

OXIDATION OF ALPHA-TOCOPHEROL BY SINGLET OXYGEN

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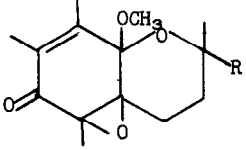
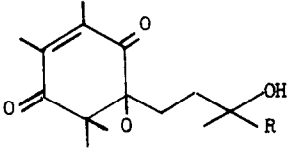
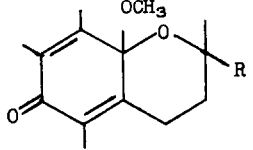
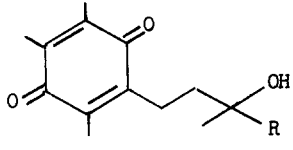
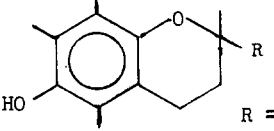
Dye-sensitized photooxidation of olefins, dienes, and heterocyclic compounds proceed via singlet oxygen (1). Such a mechanism has been proposed as the initial step in autoxidation of unsaturated lipids (2). To determine the ability of  $\alpha$ -tocopherol to protect against such oxidation, an investigation was undertaken to show that singlet oxygen was consumed by  $\alpha$ -tocopherol.

We reported previously (3) that the dye-sensitized photooxidation of  $\alpha$ -tocopherol in the presence of proflavin dye yields as the major products 4a,5-epoxy-8a-methoxy- $\alpha$ -tocopherone (I) and  $\alpha$ -tocoquinone 2,3-oxide (II), whose structures are shown in Table I. To demonstrate that these products are characteristic of the reaction of  $\alpha$ -tocopherol with singlet oxygen, the oxidation was repeated using  $\text{Ca}(\text{OCl})_2\text{-H}_2\text{O}_2$  to generate singlet oxygen chemically (4). d-Alpha-tocopherol (V, 466 mg, 1.08 mmol) was dissolved in 200 ml methanol and cooled to 3°C. To this solution was added 2.1 ml of 18.9 N  $\text{H}_2\text{O}_2$  (40 meq.), and 25 ml of 1.32 N  $\text{Ca}(\text{OCl})_2$  (33 meq.) was added dropwise over a period of 75 min. The solution was diluted with water (400 ml) and the reaction products were extracted with chloroform (3 x 100 ml). After the chloroform extract was washed with  $\text{H}_2\text{O}$  (3 x 100 ml) and dried over anhydrous  $\text{Na}_2\text{SO}_4$ , it was concentrated below 40°C. to an oily residue (535 mg). This residue was dissolved in petroleum ether (b.p. 40°-60°) and chromatographed on a basic alumina column (activity IV, 20 mm i.d.). The products separated by petroleum ether-ethyl ether gradient elution were identical with those obtained previously from the dye-sensitized photooxidation (3).

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TABLE I  
Yield of Oxidation Products of  $\alpha$ -Tocopherol

Compound	Singlet Oxygen Generated		
	Photochemically	Chemically	
	I	34	<1
	II	6	28
	III	14	17
	IV	<1	24
	V	29	9

R = C<sub>16</sub>H<sub>33</sub>

$\alpha$ -Tocoquinone (IV) was selectively reduced in the presence of II by a modification of the reductive method of Harrison et al. (5). In this way II could be purified. The mixture of quinones (220 mg) was dissolved in 10 ml of 0.5% HCl in absolute methanol. Excess ascorbic acid was added and the solution set aside for 1 hr. The solution was diluted with water (20 ml) and the products were extracted with ethyl ether (3 x 10 ml). The ether extract was washed with water (3 x 10 ml), diluted to 50 ml with petroleum ether, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The oily residue (170 mg) was separated on a silica gel column (30 ml, 15 mm i.d.) with cyclohexane-chloroform gradient and gave II (88 mg) and  $\alpha$ -tocopherol (70 mg).

In Table I, the yield of products obtained when singlet oxygen is generated chemically is compared with yields when singlet oxygen is generated photochemically. Due to the water present in the reaction solution during the chemical generation of singlet oxygen, hydrolysis of the oxidation intermediates is favored over methanolysis. The ratio of epoxy ketones to unsaturated ketones differs when singlet oxygen is generated photochemically (3:1) or chemically (3:4). This ratio change may be due to free radical side reactions, which have been observed in other systems when singlet oxygen is chemically generated (4).

For free radical oxidation of  $\alpha$ -tocopherol in methanol only the unsaturated ketone products are observed (6). The oxidation of  $\alpha$ -tocopherol with singlet oxygen generated chemically and photochemically yield characteristic epoxy ketones. Possibly,  $\alpha$ -tocopherol protects unsaturated lipids from the effects of photodynamic action by scavenging "active" oxygen.

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