ture, showing that a conformational interchange takes place readily and that the levorotatory quasi-axial conformer is the form of *higher* energy, since its concentration increases with increasing temperature. This is consistent with the second alternative discussed above, subject only to the restriction with regard to a lack of significantly large entropy differences between the conformers. In contrast, the rotation of III under the same conditions remains practically constant; in this case, therefore, the energy difference between the two possible conformations appears to be much larger than that of (−)-α-phellandrene, thus favoring one of the conformations to the practical exclusion of the other.

Since the reduced rotational strength of skewed dienes is known[2] to be ~25, as compared to ~200 for hexahelicene[7] and ~2.5 for cyclic ketones, the rotatory contributions of IA and IB, while different, should be of the same order of magnitude. Consequently, the relatively low rotation of (−)-α-phellandrene (Table I) shows that the concentration of IA does not differ from that of IB by more than an order of magnitude at room temperature; *i.e.*, a substantial concentration of the quasi-axial form exists at room temperature. An unequivocal quantitative evaluation of the composition of the conformational equilibrium mixtures would require the experimental determination or theoretical calculation[2,8] of the rotational strength of both conformers. In the case of the theoretical calculations, the determination of the skew angles would have to take into account non-bonded repulsions which cannot be determined from Dreiding models.

Acknowledgment.—We express our appreciation to Mr. Joseph Colony of the Department of Biochemistry, Walter Reed Hospital, Washington, D. C., for the gas-chromatographic purification of (−)-α-phellandrene and to Mr. H. K. Miller of this Institute for making available the Rudolph recording spectropolarimeter on which these measurements were made.

(7) A. Moscowitz, Ph.D. Thesis, Harvard University, March, 1957.

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[COMMUNICATION 281 FROM THE RESEARCH LABORATORIES OF DISTILLATION PRODUCTS INDUSTRIES, DIVISION OF EASTMAN KODAK CO., ROCHESTER 3, N. Y.]

The Oxidation Product from α-Tocopherol and Potassium Ferricyanide and Its Reaction with Ascorbic and Hydrochloric Acids

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Interest in oxidation products of vitamin E as possible metabolic intermediates has led us to investigate some of the physical and chemical properties of a product from the oxidation of α-tocopherol with potassium ferricyanide. A dimeric structure, containing a dihydropyran ring and dienone group, is postulated for the compound. Structures are also postulated for its reaction products with ascorbic and hydrochloric acids. All three compounds had less than 2% of the activity of *d*-α-tocopheryl acetate in the rat gestation–resorption bioassay.

We have oxidized α-tocopherol (both *d*- and *dl*-) with potassium ferricyanide and have investigated the structure of the oxidation product. This product had an absorption maximum in the ultraviolet at 300 mμ with a weaker broad band at 337 mμ. Such oxidation products assume interest in view of current studies on metabolic transformations of α-tocopherol (*e.g.*, Alaupovic, *et al.*[1]).

The properties of the oxidation product indicated that it has the dimeric structure I arising by coupling through *o*-methyl groups and ring closure to form a new dihydropyran ring. An analogous structure has been reported for the oxidation product from 2,4-di-*tert*-butyl-6-methylphenol and silver oxide.[2]

Martius and Eilingsfeld[3] described the oxidation of α-tocopherol with alkaline potassium ferricyanide and obtained a yellow compound of undetermined structure which was stated to have an ultraviolet absorption maximum at 235–236 mμ with a weaker band at 300 mμ. We followed the oxidation procedure described by these authors, but did not obtain a compound having this property. Issidorides[4] has reported the formation of a dimer by oxidation of α-tocopherol with potassium permanganate and Boyer[5] has described a dimer formed from α-tocopheroxide, but from the reported properties of these products, neither appears to have structure I.

The proposed structure I was based on the following findings: (1) molecular weight determination and elementary analyses determined on a sample purified by chromatography indicated a compound formed from two molecules of the parent tocopherol; (2) the infrared absorption spectrum showed bands characteristic of a conjugated unsaturated ketone, but no band characteristic of a hydroxy compound; a strong band at 9.13 μ, not shown by α-tocopherol, indicated the presence of an additional ether group; (3) the ultraviolet absorption spectrum showed maxima at wave lengths (300, 337 mμ) in the range characteristic of linearly conjugated, dieneone systems[6]; (4) further slow oxidation (Fig. 1) of the compound occurred on reaction with ferric chloride in the Emmerie–Engel assay procedure[7] suggesting the

(1) P. Alaupovic, B. C. Johnson, Q. Crider, H. N. Bhagavan and B. J. Johnson, *Am. J. Clin. Nutrition*, **9**, Part II (1961).

(2) R. F. Moore and W. A. Waters, *J. Chem. Soc.*, 243 (1954).

(3) C. Martius and H. Eilingsfeld, *Ann.*, **607**, 159 (1957).

(4) A. Issidorides, *J. Am. Chem. Soc.*, **73**, 5146 (1951).

(5) P. D. Boyer, *ibid.*, **73**, 733 (1951).

(6) H. Wynberg, *Chem. Rev.*, **59**, 169 (1959).

(7) H. Rawlings, *Oils and Soap*, **21**, 257 (1944).

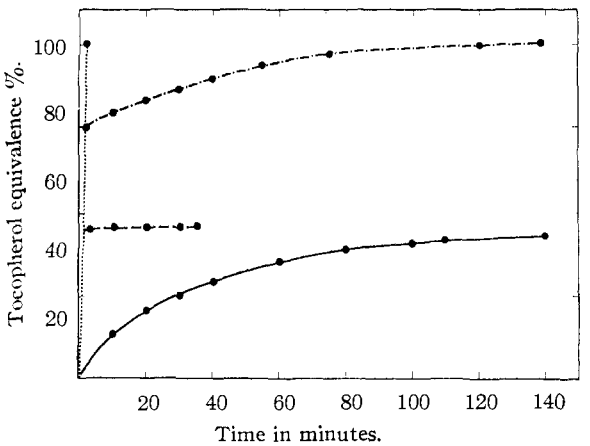


Fig. 1.—Oxidation curves of ferricyanide oxidation product I (———), product II (— —), product III (· — ·), and α-tocopherol (.....) in Emmerie–Engel assay procedure.

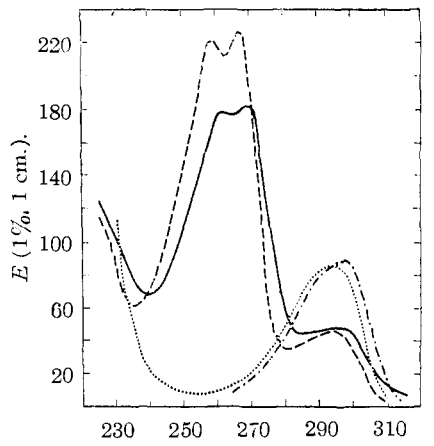
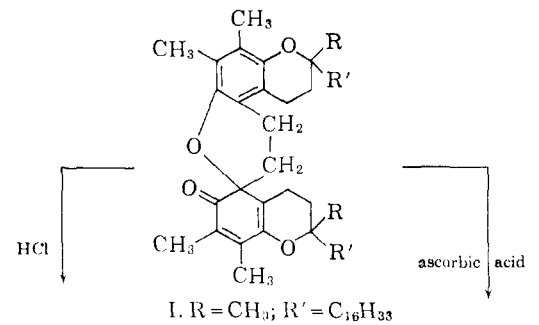
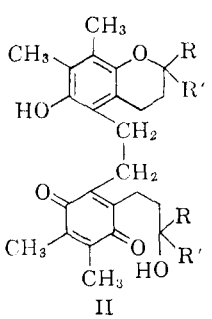


Fig. 2.—Ultraviolet absorption spectra of product II (———), 50:50 mixture of *d*-α-tocopherol and α-tocoquinone (— —), product III (— · —) and *d*-α-tocopherol (.....).

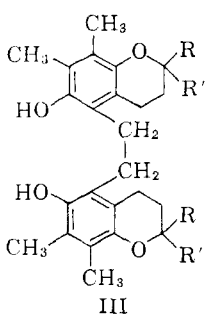
formation by alcoholysis under these conditions of a second oxidizable group, such as might arise by opening of the pyran ring; and (5) treatment with hydrochloric acid and with ascorbic acid gave, respectively, compounds with properties consistent with structures II and III.



I. R = CH_3; R′ = $C_{16}H_{33}$



II



III

The reaction of the oxidation product from α-tocopherol and potassium ferricyanide with the acid reagents to give II and III resembles similar reactions of the "α-tocopheroxide" of Boyer[5] which has been shown to have a quinone acetal structure[8] and to be converted to α-tocoquinone with acids and to α-tocopherol with ascorbic acid. The formation of II and III from I might thus be expected in view of the vinylogous acetal group in the proposed structure I.

Evidence for the structure of II was as follows: (1) the compound was found to have a molecular weight approximately twice that of α-tocopherol; (2) its ultraviolet absorption spectrum indicated that the molecule contained both a tocopherol and tocopheryl quinone moiety: the similarity of the spectrum to that of a 50:50 mixture of α-tocopherol and α-tocoquinone is shown in Fig. 2; (3) the strong band at 2.9 μ (hydroxyl) in the infrared was approximately equivalent to that of α-tocoquinone, showing, for a dimeric compound, the presence of two hydroxyl groups; (4) response in the Emmerie–Engel assay procedure was approximately equivalent to one-half that of α-tocopherol (Fig. 1), which was consistent with the presence of an oxidizable phenol group; and (5) on oxidation with ferric chloride a crystalline compound was obtained having the properties expected for the corresponding diquinone.

Evidence for the structure of III was as follows: (1) it was found to have a molecular weight approximately twice that of α-tocopherol; (2) the ultraviolet absorption curve (Fig. 2) was essentially the same as that of α-tocopherol, indicating, for a dimeric compound, the presence of two α-tocopherol moieties; (3) its infrared absorption spectrum showed the same characteristic bands as α-tocopherol (see Experimental); and (4) response in the Emmerie–Engel assay procedure (Fig. 1) was equivalent to that of α-tocopherol, when sufficient time was given to complete the reaction, further confirming the presence of two oxidizable hydroxyl groups. The fast initial oxidation of III followed by a slower second stage is considered due to partial oxidation of III to I in the first stage followed by the slower oxidation of I. Martius and Eilingsfeld[3] have reported a similar interpretation of the apparent, two-stage oxidation of monoethers of durohydroquinone with ferric chloride in alcohol in the presence of *o*,*o*′-phenanthroline.

The compounds I, II and III showed less than 1/50 of the vitamin E activity of *d*-α-tocopheryl acetate as measured by the gestation–resorption bioassay of Mason and Harris.[9] These assays were done by the Biochemistry Department of this Laboratory under the direction of Dr. P. L. Harris.

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

(9) K. E. Mason and P. L. Harris, *Biological Symp.*, **12**, 459 (1947).

The oxidation products from *d*- and *dl*-α-tocopherol differ in one property. The purified oxidation product from *d*-α-tocopherol has a relatively high specific rotation, $[\alpha]^{25}D$ +31.5°; the oxidation product from synthetic *dl*-α-tocopherol shows no optical rotation. This provides a method for distinguishing between the two which has been reported separately[10] in more detail. Such a method has been needed because *d*-α-tocopherol has 36% greater vitamin E activity than *dl*-α-tocopherol.[11]

Experimental

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. Isooctane was the solvent used for the ultraviolet determinations. Melting points were determined in capillary tubes, using 3-inch immersion thermometers. The microanalyses and molecular weight determinations (ebullioscopic, benzene) were done by the Microanalytical Laboratory of the Eastman Kodak Co. under the direction of Mr. Donald Ketchum.

Preparation of Compound I.—A sample of *d*-α-tocopherol (4.0 g.) in 500 ml. of petroleum ether (Skellysolve F) was shaken in a separatory funnel 3 minutes with a solution of potassium ferricyanide (10.0 g.) in 100 ml. of 0.2 *N* sodium hydroxide. There was a momentary dark color formed in the petroleum ether layer followed by a bright yellow color. The petroleum ether layer was separated, washed with water several times, dried over anhydrous sodium sulfate, and the solvent removed to give 4.2 g. of yellow oil, $E(1\%, 1\text{ cm.})$(300 mμ, 337 mμ) = 43.6, 15.0.

The oxidation product was dissolved in petroleum ether (Skellysolve F) and chromatographed on a column (1″ × 8″) of magnesium silicate (Florisil, 60–100 mesh, Floridin Co.). The middle portion of the main yellow zone was eluted and rechromatographed on another column of Florisil and the middle portion eluted for an analytical sample. The product was a yellow oil, 2.37 g., $E(1\%, 1\text{ cm.})$ (300, 337 mμ) = 53.8, 22.1. The infrared spectrum showed bands at 5.97, 6.03 and 6.27 μ (carbonyl and conjugated double bonds) and at 7.96, 9.13, 10.36 μ (ether).

Anal. Calcd. for $C_{58}H_{98}O_4$: mol. wt., 859; C, 81.1; H, 11.5. Found: mol. wt., 778; C, 82.3; H, 12.0.

The observed molecular weight, while lower than calculated, was in the range for a dimeric molecule. The discrepancy may be due to failure to effect complete purification by chromatography since the value for the crystalline oxidation product, m.p. 126–127°, $E(1\%, 1\text{ cm.})$(300 mμ, 337 mμ) = 108, 51, from 2,2,5,7,8-pentamethyl-6-hydroxychroman prepared by the procedure of Smith, *et al.*,[12] showed good agreement with theory.

Anal. Calcd. for $C_{28}H_{38}O_4$: mol. wt., 438; C, 76.7; H, 8.7. Found: mol. wt., 426; C, 76.6; H, 8.4.

Conversion of Compound I to Compound II.—To a solution of the oxidized product I (2.7 g.) in ether (40 ml.) and ethanol (125 ml.) was slowly added 1.5 ml. of concentrated hydrochloric acid in ethanol (15 ml.). After 0.5 hour, the solution was diluted with petroleum ether (250 ml.) and washed with water to remove the alcohol and acid. The petroleum ether solution, after drying over anhydrous sodium sulfate, was evaporated to approximately 25 cc. and chromatographed on a column of an acid clay adsorbent (Hilite, green label, Hillard Corp.). The chromatogram was developed with petroleum ether containing ethyl ether (5%) to give a strongly held brownish zone which on elution with ether yielded an amber colored oil (1.5 g.). The ultraviolet absorption spectrum showed max. 262, 269 mμ (doublet), $E(1\%, 1\text{ cm.})$ = 197, 203, with a shoulder at 295 mμ, $E(1\%, 1\text{ cm.})$ = 52. The infrared absorption spectrum showed bands characteristic of both hydroxyl (2.9 μ) and quinone (6.1 μ) groups.

Anal. Calcd. for $C_{58}H_{98}O_5$: mol. wt., 875; C, 79.6; H, 11.3. Found: mol. wt., 796; C, 79.2; H, 11.1.

Oxidation of Compound II with Ferric Chloride.—A sample of compound II (1 g.) dissolved in ether (12 ml.) was shaken successively with three portions (4 ml. each) of a solution of ferric chloride hexahydrate (1 g.) dissolved in methanol (6 ml.) and water (6 ml.). After washing the ether layer several times with water, it was dried over anhydrous sodium sulfate, and evaporated. The residue was taken up in petroleum ether (10 ml. of Skellysolve F) and chromatographed on a small column of an acid clay adsorbent (Hilite, blue label, Hillard Corp.). Elution of the adsorbed fraction with ether gave, on evaporation, a yellow oil (0.62 g.) having $E(1\%, 1\text{ cm.})$(264 mμ) = 331. On crystallization from isopropyl alcohol at 5°, yellow crystals were obtained, m.p. 45–46°, $E(1\%, 1\text{ cm.})$(264 mμ) = 389.

Anal. Calcd. for $C_{58}H_{98}O_6$: mol. wt., 891; C, 78.1; H, 11.1. Found: mol. wt., 924; C, 78.5; H, 11.8.

Conversion of Compound I to Compound III.—To a solution of the oxidized product I (1.3 g.) in ether (20 ml.), ethanol (160 ml.) and water (5 ml.) was added ascorbic acid (4 g.). After standing overnight at room temperature, the reaction was taken up in petroleum ether (300 ml.) and washed with water. The petroleum ether solution was then dried over anhydrous sodium sulfate, filtered, and evaporated to give a light yellow oil having $E(1\%, 1\text{ cm.})$(297 mμ) = 76.7. The product was further purified by chromatographing it on a column of sodium aluminum silicate adsorbent (Doucil, Philadelphia Quartz Co.) to give a fraction (0.65 g.) having $E(1\%, 1\text{ cm.})$ (297 mμ) = 87. Its infrared absorption spectrum was very similar to that of α-tocopherol with a strong band at 2.9 μ (OH) and none in the carbonyl region. When the spectrum was determined in solution (CS_2), there was a smaller shift in location of the hydroxyl band (to 2.85 μ) than occurs with α-tocopherol (to 2.77 mμ). This indicated intramolecular bonding of the –OH group.

Calcd. for $C_{58}H_{98}O_4$: mol. wt., 859. Found: mol. wt., 785.

Preparation of Diacetate of III.—To a solution of compound III (0.3 g.) in pyridine (2 ml.) was added acetic anhydride (2 ml.). After warming the mixture to 70° for 2 hours, it was diluted with petroleum ether (Skellysolve F, 50 ml.) and washed successively with dilute hydrochloric acid, sodium bicarbonate solution, and finally with water until the washings were neutral. The solution was dried over anhydrous sodium sulfate, the solvent evaporated, and the residue (0.32 g.) crystallized from isopropyl alcohol (2 ml.) at 5°. The white crystals melted at 74–75° and had $E(1\%, 1\text{ cm.})$(287 mμ) = 55.1.

Anal. Calcd. for $C_{62}H_{102}O_6$: mol. wt., 943.5; C, 78.9; H, 10.9. Found: mol. wt., 972; C, 79.3; H, 11.1.

Ferric Chloride Assay.—The oxidizable phenolic groups in compounds I, II and III were determined with ferric chloride and α,α′-dipyridyl by the Emmerie–Engel procedure as modified by Rawlings.[7] Readings were taken at intervals until the oxidations appeared to be complete. Results are shown in Fig. 1.

(10) D. R. Nelan and C. D. Robeson, *Nature*, **193**, 477 (1962).

(11) "National Formulary," 11th ed., Mack Printing Co., Easton, Pa., p. 459.

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