Reactions of Vitamin E with Peroxides. II. Reaction of Benzoyl Peroxide with *d*-α-Tocopherol in Alcohols*

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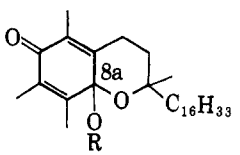
ABSTRACT: Benzoyl peroxide oxidation of *d*-α-tocopherol in the presence of alcohols resulted in the formation of 8a-alkoxy-α-tocopherones. The alkoxy groups were derived from the alcohols. Examples of straight-chain alkoxy-α-tocopherones with up to eighteen carbon atoms were made. A secondary alkoxy derivative was made, but a tertiary derivative could not be made. The use of ferric chloride–2,2′-bipyridine or bromine as oxidizing agent also resulted in the formation of the substituted α-tocopherones.

If the biological activity of vitamin E (*d*-α-tocopherol) is some function of its oxidative chemistry, a study of this chemistry under conditions which would be expected to occur *in vivo* would be biochemically pertinent. It is likely that the immediate environment of α-tocopherol is of a hydrophobic nature so that reactions of this vitamin that do not involve water might well be biologically important. Therefore we have undertaken a study of mild oxidations of α-tocopherol in dry nonaqueous solvents.

We have found that the products obtained from these oxidations depend strikingly upon the nature of the solvent. In a previous paper (Goodhue and Risley, 1964), we reported that the reaction of benzoyl peroxide with solutions of *d*-α-tocopherol in hydrocarbons resulted in benzoyloxy substitution on the 5-methyl group of α-tocopherol. We now report that benzoyl peroxide reactions carried out in anhydrous alcohols proceed differently and result in the formation of substituted chromanones which we shall call "8a-alkoxy-α-tocopherones" (compound Ia), following Dürckheimer and Cohen (1962). The alkoxy substituent is derived from the solvent alcohol. These alkoxy-α-tocopherones are homologs of 8a-ethoxy-α-tocopherone (Boyer's "tocopheroxide") (compound Ia, $n = 2$) originally made by oxidation of α-tocopherol with $FeCl_3$-2,2′-bipyridine in ethanol (Boyer, 1951; Martius and Eilingsfeld, 1957). The 8a-hydroxy-α-tocopherone (compound Ib)[1] in this series was made by Dürckheimer and Cohen (1962, 1964).



Ia, R = n-C_nH_{2n+1}
Ib, R = H
Ic, R = $CH(CH_3)_2$
Id, R = $C(CH_3)_3$

Oxidation of α-tocopherol in solvents such as hydrocarbons which cannot donate groups to the 8a position mainly yields products substituted on the 5-methyl group of α-tocopherol. We suggest that α-tocopherol oxidation *in vivo* in proximity to nucleophilic groups such as alcohols or thiols could result in formation of 8a-substituted α-tocopherones. Subsequent reduction to α-tocopherol completes an oxidation-reduction cycle that does not involve α-tocopheryl-*p*-quinone.

Results

Benzoyl Peroxide as Oxidizing Agent. Using benzoyl peroxide we made α-tocopherones with the following 8a- substituents: methoxy, ethoxy ("tocopheroxide"), 1-butoxy, 1-octoxy, 1-tetradecoxy, and 1-octadecoxy (Table I). We also made the 8a-(2-propoxy)-α-tocopherone (compound Ic), but we were not able to make the tertiary derivative, compound Id. Yields of the α-tocopherones estimated from spectral measurements at 238 mμ in hexane were of the order of 60–100% before purification. Yields of purified products varied from 28 to 78% (Table I). When water was present, the yield of α-tocopherones was reduced and α-tocopheryl-*p*-quinone was formed. In an equimolar mixture of water and methanol, the quinone was the major product isolated. Another major by-product was the α-tocopherol spirane-dienone dimer (see Nelan and Robeson 1962, for structure) which was especially noticeable in the by-products of benzoyl peroxide reactions in 2-propanol and the longer-chain normal alcohols. When the oxidation of α-tocopherol was carried out in dry *t*-butyl alcohol, the main product isolated by alumina chromatography was the spirane-dienone dimer. Reaction in dry acetonitrile yielded 5-benzoyloxymethyl-γ-tocopherol. Benzoic acid was

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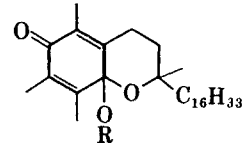
[1] Termed "9-hydroxy-α-tocopherone" by Dürckheimer and Cohen (1962).

TABLE I: 8a-Alkoxy-α-tocopherones.



R	Oxidizing Agent	Yield (%)	Molecular Formula	Molecular Weight Calcd	Molecular Weight Found[a]	Carbon Calcd	Carbon Found	Hydrogen Calcd	Hydrogen Found	ε[b]
CH_3	Benzoyl peroxide	78	$C_{30}H_{52}O_3$[c]	460.7	468	78.3	78.3	11.4	11.5	12,900
	$FeCl_3$-2,2′-bipyridine	73	$C_{30}H_{52}O_3$[d]				78.3		11.4	12,900
	Br_2	54								12,900
C_2H_5	Benzoyl peroxide		$C_{31}H_{54}O_3$	474.7						12,900
2-C_3H_7	Benzoyl peroxide	78	$C_{32}H_{56}O_3$	488.8	493	78.8	78.6	11.5	11.7	12,800
	$FeCl_3$-2,2′-bipyridine	28					78.5		11.7	13,000
1-C_4H_9	$FeCl_3$-2,2′-bipyridine	51	$C_{33}H_{56}O_3$	502.8						12,900
1-C_8H_{17}	$FeCl_3$-2,2′-bipyridine	52	$C_{37}H_{66}O_3$	558.9		79.5	79.1	11.9	12.0	12,800
1-$C_{14}H_{29}$	Benzoyl peroxide		$C_{43}H_{78}O_2$	643.1		80.3	80.1	12.2	12.2	13,000
1-$C_{18}H_{31}$	Benzoyl peroxide	48	$C_{47}H_{86}O_3$	699.2		80.7	80.3	12.4	12.8	12,900

[a] Ebulliscopic. [b] 238 mμ in hexane. [c] *Anal.* Calcd: CH_3O, 6.74. Found: CH_3O, 7.2. [d] *Anal.* Calcd: CH_3O, 6.74. Found: CH_3O, 7.0.

formed in reactions at room temperature, but very little was found in reactions at 75°.

The structures of the alkoxy-α-tocopherones were determined by comparison of their ultraviolet and infrared spectra with the spectra of authentic 8a-ethoxy-α-tocopherone (compound Ia, $n = 2$). These spectra have been published (Boyer, 1951). All the substituted α-tocopherones had identical ultraviolet spectra with a molar extinction in hexane approximating 12,900 at 238 mμ (Table I). The infrared spectra were grossly similar to that for 8a-ethoxy-α-tocopherone. Furthermore, the microanalytical data were consistent with the proposed structures.

Other Oxidizing Agents. Diacyl peroxides such as decanoyl peroxide and dodecanoyl peroxide and a mixture of cyclohexane peroxides (CXP – 85, U.S. Peroxygen Corp.) also reacted with α-tocopherol in the presence of alcohols to form alkoxy-α-tocopherones.

By using modifications of Boyer's method ($FeCl_3$-2,2′-bipyridine) we made a series of the substituted α-tocopherones (including compound Ic) with alkoxy substituents containing one to eight carbons (compound Ia, $n = 1$–8), but we could not make any of the higher analogs unless a peroxide was added. The effect of peroxides was first observed when purified bis-(2-methoxyethyl) ether was substituted for solvent containing peroxides that arose from autoxidation.

At −67°, bromine reacted with alcoholic solutions of α-tocopherol to produce alkoxy-α-tocopherones provided the reaction mixture was maintained near neutrality. We used cyclohexylamine for this purpose. In the absence of the base, α-tocopheryl-*p*-quinone and other compounds were formed. These compounds were not identified.

When α-tocopherol was reacted with bromine in hexane solutions at room temperature, 5-bromomethyl-γ-tocopherol was formed. When treated with 1 N KOH, this compound was converted rapidly to the spirane-dienone dimer of α-tocopherol. A possible mechanism of dimerization following a 1–4 elimination (in this case elimination of HBr) to give a quinone methide has been discussed (Goodhue and Risley, 1964). The formation of the dimer confirms that bromine is substituted on the 5-methyl group.

Hydrolysis and Reduction of 8a-Alkoxy-α-Tocopherones. The alkoxy-α-tocopherones were converted to α-tocopheryl-*p*-quinone by treatment with dilute aqueous HCl. Treatment of the α-tocopherones with ascorbic acid or sodium borohydride at room temperature for approximately 1 hour resulted in complete conversion to α-tocopherol. The hydrolysis and reduction methods used were essentially those published for 8a-hydroxy-α-tocopherone (Dürckheimer and Cohen, 1962) and 8a-ethoxy-α-tocopherone (Boyer, 1951; Harrison *et al.*, 1956).

Discussion

A remarkable tendency for forming 8a-substituted α-tocopherones is demonstrated by α-tocopherol when it is oxidized by benzoyl peroxide in the presence of alcohols. The alkoxy groups are donated by the alcohols during the course of the reaction. This reaction is not confined to benzoyl peroxide but proceeds with alkyl diacyl peroxides, cyclohexanone peroxides (a mixture of peroxides; see Hawkins, 1961), and the undefined mixture of peroxides found in autoxidized bis-(2-methoxyethyl) ether. Nonperoxidic oxidizing agents that react with α-tocopherol to yield α-tocopherones include the ferric chloride–2,2′-bipyridine reagent (Boyer, 1951; Martius and Eilingsfeld, 1957), tetrachloro-*o*-quinone (Dürckheimer and Cohen, 1964), and bromine.

Dürckheimer and Cohen (1962, 1964) have reported the formation of 8a-hydroxy-α-tocopherone by oxidation of α-tocopherol with tetrachloro-*o*-quinone or *n*-bromosuccinimide in aqueous acetonitrile. This reaction appears to be of the same type by which alkoxy-α-tocopherones are formed, but in this case water instead of an alcohol reacts with the oxidized α-tocopherol intermediate to form the hydroxy-α-tocopherone. These same authors also report the very interesting synthesis of 8a-acetoxy-α-tocopherone by oxidation of α-tocopherol in absolute acetonitrile with tetrachloro-*o*-quinone in the presence of tetramethylammonium acetate (Dürckheimer and Cohen, 1964). Again, this product could be viewed as the reaction of an oxidized α-tocopherol intermediate with acetate.

What seems most interesting and significant about these experiments is the finding that the products of α-tocopherol oxidation depend not so much upon the oxidizing agent as upon the availability of additional reactants that can form stable products. Thus we find that the same oxidizing agents that produce 8a-substituted α-tocopherones in alcohol solution produce 5-methyl-substituted γ-tocopherols in hydrocarbon solutions where reaction with the solvent is unlikely. For example, in hexane or benzene, diacyl peroxides produce 5-acyloxymethyl-γ-tocopherols (Goodhue and Risley, 1964) and bromine produces 5-bromomethyl-γ-tocopherol. By contrast, the use of either of these oxidizing agents in alcohols that can donate alkoxy groups to the 8a position produces alkoxy-α-tocopherones. It would be interesting to see if the reaction of peroxides and α-tocopherol in aprotic solvents containing buffered carboxylic acid salts also would yield 8a-acyloxy-α-tocopherones. We have already shown that the benzoyl peroxide oxidation of α-tocopherol in unbuffered acetonitrile yields 5-benzoyloxy-γ-tocopherol rather than 8a-substituted product.

An interesting example of the apparent competition of reaction at the 8a position with reaction at the 5-methyl position is provided by the benzoyl peroxide oxidation in *t*-butyl alcohol. We could not obtain the *t*-butoxy-α-tocopherone. Instead, we obtained the spirane-dienone dimer. This result appears to be owing to the steric hindrance of the *t*-butoxy group. Courtauld atomic models show that primary and secondary, but not tertiary, alkoxy groups fit into the 8a position. Since 8a-*t*-butoxy derivatives cannot be made, reactions in *t*-butyl alcohol take the same course as reactions in the relatively inert hydrocarbon solvents and thus a

5-methyl-substituted product (the dimer) is obtained.

This tendency of α-tocopherol to form α-tocopherones during oxidation coupled with the relative ease with which these chromanones can be reduced to α-tocopherol (e.g., by ascorbic acid) suggests mechanisms by which α-tocopherol (or chromanols in the coenzyme Q and vitamin K series) could participate catalytically in biological transfer of electrons. These mechanisms need not involve α-tocopheryl-*p*-quinone, a substance with little vitamin E activity (see Bunyan *et al.*, 1963, for recent experiments). *In vivo* oxidation of α-tocopherol in the presence of biochemical nucleophilic groups such as sulfhydryl or hydroxy groups should yield compounds analogous to the alkoxy-α-tocopherones. α-Tocopherone-forming reactions may be involved in the apparent protection by α-tocopherol of enzyme sulfhydryl sites (Ames and Risley, 1949; Corwin and Schwarz, 1963). Moreover, the oxidation to an α-tocopherone followed by reduction to α-tocopherol provides a reasonable cyclic mechanism for inhibition of *in vivo* accumulation of peroxides whether by decomposition of peroxides or by interference with oxidation by free radical chain processes. We presently have no evidence for the natural occurrence of 8a-substituted α-tocopherones, but it is conceivable that these compounds have escaped detection previously owing to their reduction to α-tocopherol or hydrolysis to α-tocopheryl-*p*-quinone during isolation.

The mechanisms by which the various oxidation products of α-tocopherol are formed is of interest. The products obtained can be viewed as the results of combinations of an electron-deficient tocopherol moiety either with itself or with reactants derived from the oxidizing agent or the solvent. Either free radical or ionic intermediates could be involved. The predominant forms of the oxidized tocopherol intermediate must involve manifestation of electron deficiency at position 8a of the ring, at the 5-methyl, and at the 6-oxygen. As we have seen, reaction at the 5-methyl group yields the 5-substituted methyl-γ-tocopherols (Goodhue and Risley, 1964) or α-tocopherol dimers (Nelan and Robeson, 1962; Skinner and Alaupovic, 1963). Compounds resulting from substitution of the 6-oxygen have been observed recently (Skinner, 1964). The various compounds resulting from reaction at position 8a were discussed. Similar ideas regarding the mechanisms of α-tocopherol oxidation have been expressed previously (more recently, Skinner and Alaupovic, 1963; Skinner, 1964).

Experimental Procedure

8a-Methoxy-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanone (8a-Methoxy-α-tocopherone)

Benzoyl Peroxide Method. Benzoyl peroxide (0.533 g, 2.32 mmole) was added during reflux under nitrogen to a solution of *d*-α-tocopherol (1 g, 2.32 mmoles) in dry methanol (200 ml distilled from CaH_2). The mixture was refluxed 30 minutes and then cooled to 5°. KOH (1 g in 20 ml methanol) was added. After the addition of 100 ml of cold water, the product was extracted with three 100-ml portions of hexane. The combined extracts were dried with Na_2SO_4. Solvent was removed and the resulting yellow oily residue was chromatographed on a column, 3.7 × 20 cm, of basic alumina (Grade III, Woelm). The product was eluted with 1 liter of purified hexane. Yield was 0.835 g. A middle fraction was taken for analysis; γ_{max}^{hexane} 238 mμ (ε 12,900); infrared bands 5.96 μ (conjugated carbonyl); 6.08 (conjugated double bonds). See Table I for analysis.

Ferric Chloride–2,2′-Bipyridine Method. A solution of *d*-α-tocopherol (1 g, 2.32 mmoles) and 2,2′-bipyridine (4.5 g, 28.8 mmoles) in dry methanol (200 ml) was cooled to −20° with a dry ice–acetone bath. Anhydrous $FeCl_3$ (0.75 g, 4.63 mmoles) dissolved in 50 ml of methanol was added rapidly with stirring. The mixture immediately turned dark red. Stirring at −20° was continued for 2 hours. Hexane (100 ml) and ice-cold water (100 ml) were added. The bottom layer was reextracted with 100 ml of hexane. The two top layers were combined and washed with dilute aqueous $FeSO_4$ until red color was no longer obtained. The hexane extract was dried with Na_2SO_4 and then concentrated by evaporation. The residue was chromatographed on alumina as described. Yield was 0.675 mg. Ultraviolet and infrared spectra were identical with those of the product obtained by use of benzoyl peroxide method. See Table I for analysis.

Bromine Method. A solution of *d*-α-tocopherol (0.5 g, 1.16 mmoles) and cyclohexylamine (0.230 g, 2.32 mmoles) in 200 ml of dry methanol (distilled from CaH_2) was cooled to −67° in a dry ice–acetone bath. Bromine (0.25 g, 1.31 mmoles) in 25 ml of dry methanol was added with stirring over a 15-minute period. The mixture was stirred for 1 hour at −67° and then allowed to come to room temperature. Cold water (100 ml) was added and the product was extracted with three 100-ml portions of hexane, dried with Na_2SO_4, and chromatographed on alumina as described above. Infrared and ultraviolet spectra of this product and the benzoyl peroxide oxidation product were superimposable.

General Methods for Preparation of 8a-alkoxy-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanones (8a-Alkoxy-α-tocopherones)

The methods used for preparation of 8a-methoxy-α-tocopherone were used with the following modifications:

Ferric Chloride–2,2′-Bipyridine Method. Ferric chloride–2,2′-bipyridine oxidations in C_1 to C_8 alcohols were run at temperatures from −20° to −10°. Low-boiling alcohols were removed by evaporation under vacuum. Higher-boiling alcohols such as 1-octanol were removed by fractional crystallization. Further purification was accomplished with alumina chromatography.

Benzoyl Peroxide Method. Reactions were run at 70° under nitrogen. Alcohols from C_1 to C_8 were used as their own solvents. In reactions of 1-tetradecanol and 1-octadecanol, the alcohol was dissolved in hexane. In these cases, the alcohol was used in a 20- to 40-fold molar excess compared to α-tocopherol. The total volume for reactions of 1 g of α-tocopherol was 200 ml. Higher-boiling alcohols were removed by fractional crystalliza-

tion. Alkoxy-α-tocopherones were obtained by hexane elution of an alumina column. Dimer products were obtained by elution of the column with 5% ether in hexane.

Oxidation of d-α-tocopherol in t-Butyl Alcohol. d-α-Tocopherol (0.5 g, 1.16 mmoles) dissolved in 100 ml of dry *t*-butyl alcohol (distilled from CaH_2) was heated to 70° under a nitrogen atmosphere. Benzoyl peroxide (0.27 g, 1.16 mmoles) was added with vigorous stirring. The peroxide dissolved immediately and the reaction mixture turned yellow. Heating at 70° was continued for 75 minutes. The ultraviolet spectrum in hexane showed a major peak at 300 mμ with shoulder at 294 mμ and a smaller peak at 338 mμ. Peaks at 266, 272, and 280 mμ disappeared after extracting the reaction mixture with 1 N KOH; the peaks at 294, 300, and 338 mμ remained unchanged. The yellow oil obtained by evaporation was purified by eluting the basic alumina (Grade III, Woelm) column (3.7 × 20 cm) with 5% ether in hexane. According to infrared spectroscopy, the product was identical with the spirane-dienone dimer of α-tocopherol.

Oxidation of α-tocopherol in Acetonitrile with Benzoyl Peroxide

The method described previously (Goodhue and Risley, 1964) was used except that purified acetonitrile was substituted for benzene. Acetonitrile was purified according to the directions of Dürckheimer and Cohen (1964). The γ_{max}^{hexane} of the product was 302 mμ. Ultraviolet spectra of product and of 5-benzoyloxymethyl-γ-tocopherol were identical. Upon shaking the acetonitrile solution of the product with 1 N KOH a product was obtained whose infrared spectrum was identical with the spectrum of an authentic sample of the spirane-dienone dimer of α-tocopherol (Nelan and Robeson, 1962).

5-Bromomethyl-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (5-Bromomethyl-γ-tocopherol)

A solution of bromine (0.235 g, 1.47 mmoles) in 15 ml of hexane (dried over sodium) was mixed rapidly with a solution of 0.5 g d-α-tocopherol (1.16 mmoles) in 200 ml dry hexane at room temperature. After 2 hours in ordinary laboratory light, the mixture became nearly colorless. Solvent was removed under vacuum. Yield of the straw-colored oil was 0.585 g (99.4%); γ_{max}^{hexane} 315 mμ (ε 3800). Infrared spectrum (neat) was similar to spectrum of α-tocopherol but the OH band was displaced to 2.83 μ.

Anal. Calcd for $C_{29}H_{49}O_2Br$: mw 509.6; C, 68.5; H, 9.7; Br, 15.7. Found: mw 513; C, 68.5; H, 9.8; Br, 15.4.

A solution of the product (0.125 g in 25 ml hexane) was shaken with 10 ml of 1 N KOH. The hexane layer became yellow instantly. Shaking was continued for 10 minutes. The hexane layer was removed, washed with water, and dried over Na_2SO_4. Solvent was removed. The ultraviolet and infrared spectra of the product and of the authentic spirane-dienone dimer of α-tocopherol were superimposable.

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We wish to thank D. N. Nelan for generous samples of the spirane-dienone dimer and 8a-ethoxy-α-tocopherone. The ultraviolet and infrared spectrographic measurements were made by Mr. W. Blum of this Laboratory using a Cary recording spectrophotometer, Model 11M, and a Perkin-Elmer spectrophotometer, Model 21, respectively. The microanalyses and molecular weight determinations were done by the Microanalytical Laboratory of the Eastman Kodak Co. under the direction of Mr. Donald Ketchum.

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