REACTIONS OF VITAMIN E WITH PEROXIDES I. REACTION OF BENZOYLPEROXIDE WITH d-α-TOCOPHEROL IN HYDROCARBONS*

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Inglett and Mattill (Inglett, 1955) reported that the reaction of benzoylperoxide with a benzene solution of α-tocopherol gave α-tocopheryl-p-quinone in 23-53% yields. They suggested that the quinone was formed by hydrolysis of an intermediate such as Ia. We have been especially interested in obtaining Ia, since it is a carboxylic ester analog of Ib, a proposed intermediate in oxidative phosphorylation (Clark, 1960; Dallam, 1961).

We were unable to obtain intermediate Ia in a series of reactions run at temperatures ranging from -40° to 80° in anhydrous benzene or hexane under nitrogen. However, these reactions yielded a compound to which we assigned the structure IIa (λ max 302 mμ). Benzoic acid was removed by crystallization from hexane. Further attempts to purify IIa by chromatography on alumina and magnesium silicate or shaking the crude reaction mixture with aqueous N KOH resulted in rapid formation of a compound previously identified as the spirane-dienone dimer of α-tocopherol (Nelan, 1962) as shown by correlation of ultraviolet and infrared spectra with the spectra of authentic dimer. The dimer may

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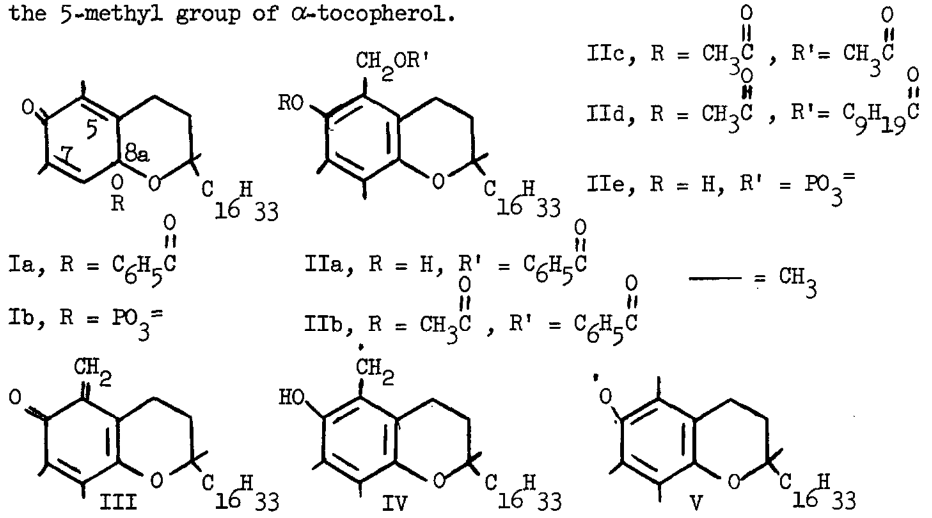
have been formed by 1,4-elimination of benzoic acid from IIa to give the o-quinone methide (III) followed by dimerization. Similar reactions of o-quinone methides have been noted by others (e.g. Merijan, 1963). Formation of the dimer --- shown to be coupled through the 5-methyl group (Nelan, 1962; McHale, 1964; Schudel, 1963) --- from IIa provided evidence that substitution of the benzoyloxy radical of IIa also was on the 5-methyl group of α-tocopherol.



IIc, $R = CH_3\overset{O}{\overset{\|}{C}}$, $R' = CH_3\overset{O}{\overset{\|}{C}}$

IId, $R = CH_3\overset{O}{\overset{\|}{C}}$, $R' = C_9H_{19}\overset{O}{\overset{\|}{C}}$

IIe, $R = H$, $R' = PO_3^{=}$

Ia, $R = C_6H_5\overset{O}{\overset{\|}{C}}$

Ib, $R = PO_3^{=}$

IIa, $R = H$, $R' = C_6H_5\overset{O}{\overset{\|}{C}}$

IIb, $R = CH_3\overset{O}{\overset{\|}{C}}$, $R' = C_6H_5\overset{O}{\overset{\|}{C}}$

—— = CH_3

The structure of IIa was proven by comparing UV, IR and NMR spectra of its acetate with those of authentic acetate IIb obtained by reaction of sodium benzoate with 5-bromomethyl-γ-tocopheryl acetate (Nelan, 1964). Mild acetylation of IIa produced the diacetate IIc as well.

We have made IIc, and the decanoate IId by reaction of α-tocopherol with the appropriate acylperoxide, followed by acetylation. The unacetylated compounds exhibited the instability characteristic of the benzoate IIa.

Inglett and Mattill obtained 5-benzoyloxymethyl-2,2,7,8-tetramethyl-6-hydroxychroman in benzene (Inglett, 1955), but they did not report the formation of IIa when α-tocopherol was substituted for the pentamethylhydroxychroman. Compound IIa may not have been observed because it decomposed and dimerized. In this connection, we have noted that the compound designated bis(dl-α-tocopher-6-one)peroxide in Inglett's thesis

(Inglett, 1952) has an infrared spectrum which appears to be identical to that of the spirane-dienone dimer. α-Tocopheryl-p-quinone, the major product identified by Inglett and Mattill may have been the result of the reaction of the α-tocopheryl radical with water, producing a hemiketal. This hemiketal rapidly decomposes to α-tocopheryl-p-quinone (Dürckheimer, 1962).

Though we have no proof that homolytic reactions are involved, we suggest that IIa was formed by coupling of a benzoyloxy radical with α-tocopheryl radical IV. An alternative mechanism would involve addition of benzoic acid to the quinone methide (III). Experiments indicate that benzoylperoxide did not react with α-tocopheryl acetate, so we believe that radical IV was formed from radical V, as previously suggested (Skinner, 1963).

Formation of IIa is an example of preferential reaction at the 5-methyl as opposed to the 7-methyl position of α-tocopherol. The major product of the benzoylperoxide reaction with α-tocopherol was IIa (50-70%) but since all by-products were not identified, reaction at the 7-methyl position was not excluded. The bulk of the identified by-products consisted of spirane-dienone dimer (5-10%) and α-tocopheryl-p-quinone (5-10%).

Biochemical mechanisms for oxidative (or photo) phosphorylation based on known chemical reactions involving either the 3-methyl group of vitamin K or the 5-methyl group of coenzyme Q have been proposed (Mamont, 1963; Erickson, 1963). The special reactivity of the analogous 5-methyl group of α-tocopherol reported herein suggests that α-tocopherol may participate in similar biochemical reactions.

Finally, we have shown that α-tocopherol dimers may be formed by an alternate pathway that probably does not involve direct coupling of two radicals such as IV, namely that IIa can decompose and dimerize. Therefore, isolation of α-tocopherol dimers from *in vivo* or *in vitro* oxidations does not exclude the existence of intermediate compounds such as

IIe that might have a function more specific than that of free radical scavengers.

5-Benzoyloxymethyl-γ-tocopherol (IIa) Benzoylperoxide (0.533 g., 2.32 mmoles) was added to 1 g of d-α-tocopherol (2.32 mmoles) in 200 ml of benzene (dried over sodium). Nitrogen was bled through the system at a moderate rate. The reaction was started at room temperature, then heated to reflux for a total reaction time of 12 minutes. 5 mg of $NaBH_4$ was added to decompose unreacted peroxide. The mixture was concentrated to 50 ml on a flash evaporator and benzoic acid crystals were removed by filtration. λ_{max}^{hexane} 302 mμ. IR bands - 3.01 μ (phenolic OH); 5.91 μ (benzoate carbonyl); 7.82 μ (benzoate); 6.24, 6.32, 14.04 μ (phenyl).

5-Benzoyloxymethyl-γ-tocopheryl acetate (IIb) Acetic anhydride (0.23 ml) and pyridine (0.38 ml) was added to the solution of IIa and the solution was allowed to stand at room temperature overnight. Solvent was removed and the oily residue was chromatographed on a column, 3.7 x 20 cm, of magnesium silicate (Florisil, 60-100 mesh Floridan Co.) by elution with 10% diethyl ether in hexane. First 90-ml fract. discarded (contains dimer--and other products). Fraction 2 (590 ml) contained IIb (847 mg). Fraction 3 (250 ml) contains α-tocopheryl-p-quinone, benzoic acid and other unidentified materials. Fraction 2 was rechromatographed on neutral alumina (Brockman Grade III, Woelm) to obtain analytical samples. IIb was eluted with second 300 ml of 4% diethyl ether in hexane. Small amounts of IIc come off in succeeding fractions. λ_{max}^{hexane} - 273 mμ (ε 2350); 291 mμ (ε 3200); 293 mμ (ε 3120). IR bands - 5.62 μ (acetate carbonyl); 5.76 μ (benzoate carbonyl); 6.24 μ, 6.31 μ, 14.09 μ (phenyl); 6.89 μ (benzoate); 7.31 μ (acetate); 9.25 μ (chroman). Calc. for $C_{38}H_{56}O_5$; sapon. eq. 296; C, 77.0; H, 9.52. Found sapon. eq. 292; C, 77.3; H, 9.5.

α-Tocopherol dimer Crude IIa in benzene solution (from above preparation) was shaken 5 minutes with 100 ml of N KOH. A bright yellow color developed immediately. Product was worked up according to Nelan and Robeson (Nelan, 1962). λ_{max}^{hexane} 302 mμ (ε 4620); 338 mμ (ε 1900). IR bands 5.97, 6.03, and 6.27 μ (conjugated carbonyl and conjugated double bond).

5-Decanoyloxymethyl-γ-tocopheryl acetate (IId) Decanoylperoxide was substituted for benzoylperoxide in the syntheses of IIa and IIb. λ_{max}^{hexane} 284 mμ (ε 2750); 291 mμ (ε 2940). IR bands 5.65 μ (acetate carbonyl); 5.73 μ (decanoate carbonyl); 7.31 μ (acetate); 8.64 μ (decanoate); 9.25 μ (chroman). Calc. for $C_{42}H_{72}O_5$; C, 76.8; H, 11.05. Found C, 76.6; H, 11.1.

5-Acetoxymethyl-γ-tocopheryl acetate (IIc) Acetylperoxide (25% in dibutylphthalate) was substituted for benzoylperoxide in the syntheses of IIa and IIb. λ_{max}^{hexane} were the same as for IId (above). IR data show presence of two acetate groups by bands at 5.66 and 5.73 μ and 8.26 and 8.34 μ. Calc. for $C_{33}H_{54}O_5$; C, 74.7; H, 10.3. Found C, 74.9; H, 10.1.

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