shift for C-5 increased from δ_C 112.2 ppm (for γ-T) to δ_C 115.2 ppm in the dimer afforded the evidence that the two γ-T molecules are linked at aromatic carbon-5 in the 5,5'-bi-γ-T as also found in the corresponding tocotrienol dimers (5,5'-bi-γ-T_3) [219]. Similarly as in 5,5'-bi-γ-T_3, upfield shifts from δ_C 22.32 ppm (for γ-T) to δ_C 20.59 ppm (for (*S*)-5,5'-bi-γ-T) and δ_C 20.66 ppm (for (*R*)-5,5'-bi-γ-T) were observed for C-4 and C-4' methylene carbons. When the ^{13}C NMR spectrum was recorded for a mixture of (*R*)-5,5'-bi-γ-T and (*S*)-5,5'-bi-γ-T, distinguishable resonance peaks have been identified, significant differences for their chemical shifts are found at some carbons such as C-9, C-4, C-3, C-2 and C-2a of the chromanyl moieties (Table 1.4). For (*R*)-5,5'-bi-γ-T_3 and (*S*)-5,5'-bi-γ-T_3, their ^{13}C NMR data also slightly differ at a few resonance peaks correspond to carbons C-9, C-3 and C-2a (Table 1.4). However, these NMR data are inadequate for the assignment of the absolute configuration of the diastereomeric compounds.



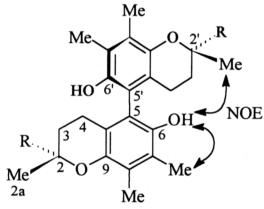
(*R*)-5,5'-bi-γ-T



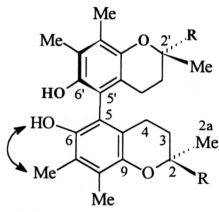
(*S*)-5,5'-bi-γ-T

Stereochemistry of the 5,5'-bi-γ-T_3 and 5,5'-bi-γ-T diastereomers

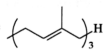
Some characteristic differences exist between the diastereomeric dimers and are given in Table 1.5. Assignments for the absolute configuration of the diastereomeric bichromanyl dimers are possible by NOE difference NMR experiments. NOE difference data (Table 1.5) show that the NOE enhancement between 6-OH and 2'-Me (or 6'-OH and 2-Me) is 5.8%, and between 6-OH and 7-Me is 3.2% for the (*R*)-5,5'-bi-γ-T; there is no NOE interaction between the 6-OH and 2'-Me protons in the (*S*)-5,5'-bi-γ-T. Similarly, NOE was observed for the (*R*)-5,5'-bi-γ-T_3, which has an enhancement of 0.2% between 6-OH and 2'-Me groups, and the NOE for 6-OH and 7-Me groups is 1.8%. Thus NOE difference data gave convincing evidence for the assignments of the absolute configurations relative to the 2*R* configuration of natural vitamin E.



(*R*)-5,5'-bi-γ-T_3 or (*R*)-5,5'-bi-γ-T



(*S*)-5,5'-bi-γ-T_3 or (*S*)-5,5'-bi-γ-T

T_3 = Tocotrienol, R = 

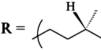
T = Tocopherol, R = 

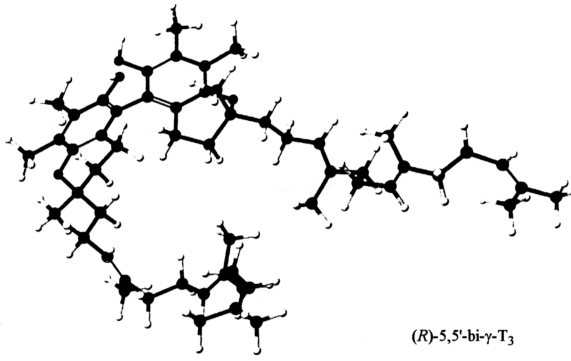
Table 1.5 Characteristics and ^{13}C NMR spectral data of bichromanyl dimers

Parameter	5,5'-bi-γ-T_3		5,5'-bi-γ-T	
	(*R*)-	(*S*)-	(*R*)-	(*S*)-
TLC R_f[#]	0.14	0.24	0.20	0.32
HPLC r.t. / min.	4.2	3.1	3.5	2.5
NOE difference				
(6-OH ⟶ 2'-Me)	0.2%	0	5.8%	0
(6-OH ⟶ 7-Me)	1.8%	0.1%	3.2%	0
Relative energy* (kcal/mol)	17.4	20.0	37.6	38.4
Steric energy (kcal/mol)	1.53	-4.06	N.A.	N.A.
Distance between 6-OH and 2'-Me (Å)	5.392	6.723	N.A.	N.A.
Carbon chemical shifts				
C-2a	23.80	23.67	23.98	23.74
C-3	31.21	31.31	31.24	31.21
C-4	20.58	20.56	20.66	20.59
C-9	144.72	144.63	144.71	144.60

* Data for the energy-minimized structures were obtained by molecular simulation using MM2 programme, CAChe-Tektronix system; N.A. = data not available.

[#] R_f values for silica gel TLC developed by 3% of ethyl acetate in *n*-hexane.

Molecular modelling is also used to explain some of the characteristics of the bichromanyl dimers including their NOE difference spectra. The C-2 and C-2' chiral centres in all bichromanyl dimers have the natural (2*R*)-configuration and the 2-methyl and 2'-methyl groups prefer an axial conformation. The energy-minimized structures of the 5,5'-bi-γ-T_3 and 5,5'-bi-γ-T bichromanyl dimers obtained by simulation are shown in Figs. 1.10 and 1.11, respectively. From the data given in Table 1.5, the dimer (*R*)-5,5'-bi-γ-T_3 has a shorter distance between 2'-methyl and 6-hydroxyl groups as compared to the (*S*)-5,5'-bi-γ-T_3, this may explain the NOE interaction observed by NMR spectroscopy. The MM2 minimizations also provide steric energy data derived from bond stretching and nonbonded interactions such as van der Waals and hydrogen bonding. The results indicate that the steric energy for (*R*)-5,5'-bi-γ-T_3 is greater than that for (*S*)-5,5'-bi-γ-T_3. The two axial C-2a methyls in (*S*)-5,5'-bi-γ-T and (*S*)-5,5'-bi-γ-T_3 are oriented such that they face each other and thus will give rise to greater steric repulsion than that in (*R*)-bichromanyl dimers. The (*S*)-bichromanyl dimers have relatively more planar arrangement in which there is relatively less steric interactions between the 2-methyl and 2'-methyl groups; however, they have less intramolecular hydrogen bonding between the phenolic groups as compared to the (*R*)-bichromanyl dimers which have a relatively more bisected conformation. As a consequence of the preferred conformational arrangement, the (*R*)-bichromanyl dimers are relatively more "polar" because the phenolic groups are more exposed for chromatographic bonding. Whereas (*S*)-bichromanyl dimers are more hydrophobic than the (*R*)-bichromanyl dimers due to the relative proximity of the long hydrocarbon side chains to the phenolic groups and thus are more surrounded by the side chains.



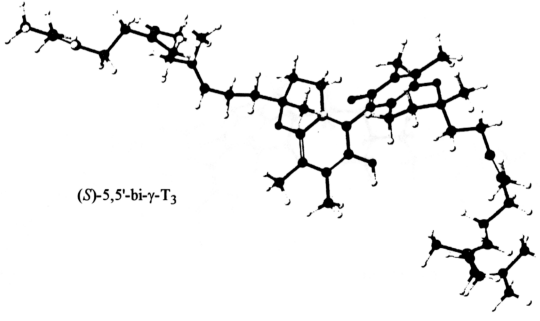


Fig. 1.10 Energy-minimized models of (*R*)- and (*S*)-5,5'-bi-γ-T_3.

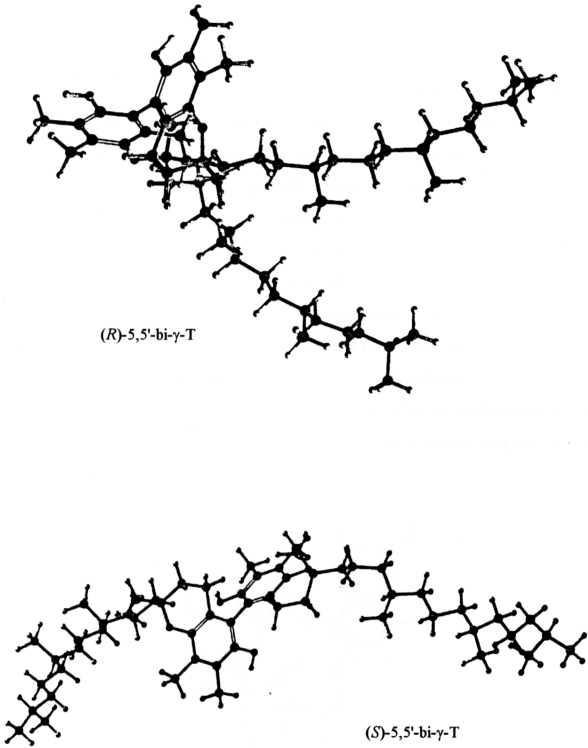
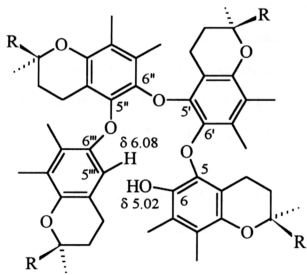


Fig. 1.11 Energy-minimized models of (*R*)- and (S)-5,5'-bi-γ-T.

Oxidation products of γ-T_3DED and γ-TDED

The oxidation of γ-T_3DED by alkaline $K_3Fe(CN)_6$ generated a stable chromanoxyl radical which has been detected by ESR and the spectrum is shown in Fig. 1.8 (page 15). A qualitative analysis for the oxidation products of γ-TDED (which has a similar chromanyl structure as γ-T_3DED) has been carried out and the results are given in Table 1.2. Prolonged oxidation of the γ-TDED caused extensive decomposition of the compound; under an optimized reaction condition, a major product of 20% yield has been obtained. As shown by mass spectroscopy, the product was found to be a tetramer of γ-T which has a molecular weight of 1659.6 (calculated 1659.41). ^{13}C NMR spectrum of the tetramer is rather complicated as too many resonance peaks fall in the aromatic quaternary region, therefore a complete 2D-NMR analysis would be necessary for exact assignment. Since the ^{13}C NMR spectrum indicates that carbonyl (C=O) and ether ($-CH_2-O-$) groups are not present, all the chromanoxy C-6 carbons (δ_C 145-147 ppm) should be retained in the tetramer. A simple structure of the new tetramer will be from O-C coupling of two radicals derived from the γ-TDED.



γ-T tetramer

Radical dimerizations of γ-vitamer

In the oxidation of γ-vitamer (γ-T_3 or γ-T), chromanoxyl radicals (γ-$T_3^{\bullet}$ or γ-$T^{\bullet}$) can be easily obtained and have been detected by ESR (Fig. 1.8). These chromanoxyl radicals are relatively unstable with a short half-life of less than 15 minutes. The chromanoxyl radicals readily undergo coupling reactions, as shown in Fig. 1.12, to give a mixture of dimeric products, i.e. the dichromanyl ether dimer and diastereomeric bichromanyl dimers. The dichromanyl ether dimer possess one phenolic group therefore can act as antioxidant; oxidation of this dimer gives rise to stable chromanoxyl radicals (Fig. 1.8) and the resulting major product is the tetramer shown above.

Oxidation products of 5,5'-bi-γ-T

Two major products with approximately similar yields have been obtained from the oxidation of (*R*)-5,5'-bi-γ-T, similar products have also been found in the oxidation of (*S*)-5,5'-bi-γ-T; some of their characteristic data are given in Table 1.2. Mass spectroscopic data show that these compounds have m/z 830.3 corresponding to the molecular weight for a dimer of γ-T (calculated 830.71). ^{1}H and ^{13}C NMR spectral data of their chromanyl moieties are given in Tables 1.3 and 1.4, respectively. The proton and carbon chemical shifts for the phytyl side chains of the products remain unchanged as compared to the starting bichromanyl dimers, indicating that the reaction centre is located at the chromanyl moieties. The products can be separated by TLC and purified by column chromatography; however, the pure compounds are relatively unstable at room temperature and each compound could be readily converted back to a racemic mixture of (*R*)-5,5'-bi-γ-T and (*S*)-5,5'-bi-γ-T. These results together with the ^{13}C NMR spectral data (Table 1.4) suggest that the new compounds are dienone dimers exist in diastereomeric forms, *viz.* (*R*)-5,5'-bi-γ-T dienone dimer and (*S*)-5,5'-bi-γ-T dienone dimer. ^{13}C NMR spectra for the two dienone dimers show similar patterns of resonance peaks with small differences of 0.03-0.40 ppm in chemical shifts, twelve non-equivalent carbons derived from two aromatic rings of the bichromanyl dimers can be assigned to twelve resonance peaks downfield from

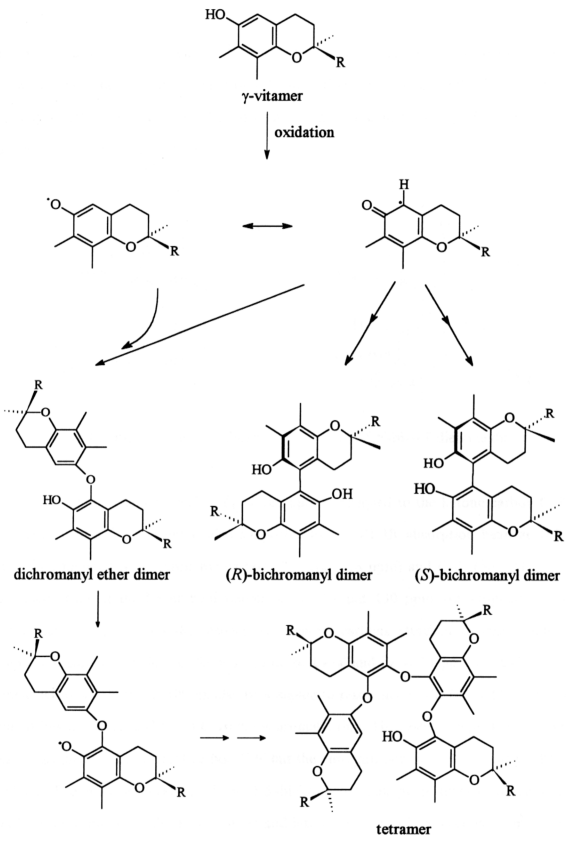
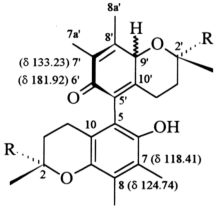
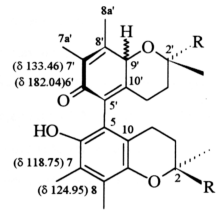


Fig. 1.12 Mechanism for the formation of dimers and tetramers of γ-vitamers.

the $CDCl_3$ peaks. As indicated by ^{13}C NMR spectroscopy, one of the aromatic rings of the bichromanyl dimer is unreacted during the oxidation and chemical shifts for its carbons are only slightly affected; the other aromatic ring of the bichromanyl dimer has been converted to a dienone structure.



(*R*)-5,5'-bi-γ-T dienone dimer



(*S*)-5,5'-bi-γ-T dienone dimer

The carbon chemical shift at $\delta_C \approx 182$ ppm is assigned to the ketone carbon C-6'; the presence of a C=O group is also supported by the FT-IR absorption band at 1625 cm^{-1}. As compared to the aromatic carbons C-7 (δ_C 118 ppm) and C-8 (δ_C 124 ppm), resonance peaks at the lower field region $\delta_C \approx 133$ and 130 ppm are assigned to the olefinic carbons C-7' and C-8', respectively; methyl carbons attached to these olefinic carbons are shifted upfield, i.e. $\delta_C \approx 15$ ppm for C-7a' and $\delta_C \approx 14$ ppm for C-8a'. The olefinic carbons C-5' and C-10' can also be assigned to resonance peaks ($\delta_C \approx 147$ and 151 ppm) at lower field than C-5 and C-10 in the aromatic ring. The resonance peak at δ_C 98.0 ppm is assigned to the ethereal carbon C-9' but the configuration at C-9' has not yet been resolved. The *RS*-configuration of the 5,5'-bichromanyl linkage is tentatively assigned based on the properties (chromatographic and intramolecular H-bonding) as occur in the *RS*-bichromanyl dimers; however, the absolute configuration of the dienone dimers can only be assigned when 2D-NMR or X-ray data are available.

Radicals generated from 5,5'-bi-γ-T_3

When (*R*)-5,5'-bi-γ-T_3 dimer was oxidized by alkaline $K_3Fe(CN)_6$, radicals have been detected by ESR and the spectrum is shown in Fig. 1.13. Simulation of the spectrum indicates that two types of radicals were detected, one of them is a chromanoxyl radical with hyperfine coupling constants $a_{7\text{-}CH_3}$ 4.80 G, $a_{4\text{-}CH_2}$ 1.20 G and $a_{8\text{-}CH_3}$ 1.10 G. The other radical had hyperfine coupling constants 1.40 G and 1.00 G for two non-equivalent protons but its structure has not been identified; it may be a radical derived from further reaction products. However, when the (*R*)-5,5'-bi-γ-T_3 dissolved in a mixture of methylcyclohexane and di-*t*-butyl peroxide was subjected to UV irradiation [77], a direct hydrogen abstraction by tert-butoxyl radical can avoid side reaction or formation of the other radical, and only the chromanoxyl radical has been detected by ESR as shown in Fig. 1.13.

Mechanisms for the reaction of bichromanyl dimers

The bichromanyl dimers 5,5'-bi-γ-T_3 and 5,5'-bi-γ-T derived from natural γ-vitamers can exist in two diastereomeric forms as a result of restricted rotation at the C-5 and C-5' biphenyl bond. In the reaction with alkaline $K_3Fe(CN)_6$, the pure *R*-diastereomer readily isomerized to *S*-diastereomer and *vice versa*, to give a mixture of *RS*-diastereomers. A possible mechanism involving radical formation, isomerization and tautomerization is exemplified in Fig. 1.14. The loss of a hydrogen atom may reduce intramolecular H-bonding and thus ease rotation at the 5,5'-biphenyl bond of the radicals. Two major products derived from the bichromanyl dimers were found to be dienone dimers existing in diastereomeric forms. Since the dienone group is relatively unstable, the dienone dimers can tautomerize slowly at room temperature to give a racemic mixture of (*RS*)-5,5'-bi-γ-T, and the process could be thermally accelerated.

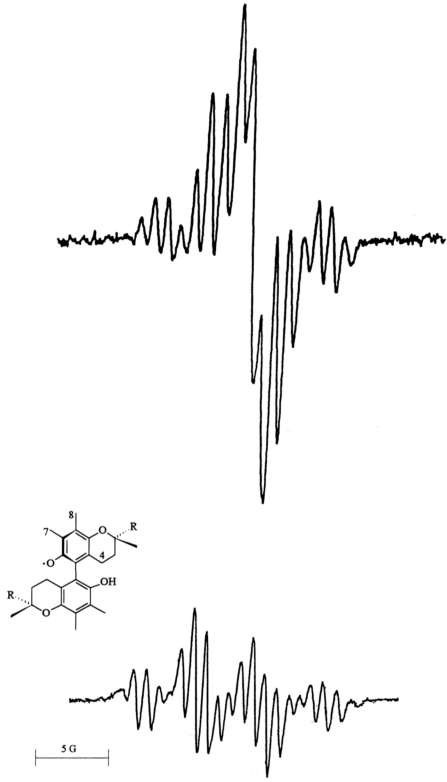


Fig. 1.13 ESR spectra recorded when (*R*)-5,5'-bi-γ-tocotrienyl dimer in hexane subjected to oxidation by $K_3Fe(CN)_6$ (top), and in methylcyclohexane mixed with di-*t*-butyl peroxide and irradiated by UV light at 300 K (bottom).

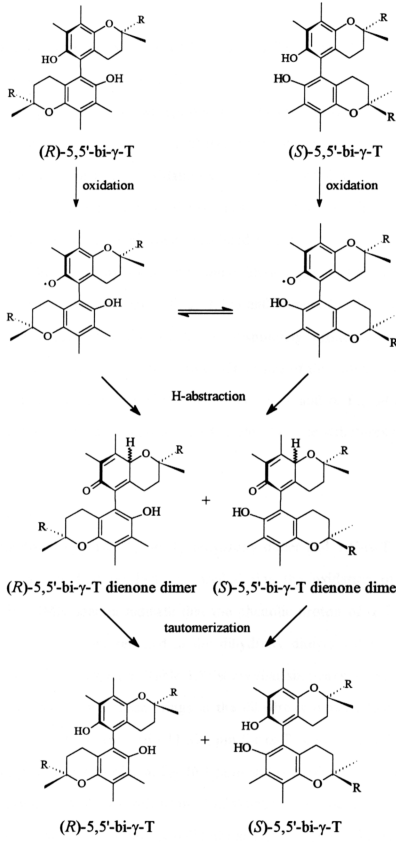


Fig. 1.14 Mechanisms for the reactions of (*R*)-5,5'-bi-γ-T and (*S*)-5,5'-bi-γ-T.

1.3.2 Oxidation Products of α-Tocotrienol and α-Tocopherol

The oxidation of α-T_3 and α-T by alkaline $K_3Fe(CN)_6$ gave rise to five major products including two trimers, two spirodienone dimers and one dihydroxy dimer. One minor dimer from α-T has also been characterized while other minor stereoisomers, quinones and products of further oxidation were not studied further. Some characteristic data are given in Table 1.6. Low resolution mass spectroscopic data of the dimers and trimers of α-T are consistent with their calculated molecular weights. ^1H and ^{13}C NMR spectral data for the chromanyl moieties of the oxidation products are given in Tables 1.7 and 1.8, respectively. All the dimers (Fig. 1.15) and trimers (p. 57) derived from α-T_3 have chromanyl structures similar to the corresponding products derived from α-T. Chemical shifts for the phytyl and isoprenoid side chains of the dimers and trimers are not significantly different from those of the monomeric α-T and α-T_3, differences in NMR spectral data for the chromanyl rings are used to elucidate the structures of the dimers and trimers.

Dihydroxy dimers

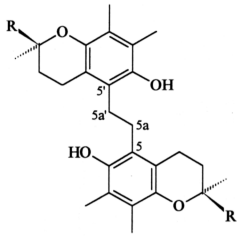
As monitored by TLC, 5,5'-α-T_3 dihydroxy dimer and 5,5'-α-T dihydroxy dimer were found to be the first product appeared during the oxidation of α-T_3 and α-T, respectively. ^1H NMR spectra indicate that the phenolic proton of α-T_3 (δ_H 4.34 ppm) and α-T (δ_H 4.21 ppm) are retained in the dihydroxy dimers but with chemical shifts at $\delta_H \approx 5.5$ ppm. ^{13}C NMR data (Table 1.8) show that six peaks in the downfield region account for two equivalent benzene rings in the dihydroxy dimers. Instead of resonance peaks for the C-5a methyl group (δ_C 11.32 ppm for α-T_3 and δ_C 11.35 ppm for α-T), a new resonance peak was recorded at δ_C 26.1 ppm and is attributed to the methylene C-5a of the dihydroxy dimers. As a result of the 5a,5a'-linkage, resonances for C-5 (δ_C 117.17 ppm for α-T_3 and δ_C 117.39 ppm for α-T) are shifted to δ_C 123.32 ppm (for 5,5'-α-T_3 dihydroxy dimer) and δ_C 122.83 ppm (for 5,5'-α-T dihydroxy dimer).

Table 1.6 Oxidation products* of α-T_3 and α-T

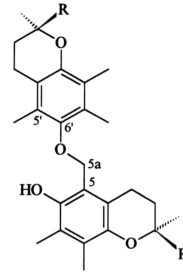
Compound	Yield (%)	TLC# R_f	m/z calculated	m/z recorded
α-Tocotrienol				
Spiroketal trimer	22	0.65	1268.97	1268
Ketal trimer	19	0.58	1268.97	1268
Spirodienone dimers	20	0.45	846.65	846
α-T_3 (unreacted)	11	0.33	424.33	424.2
Dihydroxy dimer	17	0.24	846.65	846
α-Tocopherol				
Spiroketal trimer	24	0.78	1287.11	1286
Ketal trimer	20	0.65	1287.11	1286
5*a*,*O*-α-T ether dimer	3	0.70	858.74	858
Spirodienone dimers	20	0.50	858.74	858
α-T (unreacted)	10	0.35	430.38	430
Dihydroxy dimer	18	0.27	858.74	858

* Structures of the dimers are shown in Fig. 1.15, trimers are shown in p. 57.

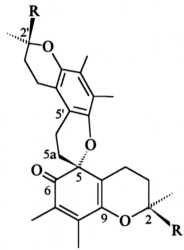
\# Solvent for silica gel TLC is 30% $CHCl_3$ in *n*-hexane.



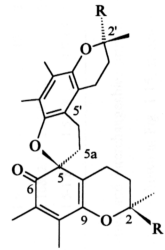
5,5'-α-T dihydroxy dimer



5*a*,*O*-α-T ether dimer



5*R*,5'-α-T spirodienone dimer



5*S*,5'-α-T spirodienone dimer

T_3 = Tocotrienol, R = 

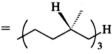
T = Tocopherol, R = 

Fig. 1.15 Structures of various dimers derived from α-T_3 and α-T.

Table 1.7 ^{1}H NMR spectral data of α-T_3, α-T, their dimers and trimers

Proton*	α-Tocotrienol					α-Tocopherol					
	α-T_3	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer	α-T	ether dimer	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer
6-OH	4.34	5.40	-	-	-	4.21	7.76	5.48	-	-	-
4-CH_2/4=CH**	2.69	2.7-2.8	2.2-2.6	-	5.2	2.60	2.65	2.7-2.8	2.2-2.6	-	5.17(t)**
4'&4"-CH_2	2.92	2.7-2.8	2.2-2.6	2.5-2.7	2.5-2.7	2.56	2.30&2.5	2.7-2.8	2.2-2.6	2.5-2.7	2.4-2.8
5a-CH_3/5a-CH_2#	2.19	2.7-2.8#	2.2-2.6#	2.5-2.7#	2.5-2.7	2.14	4.92#	2.7-2.8#	2.2-2.6#	2.5-2.7#	2.5-2.7#
5a'-CH_3/5a'-CH_2#	-	2.7-2.8#	2.2-2.6#	2.5-2.7#	2.5-2.7	-	2.24	2.7-2.8#	2.2-2.6#	2.5-2.7#	2.5-2.7#
5a"-CH_3	-	-	-	2.01	2.0	-	-	-	-	2.01	2.03
7a-CH_3	2.19	2.20	1.95	1.69	1.7	2.10	2.21	2.19	1.97	1.69	1.74
7a'&7a"-CH_3	-	2.20	2.13	2.14&2.24	2.15&2.25	-	2.17	2.19	2.12	2.15&2.25	2.25&2.30
8a-CH_3	2.19	2.15	1.84	1.6	1.6	2.10	2.12	2.13	1.84	1.59	1.66
8a'&8a"-CH_3	-	2.15	2.10	2.10& 2.18	2.10&2.20	-	2.08	2.13	2.09	2.11&2.21	2.16&2.25
3,3',3"-CH_2	1.8	1.7-1.9	1.5-1.8	1.5-1.8	1.5-1.8	1.7-1.9	1.7-1.9	1.7-1.9	1.5-1.8	1.5-1.8	1.5-1.8

* Some of the proton assignments are tentative and interchangeable, primed numbers are for dimers, primed and double-primed numbers are for trimers. Structures of dimers are shown in Fig. 1.15, structures of trimers are shown in p. 57. ** Vinylic proton in the trimers, t = triplet. # Methylene protons for the dimers or trimers.

Table 1.8 ^{13}C **NMR spectral data of α-T_3, α-T, their dimers and trimers***

Carbon	α-T_3	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer	α-T	ether dimer	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer
5	117.17	123.32	80.56 & 80.90	85.53	85.51	117.39	123.51	122.83	80.94 & 80.71	85.53	85.45
5'	-	123.32	114.67	115.14	115.06	-	116.15	122.83	114.85	115.12	115.08
5"	-	-	-	115.14	114.95	-	-	-	-	115.12	115.08
6	144.54	144.97	201.84 & 201.80	198.74	198.66	144.56	144.75	144.88	202.28 & 202.20	198.83	198.57
6'	-	144.97	145.70 & 145.62	145.73	145.63	-	147.70	144.88	145.74 & 145.64	145.71	145.59
6"	-	-	-	150.18	150.50	-	-	-	-	150.21	150.50
7	118.69	116.19	127.07 & 127.00	123.62	123.53	118.56	123.31	116.38	127.01 & 126.96	123.51	123.48
7'	-	116.19	115.60 & 115.49	129.82	129.33	-	114.76	116.38	115.70 & 115.52	129.68	129.29
7"	-	-	-	115.54	115.57	-	-	-	-	115.49	115.48
8	121.26	121.75	122.04	123.62	123.53	121.10	125.74	121.50	122.03	123.51	123.48
8'	-	121.75	114.90 & 115.00	122.04	122.00	-	117.77	121.50	115.06 & 115.00	121.92	121.88
8"	-	-	-	115.78	115.76	-	-	-	-	115.76	115.67
9	145.94	145.56	145.40 & 145.32	100.22	99.47	145.58	148.46	145.64	145.50 & 145.42	100.12	99.37
9'	-	145.56	144.56	144.74	144.70	-	147.33	145.64	144.63	144.74	144.64
9"	-	-	-	145.56	145.53	-	-	-	-	145.45	145.54
10	122.51	125.55	142.75	142.51	142.00	122.66	125.70	123.42	142.78	142.41	142.06
10'	-	125.55	123.26	122.34	122.32	-	127.65	123.42	123.28	122.24	122.21
10"	-	-	-	115.54	115.57	-	-	-	-	115.49	115.49

* Structures of dimers are shown in Fig. 1.15 and trimers are shown in p. 57. Some of the carbon assignments are tentative and interchangeable because 2D-NMR data are not available.

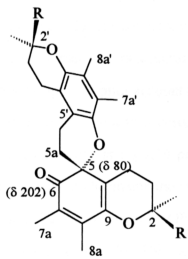
Table 1.8 (continued)

Carbon*	α-T_3	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer	α-T	ether dimer	dihydroxy dimer	spirodienone dimer (*R*)- & (*S*)-	spiroketal trimer	ketal trimer
2	74.20	74.30	75.47	75.80	75.96	74.56	74.94	74.65	75.73	77.12	76.13
2'	-	74.30	74.24	74.53	74.55	-	74.36	74.65	74.22 & 74.16	74.72	74.63
2"	-	-	-	74.18	74.24	-	-	-	-	74.30	74.36
2a	23.68	23.75	23.30 & 23.23	23.61	23.97	23.83	23.84	23.86	23.37 & 23.24	23.57	24.01
2a'	-	23.75	23.30 & 23.23	24.98	24.73	-	23.70	23.86	23.35 & 23.48	24.39	24.39
2a"	-	-	-	24.98	24.73	-	-	-	-	24.39	24.39
3	31.63	31.65	31.65	40.88	41.46	31.59	31.37	31.64	31.56	41.14	41.54
3'	-	31.65	30.95 & 30.80	31.18	31.31	-	31.27	31.64	30.93 & 30.84	31.18	31.27
3"	-	-	-	31.07	31.31	-	-	-	-	31.00	31.27
4 (-CH2-)	20.75	20.19	20.19	20.14	19.70	20.81	20.98	21.08	20.82 & 20.72	20.98	20.80
4 (=C<)	-	20.19	-	-	124.47	-	-	-	-	-	125.07
4' & 4"	-	-	21.01 & 20.90	20.14	24.06	-	20.68	21.08	21.09 & 20.97	20.98	20.66
5a	11.32	26.09	32.65	43.52	44.51	11.35	71.67(t)[#]	26.06	32.58	44.07	44.78
5a'	-	26.09	32.65	42.59	39.38	-	11.92	26.06	32.50	42.54	39.87
5a"	-	-	-	11.65	11.65	-	-	-	-	11.69	11.68
7a	11.79	12.12	13.99 & 13.90	13.98	13.98	12.27	11.86	12.19	13.82 & 13.79	14.32	14.02
7a'	-	12.12	11.82	12.00	12.00	-	13.05	12.19	11.80	12.09	12.04
7a"	-	-	-	11.85	11.85	-	-	-	-	11.69	11.68
8a	11.79	11.88	11.24	17.61	17.61	11.83	12.20	11.94	11.10	17.61	17.58
8a'	-	11.88	11.53	12.00	11.85	-	11.82	11.94	11.65	12.06	11.88
8a"	-	-	-	11.65	11.65	-	-	-	-	11.69	11.68

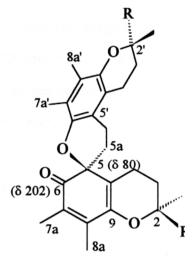
[#] t = triplet peak in OFR spectrum.

Spirodienone dimers

Another two major oxidation products of α-T_3 and α-T are spirodienone dimers existing in diastereomeric mixtures; however, resolution of these diastereomers has yet to be achieved by normal silica and reverse phase HPLC. These dimers have been characterized by ^1H and ^{13}C NMR spectroscopy to be 5*RS*,5'-α-T_3 spirodienone dimers and 5*RS*,5'-α-T spirodienone dimers. ^1H NMR data indicate that chemical shifts for 7a' and 8a' aromatic-methyl protons (δ_H 2.12 & 2.09 ppm) in one of the α-tocopheryl fragments are apparently unchanged as compared to those in α-T (δ_H 2.10 ppm), whereas the 7a- and 8a-methyl protons of the other α-tocopheryl fragment in the spirodienone dimers are shifted to higher field δ_H 1.97 and 1.85 ppm, respectively, due to the transformation to a dienone structure. The ^{13}C NMR data (Table 1.8) indicate that one chromanyl benzene ring remains intact in the spirodienone dimers, a resonance peak at δ_C 202 ppm is due the presence of an enone carbonyl (6-C=O) in the other chromanyl moiety. A resonance peak at δ_C 80 ppm is assigned to the ethereal carbon, i.e. C-5, it is proven to be a quaternary carbon by NMR DEPT experiments.



5*R*,5'-α-T spirodienone dimer



5*S*,5'-α-T spirodienone dimer

These spirodienone dimers are expected to be derived from the dihydroxy dimers through oxidation and ring closure. When pure dihydroxy dimers of α-T_3 and α-T were oxidized by alkaline $K_3Fe(CN)_6$, they were converted to a diastereomeric mixture of 5*RS*,5'-α-T_3 spirodienone dimers and 5*RS*,5'α-T spirodienone dimers, respectively. However, the cyclic ether bonds in the spirodienone dimers are labile to acid, acidification of the spirodienone dimers with dilute sulphuric acid readily resulted ring opening and converted the compounds back to dihydroxy dimers.

Radicals generated from α-T spirodienone dimers

Oxidation of the 5*RS*,5'-α-T spirodienone dimers was also carried out by alkaline $K_3Fe(CN)_6$, radical generated was detected by ESR and the spectrum is shown in Fig. 1.16. Its hyperfine coupling constants worked out by simulation are a_{CH_3} 3.80 G and a_{CH_2} 4.00 G. The structure of a chromanoxyl radical is proposed as shown in Fig. 1.16, it is relatively stable with a half-life of about one hour.

New α-T dimer

One minor product (yield 3%) obtained from the oxidation of α-T has been isolated by silica gel column chromatography. Spectroscopic data suggest that the product is 5*a*,*O*-α-T ether dimer and its structure and ^1H NMR spectrum are shown in Fig. 1.17. A singlet peak at δ_H 4.92 ppm accounts for the ethereal methylene protons at C-5a, five aromatic methyl groups from two chromanyl moieties resonate at δ_H 2.0-2.3 ppm, one phenolic proton resonance at δ_H 7.76 ppm. The ^{13}C NMR data given in Table 1.8 show that two non-equivalent chromanyl moieties present in the dimer; the carbon peak appears at δ_C 71.67 ppm has been shown by OFR NMR to be a triplet indicating it is a methylene carbon and is assigned to C-5a. This compound is a new dimer which is expected to be formed by coupling of a chromanoxyl radical and a 5a-benzylic radical derived from the α-T (Fig. 1.18).

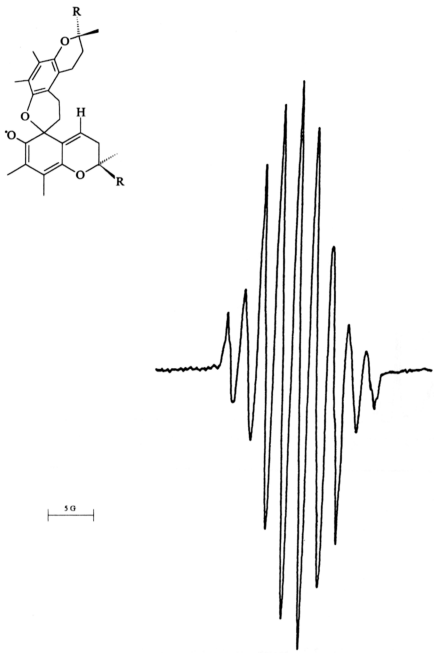


Fig. 1.16 ESR spectrum for the radicals generated from α-T spirodienone dimer.

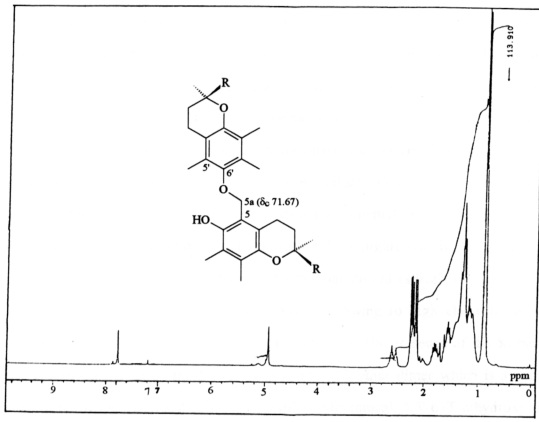


Fig. 1.17 The ^{1}H NMR spectrum of 5*a*,*O*-α-T ether dimer.

Possible mechanism for the dimerization of α-vitamers

Oxidation of α-T_3 and α-T by alkaline potassium ferricyanide produced various types of dimers and trimers. Possible mechanistic pathways for the dimerization involving various radical intermediates are exemplified in Fig. 1.18. Like α-T, oxidation of α-T_3 by alkaline potassium ferricyanide will first give rise to chromanoxyl radicals, these radicals are relatively stable and have been detected by ESR as shown in Fig. 1.1. These oxygen radicals are resonance-stabilized with the unpaired electron delocalized at carbons C-5, C-7 or C-9 as shown in Fig. 1.2; dimerization through cross-coupling of these carbon-centred radicals is unfavourable as a result of steric effects. Other carbon-centred radicals may also be formed by hydrogen abstraction from the 5-methyl or 7-methyl groups, these benzylic radicals are more exposed and reaction of these highly reactive benzylic radicals would be the dominating reaction. Among the carbon-centred radicals of α-T_3 and α-T, 5a-benzylic radical is expected to be the major one owing to high electron density at position C-5 resulted by the effect of the chromanyl ring. The presence of the 5*a*-benzylic radical is also supported by the formation of 5*a*-*O*-α-T ether dimer which is a coupling product of α-tocopheroxyl radical with the 5*a*-benzylic radical. 5,5'-α-T dihydroxy dimer can be readily formed by coupling of two 5*a*-benzylic radicals. Further oxidation of the dihydroxy dimer may give rise to chromanoxyl radicals and then lead to spirodienone dimers *via* a ring closure process. The cyclization of the dihydroxy dimer is non-stereospecific and therefore will produce diastereomeric 5*RS*,5'-α-T spirodienone dimers in approximately equal amount as determined by ^{13}C NMR. The 5*RS*,5'-α-T spirodienone dimers are stable in neutral and basic media, but acidification of the compounds caused opening of the pyran ring and afforded the 5,5'-α-T dihydroxy dimer.

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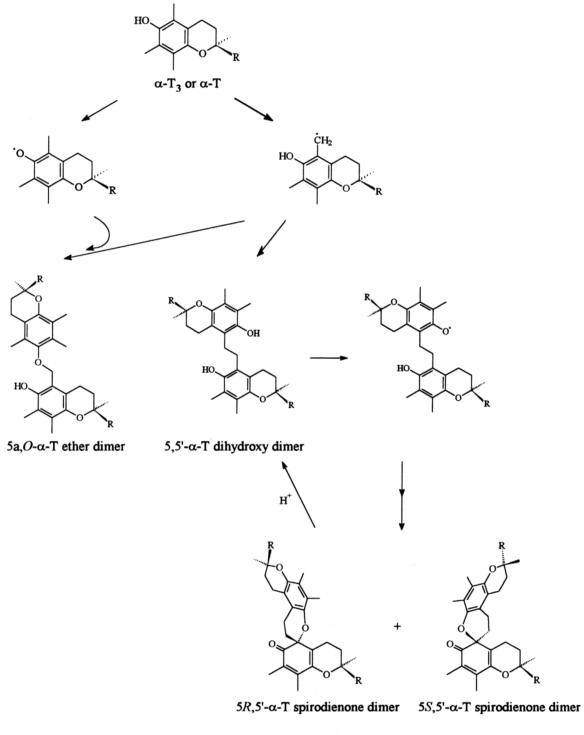
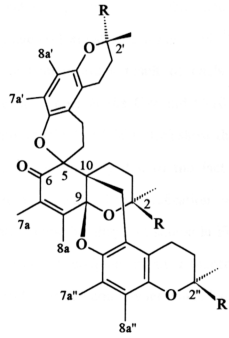


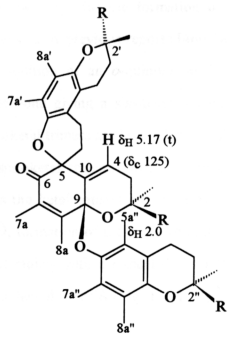
Fig. 1.18 Possible mechanistic pathways for the dimerization of α-T_3 or α-T.

Trimers of α-Vitamers

Two types of trimers obtained as major oxidation products of α-T_3 as well as α-T have been purified by silica gel column chromatography and some of their characteristic data are shown in Table 1.6. Mass spectroscopic data indicate that molecular weights of the trimers are in agreement with the calculated values. These trimers have some similarities in their NMR data which are also comparable with those of *5RS,5'*-α-T spirodienone dimers (Tables 1.7 and 1.8), suggesting that the skeleton of the spirodienone dimer is part of the trimers. Among the trimeric structures of α-T established by other workers (Fig. 1.6) [697], only the spiroketal trimer but not the spiroether trimer can be accounted for by the present ^{1}H and ^{13}C NMR data for one of the trimers isolated. However, a new structure for the other trimer is proposed in this study. Although these trimers may exist in diastereomeric forms with a chiral centre at C-5, resolution of the possible diastereomers by HPLC have not been achieved, otherwise only the pure diastereomers have been isolated.



α-T spiroketal trimer



α-T ketal trimer

^{1}H NMR spectral data (Table 1.7) show that chemical shifts for 7a' and 8a' methyl protons in the trimers are only slightly different from that in 5*RS*,5'-α-T spirodienone dimers, whereas chemical shifts for 7a and 8a methyl protons are remarkably shifted to upfield δ_H 1.7 and 1.6 ppm, as compared to δ_H 1.95 and 1.84 ppm for the spirodienone dimer. Another two singlet peaks in the aromatic methyl region are due to the 7a" and 8a" methyl protons of a third unit of α-tocopheryl fragment. A triplet peak at δ_H 5.17 ppm is only recorded for the ketal trimer, it is assigned to the olefinic proton at C-4. Considering their ^{13}C NMR data, some characteristic peaks can be easily elucidated; for instance, resonance at δ_C 198 ppm is attributed to a ketone group 6-C=O; three peaks in the region of δ_C 74-77 ppm account for the C-2, C-2' and C-2" carbons, indicating three units of α-T or α-T_3 incorporated in the trimers are non-equivalent. The resonance peak at δ_C 85 ppm is assigned to the quaternary carbon C-5 with a C-O bond; chemical shifts at δ_C 100 ppm and δ_C 99 ppm for the spiroketal trimer and ketal trimer, respectively, are assigned to C-9 carbon bearing two C-O bonds. A carbon peak for α-T ketal trimer at δ_C 125 ppm is assigned to the C-4, this carbon is proven to be protonated olefinic carbon (=CH-) by DEPT NMR experiments. Possible mechanistic pathways for the formation of trimers of α-T_3 and α-T are shown in Fig. 1.19. As suggested by previous reports [466,580,699], spiroketal trimers are a result of Diels-Alder addition of an *o*-quinone methide to a spirodienone dimer at the C-9 and C-10 double bond giving a six-membered ring. The chromatographic data (Table 1.6) show that spiroketal trimers are less polar than the ketal trimers; this is possibly due to the fact that spiroketal trimers have a more enclosed structure as a result of the cyclization, whereas the ketal trimers are more exposed for chromatographic bonding. As shown in Fig. 1.19, formation of the α-T_3 ketal trimer may involve a radical coupling of an α-tocotrienoxyl radical with a radical derived from the 5*RS*,5'-α-T_3 spirodienone dimer which has been detected by ESR (Fig. 1.17).

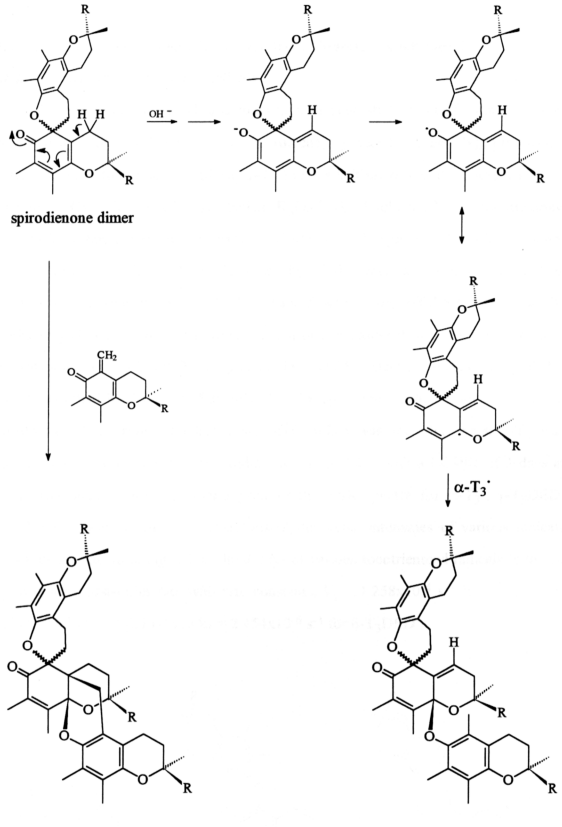
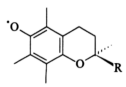


Fig. 1.19 Formation of the trimers of α-T_3 and α-T.

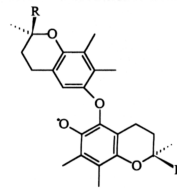
1.3.3 ESR Study on Radicals Generated from Vitamin E Compounds

Stability of various tocotrienoxyl radicals

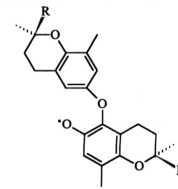
The relative stability of chromanoxyl radicals generated from α-T_3 and γ-T_3 were determined by ESR. Oxidation of α-T_3 (in toluene) by alkaline $K_3Fe(CN)_6$ produced a relatively stable α-tocotrienoxyl radical (α-$T_3^{\bullet}$) with a half-life of about 1.5 hours at room temperature. Oxidation of γ-T_3 by alkaline $K_3Fe(CN)_6$ should produce γ-tocotrienoxyl radical (γ-$T_3^{\bullet}$), but under this experimental condition ESR spectrum for γ-$T_3^{\bullet}$ was not detected; however, the radical detected by ESR was a chromanoxyl radical of γ-dichromanyl ether dimer (γ-T_3DED$^{\bullet}$). This is because the γ-$T_3^{\bullet}$ radical can rapidly dimerize to yield mainly γ-T_3DED which is then further oxidized to chromanoxyl radical. The oxidation of pure γ-T_3DED also gave an identical ESR spectrum which was stable for up to 25 days (half-life 5.6 days). Similarly, the ESR spectrum for the chromanoxyl radical of δ-tocotrienol dichromanyl ether dimer (δ-T_3DED$^{\bullet}$) was recorded when δ-T_3 was oxidized by alkaline $K_3Fe(CN)_6$, this radical was also stable with a half-life of 3 days at 300 K. The intensity for the central peak of the ESR spectra for α-$T_3^{\bullet}$, γ-T_3DED$^{\bullet}$ and δ-T_3DED$^{\bullet}$ were monitored, logarithms of the signal intensities of various radicals versus time are shown in Fig. 1.20. The decays of various tocotrienoxyl radicals followed an approximate first-order rate with rate constants, $k_1 = 1.258 \times 10^{-4}$ s^{-1} for α-$T_3^{\bullet}$, $k_1 = 1.424 \times 10^{-6}$ s^{-1} for γ-T_3DED$^{\bullet}$ and $k_1 = 2.454 \times 10^{-6}$ s^{-1} for δ-T_3DED$^{\bullet}$.



α-$T_3^{\bullet}$



γ-T_3DED$^{\bullet}$



δ-T_3DED$^{\bullet}$

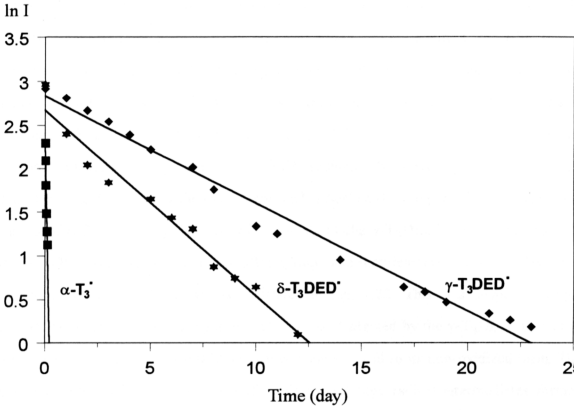
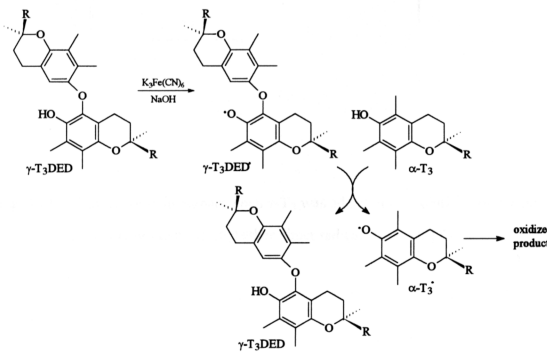


Fig. 1.20 Logarithm of the intensities of the central peak of the decaying ESR spectra. α-$T_3^{\bullet}$ = α-tocotrienoxyl radical, γ-T_3DED$^{\bullet}$ = γ-tocotrienol dichromanyl ether dimer radical, δ-T_3DED$^{\bullet}$ = δ-tocotrienol dichromanyl ether dimer radical.

Regeneration of vitamin E

When δ-tocotrienol dichromanyl ether dimer (δ-T_3DED) was oxidized by alkaline $K_3Fe(CN)_6$, a stable chromanoxyl radical (i.e. δ-T_3DED$^{\bullet}$) was detected by ESR; its signals disappeared immediately when γ-T_3 was added into the solution, and ESR signals for γ-tocotrienoxyl radical (γ-$T_3^{\bullet}$) were appeared and stable for about twenty minutes (Fig. 1.21). Similarly, oxidation of γ-T_3DED by alkaline $K_3Fe(CN)_6$ also produced a stable chromanoxyl radical γ-T_3DED$^{\bullet}$ as detected by ESR. When α-T_3 was added into the solution containing the generated γ-T_3DED$^{\bullet}$ radicals, the ESR signals of γ-T_3DED$^{\bullet}$ disappeared and signals for the α-tocotrienoxyl radical (α-$T_3^{\bullet}$) appeared immediately; the signals of α-$T_3^{\bullet}$ gradually decayed while signals of the γ-T_3DED$^{\bullet}$ reappeared and only the γ-T_3DED$^{\bullet}$ radicals were detected after 2 hours and they remained for a few days. The sequence of these ESR changes is illustrated in Fig. 1.22. This is a simple hydrogen transfer in which the phenolic hydrogen of α-T_3 is abstracted by the γ-T_3DED$^{\bullet}$ radical to produce α-$T_3^{\bullet}$ while the γ-T_3DED$^{\bullet}$ radical is being reduced to its non-oxidized form. The regenerated γ-T_3DED may play a useful role to scavenge radical intermediates formed when α-$T_3^{\bullet}$ radicals decay.



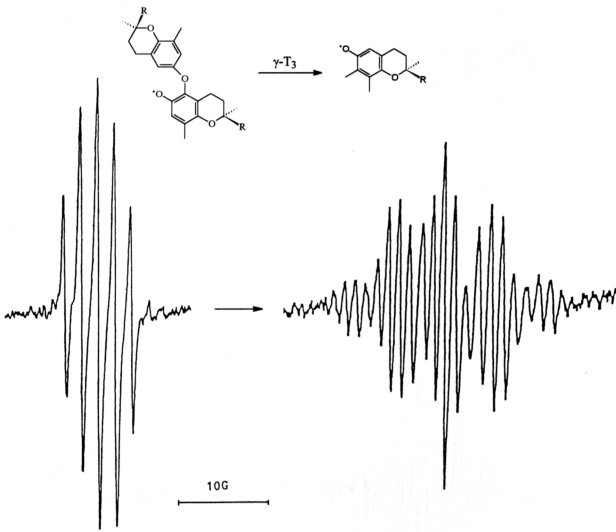


Fig. 1.21 Changes of ESR signals when γ-T_3 was added into a solution containing δ-tocotrienol dichromanyl ether dimer radicals (δ-T_3DED•).

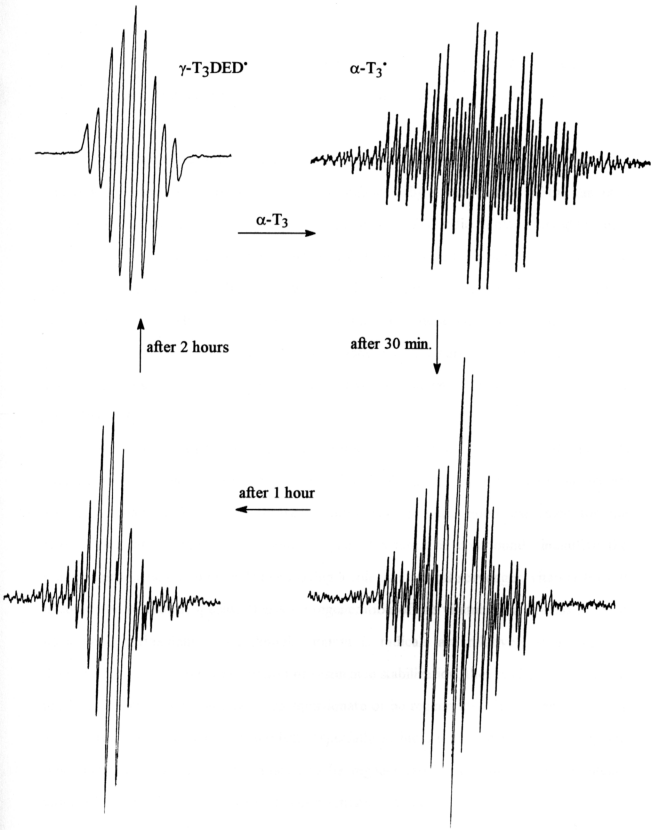


Fig. 1.22 Changes of ESR signals when α-T_3 was added into a solution containing γ-tocotrienol dichromanyl ether dimer radicals (γ-T_3DED•).

Fig. 1.22 shows that although many monomeric radicals can be generated, these are much less stable than the dimer radicals which can (in the absence of O_2) remain for long periods of time.

Oxidation of various vitamin E components by alkaline $K_3Fe(CN)_6$ will first form monomeric chromanoxyl radicals which are stabilized by delocalization of the unpaired electron around the aromatic ring and the para-oxygen. The radicals may combine to give a mixture of non-radical products particularly as dimers and further reaction leads to trimers. Oxidation of the dimers possessing the phenolic group can also give rise to radicals which are relatively more stable than the monomeric chromanoxyl radicals. For instance, the γ-T_3DED$^{\bullet}$ radical is much more stable than the parent radical generated from the γ-T_3, this may partly explain the high antioxidant capacity of γ-vitamers [201,294,318].

It is now known that the synergistic effect of vitamin C in the action of vitamin E may be based on the fact that the vitamin E radical can be regenerated by the water-soluble vitamin [435,460,495]. Present experiments provide good examples for the regeneration of a vitamin E by another type of vitamin E compounds including the oxidized products, e.g. dimers. While playing a role as a radical scavenger, vitamin E itself is converted to radical species, therefore regeneration of the vitamin E is crucial for it to be a good antioxidant. Even though vitamin E radicals are relatively stable due to delocalization of the unpaired electron or resonance stabilization, they lead to other radical products if they do not dimerize, disproportionate or be regenerated for use again. This is an important feature of the antioxidant, especially in biological systems where long lag times may be required for the radicals to be regenerated by a chain process involving vitamin C, glutathione or other reductive-enzymatic system.