Studies on the Oxidation Mechanism of Vitamin E

Part I. The Oxidation of 2,2,5,7,8-Pentamethyl-6-hydroxychroman

By Masao FUJIMAKI, Koichiro KANAMARU, Tadao KURATA
and Osamu IGARASHI*

Department of Agricultural Chemistry, The University of Tokyo, Tokyo

**Research Institute of Food Chemistry, The Ochanomizu University, Tokyo*

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α -Tocopherol model compound, 2,2,5,7,8-pentamethyl-6-hydroxychroman was oxidized under oxygen bubbling. Four oxidation products of 2-(γ,γ -dimethylallyl)-3,5,6-trimethyl-1,4-benzoquinone (B), 2,2,7,8-tetramethyl-5-formyl-6-hydroxychroman (C), trimer (D) and tocopherylethane (G) were identified, and spiroidimer (E) was tentatively identified by TLC. Two of them, (B) and (C) have not been obtained in the oxidation of α -tocopherol model compound with *p*-quinone, alkaline ferricyanide and other compounds as oxidizing agent. A scheme of oxidation mechanism of α -tocopherol model compound was also proposed.

It is considered that α -tocopherol in food is mainly oxidized through the reactions with reactive radicals which are produced by autoxidation of some oxidation sensitive food components, and that the antioxidant ability of α -tocopherol is shown in its oxidation process. Therefore it is necessary for the elucidation of the mechanism of the antioxidative action and the development of new antioxidants to explain the oxidation mechanism of α -tocopherol. Hitherto relatively strong oxidizing agents have been used in many researches¹⁻¹³⁾ on the oxidation mech-

anism of α -tocopherol. But it is quite unusual that such oxidizing agents are contained in a large amount in food. It may be considered that the concentration of reactive intermediates in the oxidation reaction is larger with strong oxidizing agents than with weak one and that the oxidation mechanism, quantities and kinds of oxidation products are different from both cases.

As the first step in the elucidation of the oxidation mechanism of α -tocopherol in food, isolation and identification of oxidation products formed during the oxidative degradation of α -tocopherol model compound (S) under

1) L. I. Smith, W. B. Irwin and H. E. Ungnade, *J. Am. Chem. Soc.*, **61**, 2424 (1939).

2) L. I. Smith, W. B. Irwin and H. E. Ungnade, *Science*, **90**, 334 (1939).

3) P. Karrer, H. Fritzsche and R. Escher, *Helv. Chim. Acta*, **22**, 661 (1939).

4) V. L. Frampton, W. A. Skinner, Jr., and P. S. Bailey, *J. Am. Chem. Soc.*, **76**, 282 (1954).

5) V. L. Frampton, W. A. Skinner, P. Cambour and P. S. Bailey, *ibid.*, **82**, 4632 (1960).

6) P. O. Boyer, *ibid.*, **73**, 733 (1951).

7) C. Martius and H. Eilingsfeld, *Biochem. Z.* **328**, 507 (1957).

8) G. E. Inglett and H. A. Mattill, *J. Am. Chem. Soc.*, **77**, 6552 (1955).

9) C. T. Goodhue and H. A. Bailey, *Biochemistry*, **4**, 854 (1965).

10) D. R. Nelan and C. D. Robeson, *J. Am. Chem. Soc.*, **84**, 2963 (1962).

11) A. S. Csallany and H. H. Draper, *J. Biol. Chem.*, **238**, 2912 (1963).

12) P. Schudel, H. Mayer, J. Metzgen, R. Rügge and O. Isler, *Helv. Chim. Acta*, **46**, 636 (1963).

13) D. McHale and J. Green, *Chem. Ind.*, **1964**, 366.

oxygen bubbling are carried out in the present investigation.

MATERIALS AND METHODS

Materials

2,2,5,7,8-Pentamethyl-6-hydroxychroman. Trimethyl hydroquinone, isoprene and zinc chloride were dissolved in acetic acid. The solution was refluxed and 2,2,5,7,8-pentamethyl-6-hydroxychroman was synthesized by the method of L. I. Smith.¹⁴⁾

n-Heptane. Extra pure reagent *n*-heptane was shaken with conc. sulfuric acid for 24 hr, then washed with water, and shaken with a 0.1N solution of potassium permanganate in 10% sodium hydroxide. The *n*-heptane was then washed with water, dried over anhydrous sodium sulfate, distilled, and the fraction boiling between 98~99°C was used.

Oxygen. Oxygen from commercial source was used without further purification.

Other reagents. Guaranteed reagents were used without further purification.

Methods

Oxidation condition. A solution of 2,2,5,7,8-pentamethyl-6-hydroxychroman (20 g) in 400 ml of *n*-heptane was oxidized at 90°C under constant stirring and bubbling of oxygen (50 ml/min) for 40 days. A certain volume of reaction solution was sampled every two days, and subjected to thin-layer chromatography (TLC).

Column chromatography. Sample solutions were evaporated, adsorbed with a certain amount of Silica gel (Kanto Chemical Co., Tokyo, for chromatography, 100 mesh), dried, applied to a silica gel column (50×1000 mm) and successively eluted. The eluate was fractionated using a fraction collector (20 ml/20 min), and analyzed by TLC. Development was carried out as follows: *n*-hexane→2% benzene in *n*-hexane→5%→10%→15%→20%→30%→50%→benzene→toluene→chloroform→ethyl acetate→methanol.

Thin-layer chromatography (TLC). Kieselgel G (Merck) was used as adsorbent and activated at 110°C

for 3 hr. Development was performed by the usual ascending method using the following solvent systems: solvent system I, chloroform-benzene 2 : 1 (v/v); solvent system II, chloroform. Oxidation products were detected by the color development of yellow, blue, green, brown, pink and orange on heating (at 150°C) after spraying conc. H₂SO₄.

Preparative TLC was carried out by using glass plates (20×20 cm) coated with Kieselgel G layers at 0.25~1 mm in thickness. After development, each component was extracted with ethyl acetate, and the excess solvent was removed by evaporation with a rotary evaporator.

Sublimation. The sample was sublimated under the pressure of 1 mm Hg at 60°C.

Spectroscopy. IR spectra were measured in nujol mulls and film with JASCO Model IR-S. NMR spectra were measured with Japan Electron Optics Model JNM-4H-100 spectrometer at 100 MHz, using tetramethylsilane as an internal standard. UV spectra were measured with Hitachi Model EPS-3 and Cary 14-PM spectrometer. Mass spectra were measured with Hitachi Model RMU-6 and RMS-4 spectrometer at ca. 70 eV of ionization energy.

Oxidation with oxidizing agents. i) Ferric chloride;¹⁵⁾ α -Tocopherol model compound (S) and ferric chloride were dissolved in methanol, and the mixture was heated under reflux for 5 hr, water was then added, and the resulting mixture was extracted with diethyl ether.

ii) Alkaline ferricyanide;¹⁵⁾ A solution of the compound (S) in *n*-hexane was vigorously shaken with a solution of 3 equiv. of K₃Fe(CN)₆ in 0.2N aqueous NaOH for 30 min. Then *n*-hexane layer was washed with water and dried over anhydrous sodium sulfate.

iii) *p*-Benzoquinone;¹⁶⁾ A solution of the compound (S) and *p*-benzoquinone in benzene was heated under reflux for 24 hr. The solvent was removed, and the residue was triturated with *n*-hexane and the products were collected by filtration.

RESULTS AND DISCUSSION

Solution of model compound (S) developed

15) J. L. G. Nilsson, H. Sievertson and H. Selender, *Tetrahedron Letters*, **1968**, 5023.

14) L. I. Smith, H. E. Vngnade, H. H. Hoehn and S. Wawzonek, *J. Org. Chem.*, **4**, 311 (1939).

16) J. L. G. Nilsson, G. D. Daves, Jr. and K. Folkers, *Acta Chem. Scand.*, **22**, 207 (1968).

FIG. 1. Thin-layer Chromatograms of Oxidation Products.

- I: Oxidation under oxygen bubbling
 II: Oxidation with $K_3Fe(CN)_6$
 III: Oxidation with $FeCl_3$
 IV: Oxidation with *p*-benzoquinone
 Development: solvent system I
 Detection: conc. H_2SO_4 (150°C)

light yellow color after about forty days of reaction, and more than ten oxidation products and unreacted model compound (S) were detected by TLC. Thin-layer chromatograms of oxidation products formed during oxygen bubbling or by other oxidizing agents are shown in Fig. 1. Although there were some similarities in the pattern of these four thin-layer chromatograms, marked differences in quantity and quality of oxidation products were observed. Of these three oxidizing agents the oxidation with *p*-benzoquinone gave the most similar pattern of oxidation products to that of oxidation under oxygen bubbling. Oxidation products were separated, isolated and purified by silica gel column chromatography, preparative thin-layer chromatography, recrystallization and sublimation. Yields of each oxidation product are shown as follows.

A (in Fig. 1): 25 mg, B: 340 mg, C: 330 mg, D: 1.78 g, E: trace, S: 4.47 g, F (in Fig. 1): 1.385 g, G: 25 mg.

a) Identification of oxidation products

The compound (B) has been obtained as yellow oil. The infrared spectrum showed the presence of a carbonyl (1640 cm^{-1}) and the absence of a hydroxy group as shown in Fig. 2. The ultraviolet spectrum in ethanol showed two absorption bands (λ_{max} 258 $m\mu$, $E_{1\text{cm}}^{1\%}=700$ and λ_{max} 267 $m\mu$, $E_{1\text{cm}}^{1\%}=720$). The NMR and mass spectra are shown in Fig. 3

FIG. 2. Infrared Spectrum of the Compound (B). (Film)

FIG. 3. NMR Spectrum of the Compound (B).

FIG. 4. Mass Spectrum of the Compound (B).

and Fig. 4 respectively. The molecular formula was assigned as $C_{14}H_{20}O_2$ from the results of the elementary analysis and mass spectra. Thus the compound (B) was identified as 2-(γ,γ -dimethylallyl)-3,5,6-trimethyl-1,4-benzoquinone, and the spectroscopic data are

FIG. 5. Infrared Spectrum of the Compound (C). (Nujol mull)

Dotted line, ν_{OD} absorption appeared after deuteration with D_2O .

FIG. 6. NMR Spectrum of the Compound (C).

FIG. 7. Mass Spectrum of the Compound (C).

identical with those of the authentic sample.^{17,18)}

The compound (C) has been obtained as yellow needles of sublimity, mp 116~118°C, and its infrared spectrum showed the presence of a carbonyl and a hydroxy group (1635 cm^{-1} and 3090 cm^{-1} *) as shown in Fig. 5. The ultraviolet spectrum in ethanol showed the absorption at $240\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}=218$), $287\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}=353$) and $387\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}=109$). The NMR and mass spectra are shown in Fig. 6 and 7 respectively. The molecular formula was assigned as $C_{14}H_{20}O_3$ from the results of the elementary analysis and mass spectra. Thus the compound (C) was identified as 2,2,7,8-tetramethyl-5-formyl-6-hydroxychroman, and the spectroscopic data are identical with those of the authentic sample.¹⁹⁾

The compound (D) has been isolated as white plates, mp 209~210°C. As this coincided with the authentic sample** in IR ($\nu_{C=O}$: 1690 cm^{-1}), MS (M^+ =654, $m/e=218$), UV (λ_{max} $295\text{ m}\mu$, $E_{1\text{cm}}^{1\%}=159$), the elementary analysis (C, 77.13%, H, 7.93%) and the behaviors on thin-layer chromatogram, it was identified as the trimer of α -tocopherol model compound. The infrared spectra are shown in Fig. 8.

As the compound (E) coincided with the authentic sample** in the behaviors on thin-layer chromatogram, it was tentatively identified as the spirodime of α -tocopherol model compound.

The compound (G) has been isolated as white plates, mp 184.0~184.5°C. As its IR (ν_{O-H} 3440 cm^{-1}), MS (M^+ =438, $m/e=219$), UV (λ_{max} $295\text{ m}\mu$, $E_{1\text{cm}}^{1\%}=159$), NMR: $\tau=8.67$ (12H, $-\text{CH}_3$), $7.84\sim 7.91$ (12H, $\phi-\text{CH}_3$), 8.21 (4H,

17) P. M. Scott, *J. Biol. Chem.* **240**, 1374 (1965).

18) P. Mamont, P. Cohen and R. Azerad, *Bull. Soc. Chim.*, **1967**, 1485.

19) W. A. Skinner and R. M. Parkhurst, *J. Org. Chem.*, **29**, 3601 (1964).

* The appearance of OD absorption at 2260 cm^{-1} in IR spectra of the sample treated with D_2O indicated the presence of OH absorption at 3090 cm^{-1} .

** These three samples were offered by Dr. Nilsson.

$-\text{CH}_2-$), 7.32 (8H, $\phi-\text{CH}_2-$) 4.50~4.90 (2H, $-\text{OH}$) and the behaviors on thin-layer chromatogram are quite identical with the authentic sample,** it was identified as the tocopherylethane derived from α -tocopherol model compound. The infrared spectra are shown in Fig. 9.

The compound (A) and (F) have been isolated as white rods and white plates respectively,

but have not been identified yet.

b) Formation mechanism of oxidation products

From the results of these experiments the oxidation mechanism of α -tocopherol model compound (S) under oxygen bubbling may be considered as follows. A hydrogen radical is abstracted from the phenolic hydroxy group of the model compound (S), and phenoxy

FIG. 8. Infrared Spectra of the Compound (D) and the Authentic Sample (Nujol mull).

I: The authentic sample
II: The compound (D)

FIG. 9. Infrared Spectra of the Compound (G) and the Authentic Sample (Nujol mull).

I: The authentic sample
II: The compound (G)

SCHEME. Formation Mechanism of the Compound (B), (C), (D), (E) and (G).

radical (I) is formed. The radical (II) is a resonance structure of phenoxy radical (I), and the unpaired electron on the carbon atom attached to oxygen is considered to take an important part in β -scission of the radical (II). The same kind of β -scission was observed in the case of 2-methoxy pyran.²⁰⁾ As a hydrogen atom of methylene group adjacent to radical site is apt to be abstracted, the radical (III) is easily converted to the compound (B) by the attack of some reactive radicals. The radical (IV) which is a tautomer of the radical (I) changes into the peroxy radical (V) through the reaction with oxygen, and two molecules of the radical (V) will undergo disproportionation²¹⁾ to form the compound (C). The tocopheryl-ethane (G) is produced through a coupling reaction between two benzyl radicals (IV)

which have been suggested as intermediates in the oxidation of α -tocopherol with benzoyl peroxide.⁸⁾ The tocopherylethane can be oxidized to the spirodimer (E) probably through two one-electron oxidations followed by an intramolecular coupling²²⁾ reaction. The quinone methide (VIII) can react with one molecule of the spirodimer (E) to form the trimer via a Diels-Alder reaction. And also two molecules of the quinone methide (VIII) form the spirodimer (E) via a Diels-Alder type reaction and the mechanism is supported by Nilsson *et al.*²³⁾

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20) E. S. Huyser, *J. Org. Chem.*, **25**, 1820 (1960).

21) G. A. Russell, *J. Am. Chem. Soc.*, **77**, 4583 (1955).

22) W. Bouguth, R. Repges and M. Sernetz, *Ber. Bunsenges. Phys. Chem.*, **70**, 34 (1966).

23) J. L. G. Nilsson, J.-O. Branstad and H. Sievertson, *Acta Pharm. Suecica*, **5**, 509 (1969).