Oxidation of *d*-α-Tocopherol in Aqueous Solution. Formation of Colored Products

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An aqueous solution of *d*-α-tocopherol solubilized with sodium deoxycholate became colored on standing, probably due to air oxidation. 5-Formyl-7,8-dimethyltocol (1) and 7-formyl-5,8-dimethyltocol (2) were isolated from the solution and concluded to be responsible for the coloration. In oxidation of aqueous solutions of *d*-α-tocopherol, the time course of formation of products was followed by analytical HPLC. The main oxidation product was 1 in solutions solubilized with sodium deoxycholate and sodium cholate, whereas α-tocoquinone was the main product in those solubilized with 2,6-di-O-methyl-β-cyclodextrin.

Key words *d*-α-tocopherol; 5-formyl-7,8-dimethyltocol; 7-formyl-5,8-dimethyltocol; α-tocoquinone; sodium deoxycholate; sodium cholate

d-α-Tocopherol (vitamin E, α-Toc) is a biological antioxidant that is currently receiving attention concerning its efficacy in preventing and reducing oxidative stress.[1—4] However, when rapid onset of action is required *via* parenteral administration, significant problems arise from its practical insolubility in water. In an effort to develop water-soluble drugs related to α-Toc, we have prepared several water-soluble derivatives.[5] Among these derivatives, *d*-α-tocopheryl *N*,*N*,-dimethylaminoacetate is a potential candidate as a parenteral prodrug for the systemic liver specific delivery of α-Toc.[5,6]

Another way to solubilize α-Toc is by the use of surfactants. Increased solubility and bioavailability can be expected by solubilizing α-Toc with deoxycholic acid and related steroids. In the course of this study, we were confronted by a problem of coloration of the solutions on standing, probably due to autooxidation of α-Toc.

In order to clarify this phenomenon, the process of oxidation of α-Toc by oxygen was monitored in water solutions of a surfactant by HPLC with a multi-wavelength UV-visible detector. Among many compounds present in the solutions, two products with absorption at visible wavelengths were separated. They were isolated and purified by preparative chromatography and characterized. They were concluded to be responsible for the coloration.

Formation of oxidized products was studied in solutions of α-Toc solubilized with sodium deoxycholate (DOC), sodium cholate (CO), and 2,6-di-*O*-methyl-β-cyclodextrin (DMCD). The rates of oxidation and the yields of the products were greatly dependent on the solubilizing agents and other conditions. The present paper describes these results in detail.

Results and Discussion

Oxidation Products with Absorption at Visible Wavelengths α-Toc (5×10^{-3} M) was dissolved in 10% aqueous DOC and kept for 24 h at 80 °C under occasional oxygen bubbling. The multi-wavelength HPLC chromatogram shown in Fig. 1 was obtained by injecting a sample of the solution, which indicated that a number of products were formed. The chromatogram had two separate peaks with absorptions in the visible region at retention times of 20.6 min (A) and 19.9 min (B), along with several peaks absorbing in the UV region. Fig. 1 also shows chromatograms monitored at 267, 292 and 386 nm. One of the UV-absorbing peaks was assigned to α-tocoquinone (α-TQ) by comparison with authentic substance. Absorption spectra corresponding to peaks A and B shown in Fig. 2 had absorption maxima at 384 nm and 386 nm, respectively. No other product had visible absorption.

The reaction mixture containing visible-absorbing substances was extracted three times with hexane and the extract was submitted to preparative thin-layer chromatography (TLC). Six spots were present at *Rf* values of 0.25, 0.34, 0.41(α-TQ), 0.50(A), 0.59(B), and 0.72. The spots for A (*Rf* 0.50) and B (*Rf* 0.59) were separated and extracted with ether. The ether solutions were evaporated and the residues were further purified by preparative HPLC. The spectral data of the isolated compounds are listed in Table 1. From the

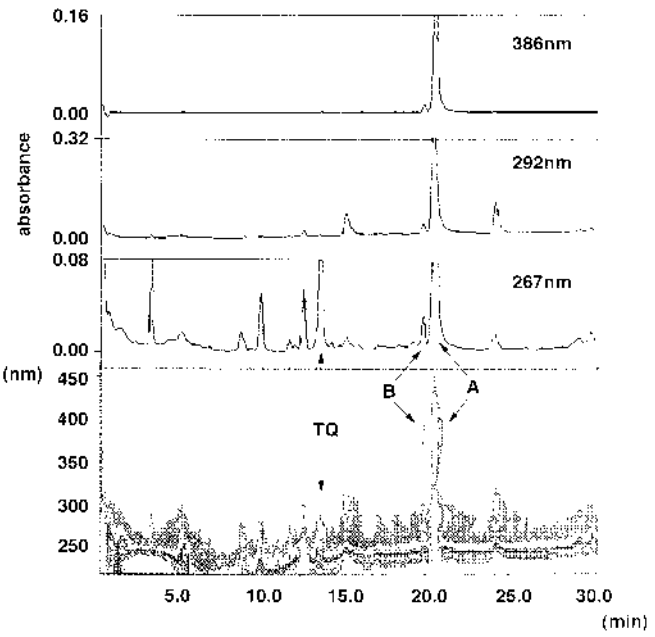

Fig. 1. Chromatograms of Oxidized Products in Aqueous Solution of α-Toc Solubilized with DOC

Contour diagram and chromatograms monitored at 386, 292 and 267 nm of reaction products of α-Toc in aqueous DOC with O_2 at 80 °C for 24 h.

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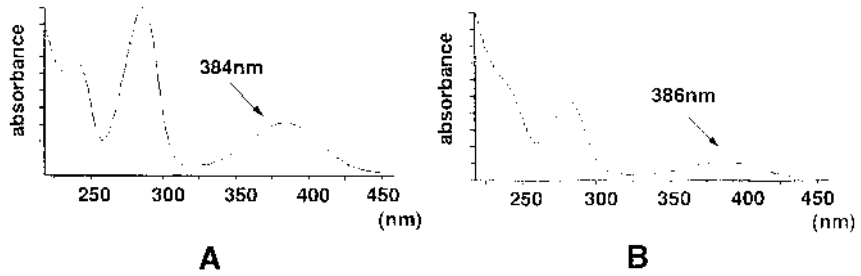
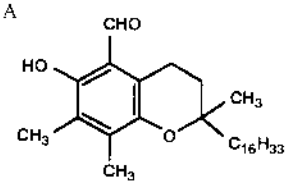


Fig. 2. Absorption Spectra of Peaks A and B

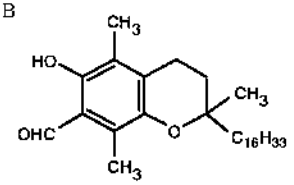
Table 1. Spectral Data of Compounds A and B

A



5-Formyl-7,8-dimethyltocol

B



7-Formyl-5,8-dimethyltocol

	A	B
UV λ_{max}^{EtOH} nm (ε)	239 (8786), 286 (13013), 388 (4516)	240 (4769), 282 (5412), 392 (1398)
IR (neat) cm^{-1}	2928 (CH_3), 1635 (C=O), 1462, 1379, 1301, 1098 (C–O–C)	2926 (CH_3) 1639 (C=O), 1462, 1377, 1300, 1055 (C–O–C)
MS (EI) *m/z* (*rel. int.*)	444 (M^+ 100), 426 (12), 243 (10), 220 (34), 217 (38), 201 (66), 191 (29), 179 (34), 151 (25)	444 (M^+ 100), 346 (10), 219 (16), 191 (6), 178 (42), 151 (16)
MS (FAB(PSO)) *m/z*	445 (M+1)	
^{1}H-NMR ($CDCl_3$) δ	0.83—0.87 (m, 12H), 1.06—1.65 (m, 24H), 1.73—1.85 (m, 2H, 3-CH_2), 2.15 (s, 3H, Ar-CH_3), 2.17 (s, 3H, Ar-CH_3), 3.03 (t, 2H, 4-CH_2, J=6.6 Hz), 10.19 (s, 1H, CHO), 12.10 (s, 1H, OH)	0.83—0.87 (m, 12H), 1.02—1.54 (m, 24H), 1.78—1.85 (m, 2H, 3-CH_2), 2.11 (s, 3H, Ar-CH_3), 2.41 (s, 3H, Ar-CH_3), 2.68 (t, 2H, 4-CH_2, J=7.0 Hz), 10.29 (s, 1H, CHO), 11.81 (s, 1H, OH)
^{13}C-NMR ($CDCl_3$) δ	11.01 (CH_3), 13.17 (CH_3), 18.43 (CH_2), 19.65 (CH_3), 19.73 (CH_3), 20.93 (CH_2), 22.61 (CH_3), 22.71 (CH_3), 23.63 (CH_3), 24.44 (CH_2), 24.80 (CH_2), 27.98 (CH), 30.79 (CH_2), 32.66 (CH), 32.80 (CH), 37.29 (CH_2), 37.39 (CH_2), 37.42 (CH_2), 37.45 (CH_2), 39.38 (CH_2), 39.59 (CH_2), 75.16 (C), 114.51 (C), 117.55 (C), 124.23 (C), 138.41 (C), 144.04 (C), 155.82 (C), 193.97 (CHO)	9.34 (CH_3), 10.13 (CH_3), 19.62 (CH_3), 19.72 (CH_3), 20.98 (CH_2), 21.84 (CH_2), 22.30 (CH_3), 22.68 (CH_3), 23.81 (CH_3), 24.42 (CH_2), 24.77 (CH_2), 27.96 (CH), 31.07 (CH_2), 32.66 (CH), 32.78 (CH), 37.27 (CH_2), 37.40 (CH_2), 39.36 (CH_2), 39.64 (CH_2), 75.19 (C), 116.84 (C), 121.60 (C), 125.40 (C), 132.11 (C), 144.10 (C), 154.09 (C), 195.38 (CHO)

data, compounds A and B were identified as 5-formyl-7,8-dimethyltocol (**1**) and 7- formyl-5,8-dimethyltocol (**2**), respectively.[7,8] Purification of the TLC spot at *Rf* 0.41 gave α-TQ.

There are three reports that have described **1** and/or **2** in the literature. Ishikawa[8] showed that α-Toc gave these compounds by the reaction with trimethylamine *N*-oxide at 180 °C in liquid paraffin under N_2 for 2 h. Suarna and coworkers[9] reported that oxidation of α-Toc with *tert*-butylhydroperoxide afforded **1** in ethanolic chloroform at 60 °C for 3 h. Molnar and Koswig[10] reported that **1** was formed by γ-irradiation of α-Toc in chloroform. The present results are the first to show that the methyl groups of α-Toc are oxidized to a formyl group under very mild conditions such as air oxidation in water.

Determination of α-Toc and the Oxidation Products α-Toc was dissolved in 10% DOC, CO or DMCD aqueous solutions at 5×10^{-3} M. The solutions were incubated in a thermostated bath under bubbling of oxygen. Samples were withdrawn at predetermined intervals and α-Toc, α-TQ and **1** were determined by analytical HPLC. Only a trace amount of **2** was formed under the conditions of the present study.

HPLC determination was established for α-Toc, α-TQ and **1**. Calibration curves between the peak areas and the concentrations were obtained from the standard solutions of known concentrations. They were linear in the ranges of 2.75×10^{-5}—4.26×10^{-3} M for α-Toc, 5.0×10^{-5}—1.0×10^{-3} M for **1** and 1.0×10^{-5}—1.0×10^{-3} M for α-TQ .

Effects of Reaction Temperature The oxidation reaction was carried out at 80 °C, 60 °C, and 40 °C using DOC solution at 5×10^{-3} M . Figure 3 shows the consumption of α-Toc, and the formation of **1** and α-TQ at various temperatures. The oxidation reaction became faster at higher temperature. The amount of **1** formed was about seven times more than that of α-TQ at any temperature. The change in the reaction temperature did not significantly affect the ratio of the two products.

Effects of Solubilizing Agents and pH The reaction was followed in solutions solubilized by DOC, CO, or DMCD. Though the rates of consumption of α-Toc were almost the same both in 10% DOC and in 10% CO solutions, the ratio of the product was different, as shown in Fig. 4. In both solutions, **1** was the main oxidation product, whereas α-TQ, a well-known oxidation product of α-Toc, was formed in only trace amounts in CO solution. The pH's of 10% DOC

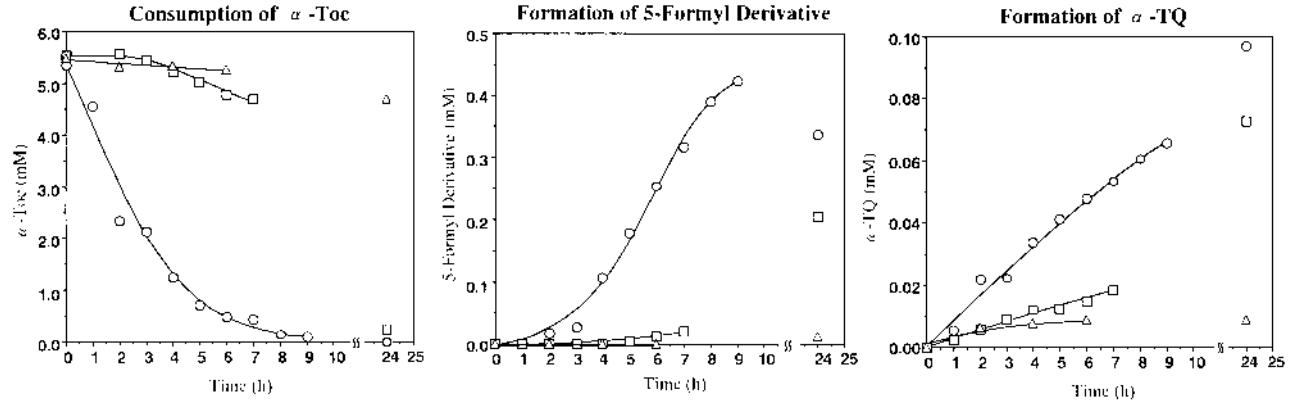


Fig. 3. Consumption of α-Toc, and Formation of **1** and α-TQ in DOC Solution

Initial concentration of α-Toc, 5×10^{-3} M. Reaction temperature, △, 40 °C; □, 60 °C; ○, 80 °C.

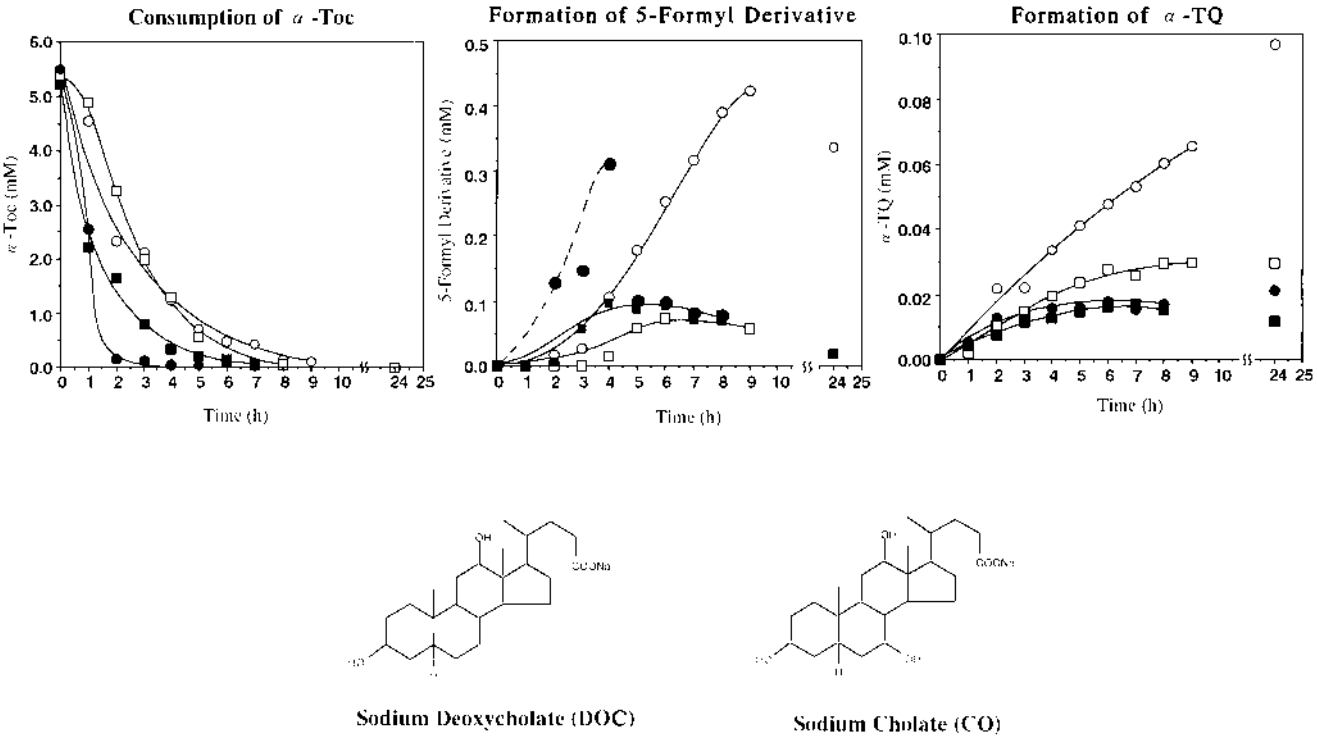


Fig. 4. Consumption of α-Toc, and Formation of **1** and α-TQ in DOC and CO Solution

Initial concentration of α-Toc, 5×10^{-3} M. Reaction temperature, 80 °C. Solubilizing agents and pH of the solution, ○, DOC, 8.3; □, CO, 8.3; ●, DOC, 10.0; ■, CO, 10.0.

and 10% CO solutions were 8.3. When pH was raised to 10 in DOC and CO solutions, the rates of oxidation of α-Toc and formation of **1** were enhanced and **1** formed at an early stage disappeared later. The results suggest that dissociation of the proton from the phenol group of α-Toc should be the predominant path for the formation of **1**, and that **1** once formed, undergoes further oxidation. The yield of α-TQ was reduced in these solutions, which shows that the reaction paths toward **1** and α-TQ were different processes.

The reaction was carried out at 45 °C with a DMCD solubilized solution, since DMCD becomes insoluble at higher temperatures. In 15% DMCD solution (pH, 3.2), **1** was not formed in a detectable amount and much more α-TQ was produced than in DOC and CO solutions, as shown in Fig. 5. In order to investigate the effect of pH in DMCD solution on formation of **1**, the pH was raised to a pH comparable to DOC and CO solution. The amount of α-TQ formed was increased in DMCD solution at pH 8.1. The formation of **1** was also unobservable in the solution at elevated pH. The difference in the products in DOC and DMCD solutions may be due to the different inclusion properties toward α-Toc.

Isotope Effect The reactions of α-Toc with oxygen were examined in a deuterium oxide (D_2O) solution of DOC at 80 °C and compared with those in H_2O solution. The results are summarized in Table 2. The yields of **1** in D_2O were almost two fifths of those in H_2O 4 h after the initiation of the reaction. This suggests that hydrogen abstraction from the hydroxyl group of α-Toc is involved in the formation of **1**.

Implications for the Reaction Mechanism Though many chemical studies have been reported on α-Toc in water,[11—19] most were concerned with its antioxidant activity and payed little attention to the oxidation products. Most re-

ports dealing with the products regarded α-TQ as the major product.

The present study showed that oxidation of α-Toc by oxygen in aqueous DOC solution gave **1** as the main product rather than α-TQ. The acceleration of the reaction at high pH, and the deuterium isotope effect suggest that deprotonation or dehydrogenation of the OH group at the C-6 position were involved as a rate determining step. (Chart 1) The anionic species of α-Toc (**3**) may be rapidly oxidized by oxygen to form the dehydrogenated species, a phenoxyradical (**4**). As shown in Chart 1, we assume two paths for transformation of the radical (**4**), one of which leads to **1** and the

Table 2. Consumption of α-Toc, and Formation of **1** and α-TQ in DOC Solutions in H_2O and D_2O

Time (h)	α-Toc (mmol/l)		α-Toc (%)		α-TQ (mmol/l)		5-Formyl deriv. (mmol/l)	
	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
0	3.45	3.66	100.0	100.0	0.00	0.00	0.00	0.00
2	2.30	2.65	65.9	71.8	0.032	0.019	0.027	0.013
4	1.06	1.68	29.1	44.9	0.051	0.032	0.098	0.039
24	0.09	0.00	0.5	0.0	0.076	0.051	0.153	0.109

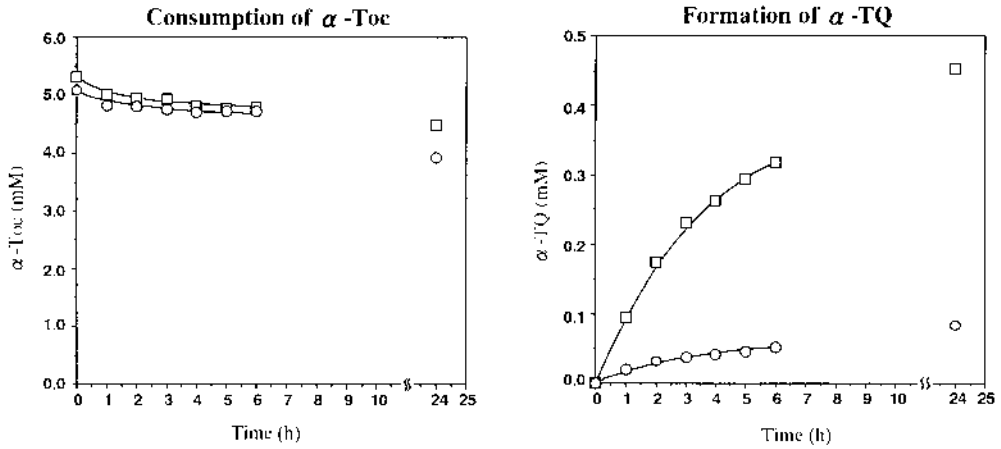


Fig. 5. Consumption of α-Toc, and Formation of α-TQ in DMCD Solution

Initial concentration of α-Toc, 5×10^{-3} M. Reaction temperature, 45 °C. pH of the solution, ○, 3.2; □, 8.1.

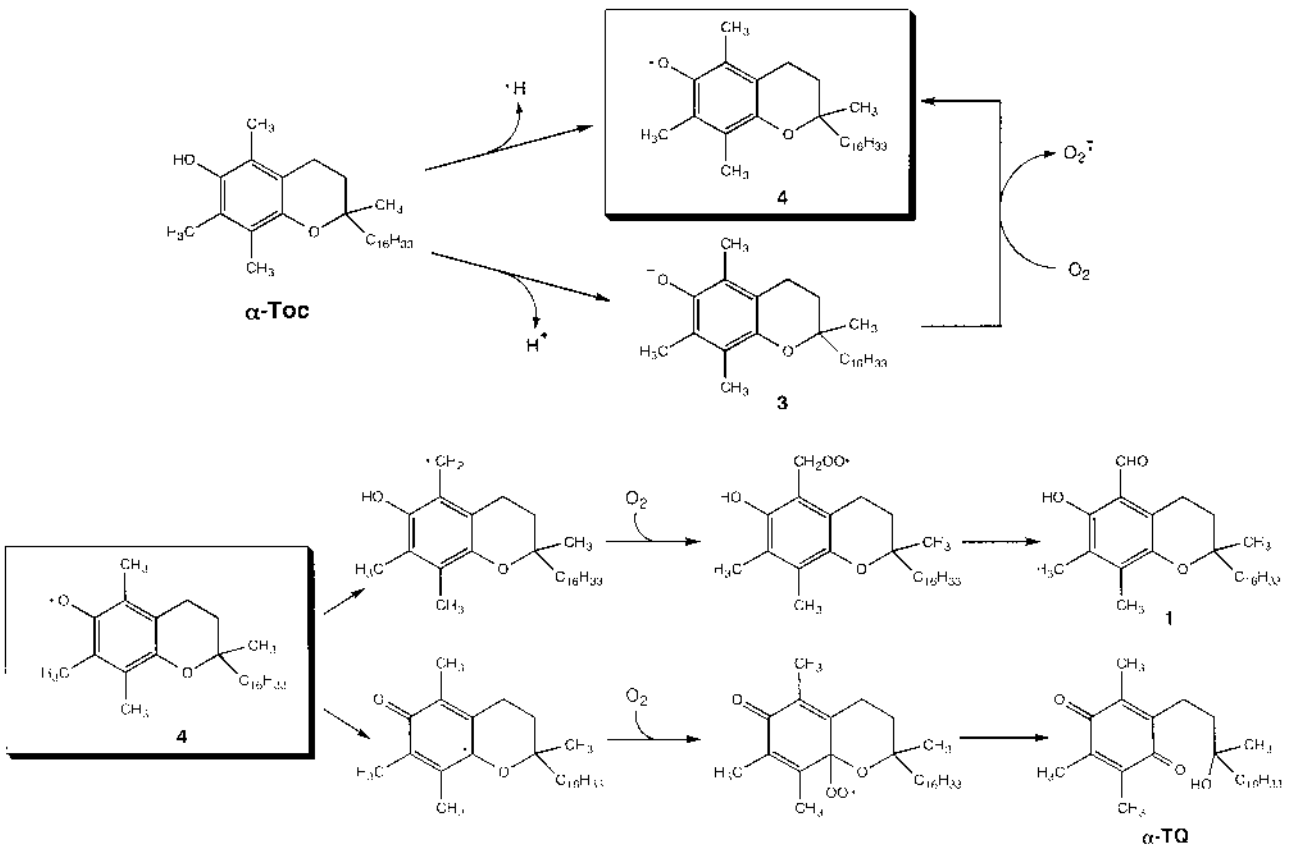


Chart 1

other to α-TQ. To form α-TQ, **4** must undergo oxygen addition at the 8a position and be followed by ring opening. DOC might protect the 8a position of α-Toc from oxygen oxidation by inclusion and **4** might adopt an alternative pathway, in which a hydrogen is abstracted from the methyl group at C-5 and oxygen oxidation at the group leads to **1**. The mode of inclusion of the solubilizing agents should affect the path and hence the oxidation products. Studies are in progress to solve this problem.

Experimental

Procedure α-Toc was dissolved in 10% DOC, CO or DMCD aqueous solutions at a concentration of 5×10^{-3} M. The solution was filtered with a membrane filter and the filtrate was kept in the dark in a bath thermostated at 40, 45, 60, or 80 °C. Oxygen was bubbled into the solution for 3 min every hour. Samples were withdrawn at intervals and a 50 μl volume was injected into the HPLC equipped with a multi-wavelength UV-visible detector.

Materials α-Toc was purchased from Sigma Chemical Co. (MO, U.S.A.), and purified by silica gel column chromatography. α-TQ was obtained from ICN Biochemicals (Cleveland OH, U.S.A.). Biochemical grade of DOC, CO, DMCD, acetonitrile for HPLC, diethyl ether and hexane were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), D_2O and $CDCl_3$ from Aldrich Chemical Co., Inc. and silica gel plates from E. Merck. Other chemicals were of reagent grade and obtained commercially. Water was used after ion exchange treatment with a Milli-Q SP Reagent Water System.

Analytical HPLC Conditions Column: Hitachi gel #3056 (silica-ODS) (4 mm i.d.$\times$250 mm), eluent: linear gradient from $CH_3CN:H_2O$ (9 : 1) to $CH_3CN:(C_2H_5)_2O$ (3 : 2), flow rate: 1.0 ml/min, detector: Hitachi L-4000 UV Detector (268 nm) and Hitachi L-4500 Diode Array Detector (200—500 nm).

Preparative HPLC Conditions Column: Neopack 120-Å 5C18 (20 mm i.d.$\times$300 mm), eluent: $CH_3OH:H_2O:(C_2H_5)_2O$ (55 : 7 : 18), flow rate: 20 ml/min, detector: TOSOH UV-8010 (290 nm).

Preparative TLC Conditions Silica gel $60F_{254}$ (layer thickness 2 mm), developing solvent : *n*-hexane : $(C_2H_5)_2O$ (9 : 1).

Spectral Measurements ^{1}H-and ^{13}C-NMR spectra were measured with a JEOL α-500 spectrometer. Mass spectra were recorded on a JEOL JNM-GX270 spectrometer. IR and UV spectra were measured with a JASCO FT/IR-5300, and Shimadzu UV-240 UV-visible recording spectrometer, respectively.

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