and the aqueous phase further extracted with two 25-ml. portions of ether. The combined ether solution was dried over Drierite and then acidified to the congo red end point with aqueous ethanolic perchloric acid. The crystalline material (platelets) which separated represented a quantitative yield of N-(1-benzyloxymethylcyclohexyl)pyrrolidine perchlorate, recrystallized from ethanol–ether, m.p. 114–115°; ν_{max}^{KB} 3100 cm.$^{-1}$; n.m.r. τ values (deuterichloroform), 1.8 to 2.5 (unresolved), 2.56, 5.39 (singlet, $C_6H_5C_2\underline{H}O$), 6.27 (singlet, $OC\underline{H}_2C\leq$), 6.35 to 6.90 (unresolved multiplet), 7.65 to 9.05 (unresolved multiplet).

Anal. Calcd. for $C_{18}H_{28}ClNO_5$: C, 57.82; H, 7.55; N, 3.75. Found: C, 57.81; H, 7.43; N, 3.62.

Oxidation Products of Vitamin E and Its Model, 6-Hydroxy-2,2,5,7,8-pentamethylchroman. V. Studies of the Products of Alkaline Ferricyanide Oxidation

W. A. SKINNER AND P. ALAUPOVIC

Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, California, and the Cardiovascular Section, Oklahoma Medical Research Institute, and Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma

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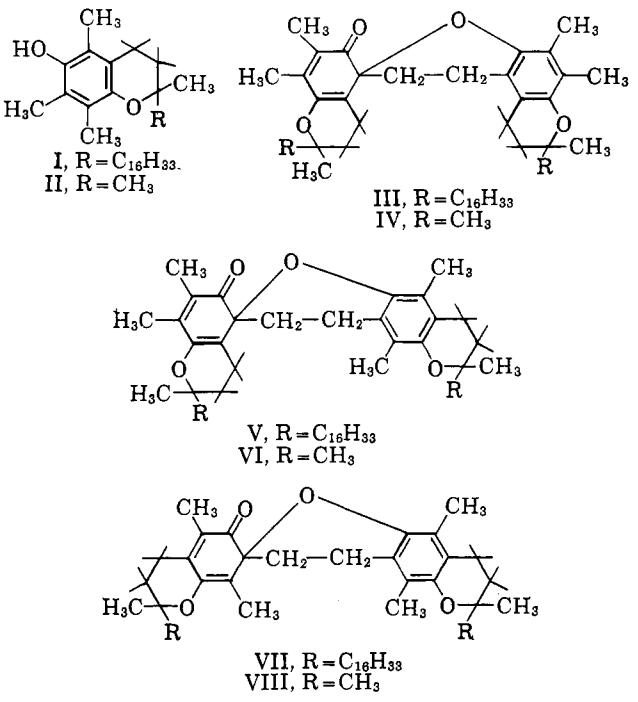
In addition to the previously reported dimeric, keto ether III or IV, other products of the alkaline ferricyanide oxidation of *dl*-α-tocopherol and its model, 6-hydroxy-2,2,5,7,8-pentamethylchroman, were isolated and one of them, compound A, a trimer, was proposed to be XIV or XV. From the spectral and chromatographic characteristics of compound A it appears to be the same as one of the liver metabolites (compound "O") previously obtained from *dl*-α-tocopherol. The free radical initiated oxidation of 6-hydroxy-2,2,5,7,8-pentamethylchroman was also studied and the products compared with those from the alkaline ferricyanide oxidation.

With increasing interest in the biological role of vitamin E, chemical and metabolic studies of the tocopherols are assuming major importance. The chemical oxidation of *dl*-α-tocopherol (I) and comparison of the oxidation products with the metabolities formed in the body would appear to be a promising approach to the identification of these metabolities and for obtaining sufficient quantities of them for their biological evaluation. With this approach in mind, studies on the oxidation of *dl*-α-tocopherol (I) and its model, 6-hydroxy-2,2,5,7,8-pentamethylchroman (II), with alkaline ferricyanide were undertaken.

Martius and Eilingsfeld,[1] Draper, *et al.*,[2] Nelan and Robeson,[3,4] and Schudel, *et al.*,[5] have reported on the alkaline ferricyanide oxidation of *d*- or *dl*-α-tocopherol and in some cases the model chroman II. Some confusion arose due to the difficulty of purifying the oxidation products from one another. Martius reported an absorption maximum of the yellow oily product from *dl*-α-tocopherol oxidation to be at 235–236 mμ with a weaker band at 300 mμ. Draper, *et al.*, reported a maximum at 295 mμ, $E_{1cm}^{1\%}$ 36, while Nelan and Robeson reported the maximum to be at 300 mμ, $E_{1cm}^{1\%}$ 53.8. Our spectral data on this product gave a maximum at 300 mμ, $E_{1cm}^{1\%}$ 56. Chemical studies on this yellow oil from the alkaline ferricyanide oxidation of *dl*-α-tocopherol led Nelan and Robeson to postulate structure III for it. However, three other isomers also would fit the chemical data. They are V, VII, and IX and differ only by positions of methyl groups. Nelan and Robeson[3,4] also reported that the oxidation of II yielded a yellow crystalline compound, m.p. 126–127°, that was analyzed for the dimer IV.

In our laboratories[6] we have found the yellow, crystalline dimer from the oxidation of II and the oils from the oxidation of I to be extremely difficult to purify from



other isomeric materials, especially trimer XV. After repeated chromatography on a silica gel (Schlesinger) column and fractional crystallization from methanol–water, an analytical sample of the dimer [($C_{28}H_{36}O_4$), m.p. 77–79°, mol. wt. (in benzene), 433 (calcd. 436)] was obtained from the oxidation of II with alkaline ferricyanide. This compound no longer showed even trace spots due to isomeric impurities on silica gel thin-layer chromatography. The infrared and ultraviolet absorption maxima agreed with those reported by Nelan and Robeson, *i.e.*, ultraviolet maximum at 300 mμ, and in the infrared the absence of OH (2.90–3.00 μ) and presence of α,β-unsaturated C=O (5.98), aryl (6.05), C=C (6.28), and chroman C—O—C (9.15 μ).

Since the nuclear magnetic resonance spectra of the yellow oil from the oxidation of I and the crystalline solid from the oxidation of II do not enable one to distinguish between the four possible structures of the di-

(1) C. Martius and H. Eilingsfeld, *Ann.*, **607**, 159 (1959).
(2) H. H. Draper, A. S. Csallany, and S. N. Shah, *Biochim. Biophys. Acta*, **59**, 527 (1962).
(3) D. R. Nelan and C. D. Robeson, *Nature*, **193**, 477 (1962).
(4) D. R. Nelan and C. D. Robeson, *J. Am. Chem. Soc.*, **84**, 2963 (1962).
(5) P. Schudel, H. Mayer, R. Rüegg, and O. Isler, *Chimia* (Aarau), **16**, 368 (1962).
(6) W. A. Skinner and P. Alaupovic, *Science*, **140**, 803 (1963).

meric keto ether, a colorless, maleic anhydride adduct, m.p. 143–145°, of the yellow crystalline dimer from the oxidation of II was prepared. Nuclear magnetic resonance spectroscopy of this adduct showed that it possessed a single methyl group on an isolated carbon–carbon double bond (τ 8.38) proving the correct structure to be IV or VI rather than VIII or X.

While our studies were under way, Schudel, *et al.*,[5] reported a clever structural proof for the dimeric keto ether being IV in the case of the model chroman and III in the case of the tocopherol derivative as postulated by Nelan and Robeson. The maleic adduct would possess structure XI.

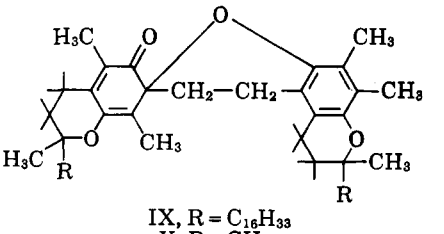
In our studies of the alkaline ferricyanide oxidation of I and II we have found that a number of products are produced in addition to the previously reported keto ethers III and IV. These products could be separated on silica gel thin-layer chromatography using hexane–chloroform (2:1) for the tocopherol derivatives and chloroform as the developing solvent for the chromatograms of the model derivatives. Column chromatography utilizing Florisil, neutral alumina (Brockmann activity I), or silica gel (Schlesinger) was used for separation of larger quantities of materials. In addition to III or IV, at least three other products are produced by the oxidation. These compounds are colorless oils in the case of the tocopherol derivatives and colorless solids in the case of the model derivatives.

Identification of one of the products as the dihydroxy dimer (XII) previously prepared[3,4] by ascorbic acid reduction of III was accomplished by comparison of its spectral characteristics and silica gel thin-layer characteristics with an authentic sample produced by lithium aluminum hydride reduction of III. A diacetate, m.p. 88–89°, prepared from this reduction product gave an excellent elementary analysis. In the case of the oxidation of the model compound II a colorless crystalline dihydroxy dimer XIII, m.p. 188°, was obtained. The same compound was prepared by the ascorbic acid reduction of IV.

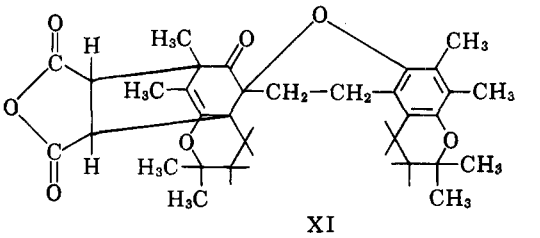
The two other products isolated from the alkaline ferricyanide oxidation of I were colorless oils and were designated as compounds A and B. Compounds A and B, which were found to be trimeric, were inseparable in a variety of paper and thin-layer chromatographic systems but could be separated by silica gel thin-layer chromatography using hexane–chloroform (2:1) or (3:1) as the developing solvent. The infrared and ultraviolet absorption spectra (of compounds A and B) were quite similar; maxima 294 mμ, absence of OH (2.90–3.00 μ), presence of α,β-unsaturated C=O (5.92), aryl (6.10), and chroman C—O—C (9.15 μ). Compounds A and B are trimeric, mol. wt. in benzene by vapor pressure (Mechrolab), 1200 and 1100 (calcd. 1284), with empirical formulae of $C_{87}H_{144}O_6$.

From the oxidation of the model compound only compound A, m.p. 228–230°, $C_{42}H_{54}O_6$, mol. wt. of 659 (calcd. 654) with a maximum at 294 mμ in the ultraviolet has thus far been isolated.

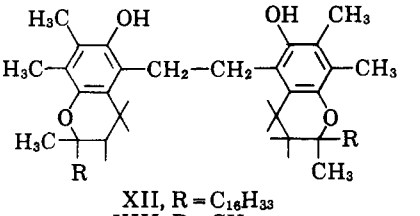
The trimers A from *dl*-α-tocopherol and the model chroman also can be obtained by heating I and III, or II and IV. In addition, XII and XIII also are produced. Compound B can be produced by heating I and III. In view of these results and by analogy with the known structure of quinone methide trimers,[7] structures XIV and XV are proposed for compound A. The strong carbonyl band (5.92 μ) of compound A and a comparison of its ultraviolet spectrum with that of the dimer and the starting chroman indicates that it possesses an α,β-unsaturated ketone function. The structure



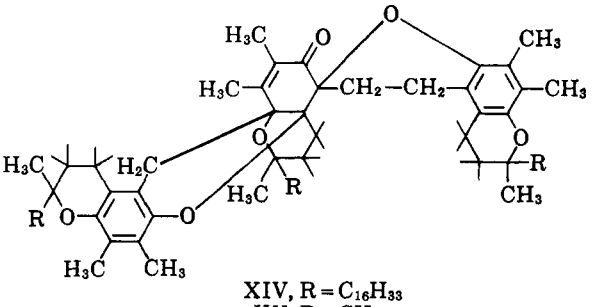
IX, R = $C_{16}H_{33}$
X, R = CH_3



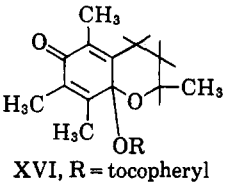
XI



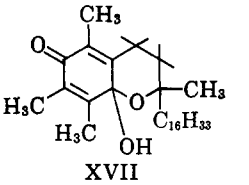
XII, R = $C_{16}H_{33}$
XIII, R = CH_3



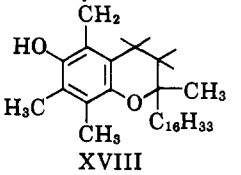
XIV, R = $C_{16}H_{33}$
XV, R = CH_3



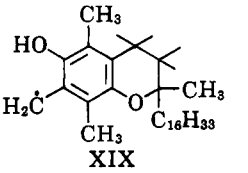
XVI, R = tocopheryl



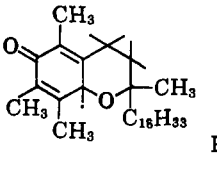
XVII



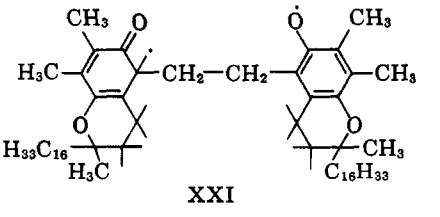
XVIII



XIX



XX



XXI

(7) S. B. Covitt, H. Sarrafizadeh R, and P. D. Gardner, *J. Org. Chem.*, **27**, 1211 (1962).

for A, wherein the newly formed ring system has the oxygen and methylene groups reversed, is also possible as are the isomers in which the methyls on the added aromatic ring are *para* to one another. The resistance of compound A towards acid hydrolysis would tend to eliminate the ring system containing the reversed oxygen and methylene groups. The paper chromatographic behavior of compounds A (XIV) and compound B resembles that of the liver metabolite "O" previously reported by Alaupovic, *et al.*[8] (Recently, Csallany and Draper[16] isolated a metabolite similar to metabolite "O" and on the basis of its chromatographic behavior suggested that it was dimer III. However, it is not possible to differentiate between the dimer III and trimers A or B with paper chromatographic systems used by these workers.) Further work on the structure of the compound A from I and II are under way.

The mechanism of the alkaline ferricyanide oxidation of I and II is of interest. Martius[1] reported that the oxidation of methyl-substituted hydroquinone monoethers yielded quinone ketals; however, the analogous compound XVI was not produced from *dl*-α-tocopherol. Studies in our laboratories employing Stuart atomic models have shown that compound XVI is sterically improbable. A recent study by Dürckheimer and Cohen[9] reported a dimer of unknown structure, in addition to the hemiketal XVII as being formed by oxidation of I with tetrachloro-*o*-quinone. This dimer was not XVI; thus, XVI has not been obtained by oxidation of I. These workers also found that reaction of *dl*-α-tocopherol with tri-*t*-butylphenoxy radicals yielded III as the only product.

We have treated the model chroman II with azobisisobutyronitrile in both dioxane and benzene, obtaiaing, in each case, the dihydroxy dimer XIII. Other products were formed, but no IV has yet been identified. Alkaline ferricyanide oxidation of XIII under the conditions used by Martius[1] afforded IV in excellent yield. The oxidation of I or II with benzoyl peroxide was shown by Inglett and Mattill[10] to yield 6-hydroxychroman benzoates and, presumably by their hydrolysis, *dl*-α-tocopherylquinone or its model. Other products were also produced but were not identified. Air oxidation of methylphenols in cumene containing ferric stearate resulted in the production of dimers coupled through benzyl groups.[11] Fries and Brandes[12] reported the alkaline ferricyanide oxidation of 2,2′-dihydroxy-3,5,3′,5′-tetramethyldibenzyl to yield a keto ether analogous to dimers III and IV. Evidence for the rearrangement of phenoxy radicals containing *ortho* methyl groups to benzyl radicals is given by Cook, *et al.*[13]

It is instructive to consider the mechanism of alkaline ferricyanide oxidation of I to III *via* initial transfer of an electron from the ionized I to $Fe(CN)_6^{-3}$ to yield a phenoxy radical which would be expected to rearrange to the benzyl radical XVIII or XIX by hydrogen transfer from the neighboring methyl group or to its resonance hybrid (XX). Dimerization of XVIII would lead to the dihydroxy dimer XII, whereas removal of another electron from XX would lead to a carbonium ion that upon hydroxylation would yield XVII and, *via* hydrolysis, α-tocopherylquinone. Removal of the electrons by $Fe(CN)_6^{-3}$ from ionized XII followed by rearrangement to the keto diradical (XXI) and ring closure would lead to III. It is not known why products from the dimerization of XIX have not been found.

Experimental[14]

Oxidation of *dl*-α-Tocopherol with Alkaline Ferricyanide.—A sample of *dl*-α-tocopherol (11.0 g.) was dissolved in 500 ml. of isooctane and shaken in a separatory funnel with a solution of potassium ferricyanide (32.6 g.) in 320 ml of 0.2 *N* potassium hydroxide for 3 min. The isooctane layer was separated, washed with water three times, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo*, leaving 10.9 g. of bright yellow oil. The chromatography of the crude oxidation products on Whatman 3 MM paper impregnated with paraffin oil revealed two spots with R_f values of 0.02 and 0.30 (95% *n*-PrOH). Silica gel thin-layer chromatography showed the presence of several separate products when a mixture of hexane–chloroform (2:1, v./v.) was used as developing solvent.

The yellow oil (10.9 g.) was dissolved in 100 ml. of petroleum ether (b.p. 50–70°) and chromatographed on a column (5 × 25 cm.) of neutral alumina (450 g., Brockmann activity I, "Bio-Rad" Co.). Elution with 250 ml. of petroleum ether yielded 102 mg. (1%) of a colorless oil (fraction 1). Thin-layer chromatography showed one major spot when developed with a mixture of hexane–chloroform (4:1, v./v.), R_f 0.98, and five minor spots, R_f 0.05–0.4.

The further elution of the alumina column with 300 ml. of petroleum ether–diethyl ether (9:1, v./v.) gave 1034 mg. (10.2%) of a slightly yellow colored oil (fraction 2). The chromatography on paraffin-oil-impregnated paper showed only one spot when developed with 95% *n*-PrOH, R_f 0.02, while thin-layer chromatography (hexane–chloroform, 2:1) showed two separate spots, R_f 0.64 (compound A, trimer) and R_f 0.34 (compound B). The next fraction eluted with 500 ml. of petroleum ether–diethyl ether (9:1) yielded 3135 mg. (31%) of a yellow oil (fraction 3) which consisted of traces of compounds A and B, and of the yellow colored dimer. Continuing elution with 1500 ml. of diethyl ether yielded 3650 mg. (36.5%) of dimer (fraction 4), devoid of minor oxidation products or other impurities. During the elution procedure a light brown colored zone developed at the top of the alumina column. The elution of this zone with 1200 ml. of diethyl ether–methanol (1:1) resulted in recovery of 2110 mg. (21%) of brown degradation products (fraction 5) formed on the column. The total column recovery consisted of 10.03 g. (92%) of oily products.

Isolation of Compound A. Trimer XIV.—The mixture of minor oxidation compounds obtained in fraction 2 from the alumina column was separated by thin-layer chromatography. Samples of 30 mg. of slightly yellow oil were dissolved in 0.5 ml. of hexane and applied on silica gel thin-layer chromatoplates (20 × 20 cm.). Plates were developed (solvent front 10 cm.) with a mixture of hexane–chloroform (2:1), and the two major bands viewed under ultraviolet light. The front band was clearly separated from the slower moving band unless the plate was overloaded. The silica gel containing the front band was scratched off the plate and extracted with 25 ml. of diethyl ether. The diethyl ether extract was filtered, solvent removed *in vacuo*, and the slightly yellow residual oil, compound A (10–15 mg.), was redissolved in hexane and further purified by a repeated thin-layer chromatography to eliminate traces of compound B. Final purification of compound A was achieved by neutral alumina (Brockmann activity I) column (10 × 1 cm.) chromatography. Elution with a mixture of hexane–diethyl ether (9:1) yielded, after removal of solvent, a colorless oil, soluble in hexane and diethyl ether, insoluble in ethanol: R_f

(8) P. Alaupovic, B. C. Johnson, Q. Crider, H. N. Bhogavan, and B. J. Johnson, *Am. J. Clin. Nutr.*, **9**, part II, 76 (1961).

(9) W. Dürckheimer and L. A. Cohen, *Biochem. Biophys. Res. Commun.*, **9**, No. 3, 262 (1962).

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

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

(12) K. Fries and E. Brandes, *Ann.*, **542**, 48 (1939).

(13) C. D. Cook, N. G. Nash, and H. R. Flanagan, *J. Am. Chem. Soc.*, **77**, 1783 (1955).

(14) Melting points are uncorrected and were obtained with the Fischer-Johns apparatus. Molecular weights were determined in benzene using the Mechrolab vapor pressure osmometer.

paraffin-oil-impregnated paper, 0.02–0.05 (95% *n*-PrOH); R_f, silica gel thin-layer chromatography, 0.64 (hexane–chloroform, 2:1); λ_{max}^{Nujol} 5.80, 5.93 (α,β-unsaturated C=O), 6.10 (aryl), and 9.15 μ (chroman C—O—C); ultraviolet (isooctane), $E_{1cm}^{1\%}$ 59 (294 mμ).

Anal. Calcd. for $C_{87}H_{144}O_6$: C, 81.2; H, 11.3; mol. wt., 1286. Found: C, 81.2; H, 11.2: mol. wt., 1200.

Isolation of Compound B.—Silica gel containing the slower moving band was scratched off the plate and extracted with diethyl ether. The diethyl ether extract was filtered, the solvent was removed *in vacuo*, and the residual oil (8–12 mg.) was submitted to a repeated thin-layer chromatography and extraction with diethyl ether. Chromatography on a column (10 × 1 cm.) of neutral alumina (Brockmann activity I) and elution with a mixture of hexane–diethyl ether (9:1) gave a nearly colorless oil, soluble in hexane and diethyl ether, insoluble in ethanol: R_f, paraffin-oil impregnated paper, 0.02–0.05 (95% *n*-PrOH); R_f, silica gel thin-layer chromatography, 0.34 (hexane–chloroform, 2:1); λ_{max}^{Nujol} 5.80, 5.93 (α,β-unsaturated C=O), 6.10 (aryl), 9.15 μ (chroman C—O—C); ultraviolet (isooctane), $E_{1cm}^{1\%}$ 53 (294 mμ).

Anal. Calcd. for $C_{87}H_{144}O_6$: mol. wt., 1286. Found: mol. wt., 1177.

Isolation of Keto Ether. Dimer III.—Fraction 4 obtained from the column chromatography of the oxidation products was submitted to repeated chromatography on neutral alumina (Brockmann activity I) and the middle portion of the yellow zone eluted with a mixture of petroleum ether–diethyl ether (1:1). Upon removal of solvent *in vacuo*, a bright yellow oil, soluble in hexane and in ethanol, was obtained: R_f, paraffin-oil impregnated paper, 0.30–0.33 (95% *n*-PrOH); R_f, silica gel thin-layer chromatography, 0.20–0.25 (hexane–chloroform, 2:1); λ_{max}^{Nujol} 5.98 (α,β-unsaturated C=O), 6.05 (aryl), 6.28 (C=C), 9.15 μ (chroman C—O—C); ultraviolet (isooctane), $E_{1cm}^{1\%}$ 56, 28 (300 and 337 mμ).

Anal. Calcd. for $C_{58}H_{96}O_4$: C, 81.2; H, 11.3; mol. wt., 857. Found: C, 81.4; H, 11.3: mol. wt., 860.

Preparation of Dihydroxy Dimer XII.—A sample of 860 mg. (1 mmole) of III was dissolved in diethyl ether (100 ml.) and refluxed with lithium aluminum hydride (1.5 g.) for 30 min. The excess of the reagent was decomposed with water, the diethyl ether layer filtered, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a light yellow oil (580 mg., 67%): R_f, paraffin-oil-impregnated paper, 0.64–0.68 (95% *n*-PrOH); ultraviolet (isooctane), $E_{1cm}^{1\%}$ 92 (299 mμ). The product was further purified by chromatography on neutral alumina (Brockmann activity I) column (15 × 2 cm.) developed with 200 ml. of a mixture of petroleum ether–diethyl ether (4:1). Elution of the column with 200 ml. of diethyl ether–methanol (1:1) yielded a nearly colorless oil having an unchanged R_f value on paper chromatogram, and ultraviolet (isooctane), $E_{1cm}^{1\%}$ 95 (299 mμ).

Anal. Calcd. for $C_{58}H_{98}O_4$: C, 81.0; H, 11.5. Found: C, 80.9; H, 11.3.

Diacetate of XII was prepared by heating a solution of 500 mg. of XII in pyridine (5 ml.) and acetic anhydride (5 ml.) to 70° for 2 hr. according to procedure by Nelan and Robeson.[3,4] The residual oil was recrystallized from isopropyl alcohol, m.p. 50–60°. Five crystallizations from ethyl alcohol yielded white crystals, m.p. 88–89°; ultraviolet (isooctane), $E_{1cm}^{1\%}$ 55, 72 (281, 291 mμ).

Anal. Calcd. for $C_{62}H_{102}O_6$: C, 78.9; H, 10.9. Found: C, 78.8; H, 10.8.

Diacetate agreed in ultraviolet absorption spectrum and paper chromatographic behavior, and gave no melting point depression (88–89°) when mixed with a diacetate derivative of XII prepared by the ascorbic acid or stannous chloride reduction of III.

Preparation of Compounds A (Trimer XIV) and B, and Dihydroxy Dimer XII from Dimer III and *dl*-α-Tocopherol.—A mixture of 858 mg. (1 mmole) of III and 430 mg. (1 mmole) of *dl*-α-tocopherol was dissolved in isooctane (50 ml.) and refluxed for 2 hr. The paper and thin-layer chromatography data showed the presence of minor oxidation products A and B, and in addition to some unchanged dimer, two reducing compounds (Emmerie–Engel reaction was positive). The reaction mixture was placed onto a neutral alumina (Brockmann activity I) column (20 × 3 cm.) and eluted first with 300 ml. of petroleum ether–diethyl ether (9:1) yielding a colorless oil (58 mg.) consisting mainly of trimer XIV. Elution with 250 ml. of petroleum ether–diethyl ether (8:2) gave 385 mg. of a mixture of compounds A (trimer XIV) and B (yield 35%). Compound A was separated from compound B according to the procedure described under isolation of trimer XIV by thin-layer chromatography. Its ultraviolet and infrared absorption spectra were identical to those of an analytically pure sample of XIV obtained by the oxidation of *dl*-α-tocopherol by alkaline potassium ferricyanide.

After obtaining an intermediate fraction (85 mg.) consisting of traces of compounds A and B of unchanged keto ether, the elution of the column with 1000 ml. of diethyl ether–methanol (1:1) gave 760 mg. of a slightly brown colored oil. The chromatography on paraffin-oil-impregnated paper showed two spots, R_f 0.68 and 0.88 (95% *n*-PrOH). For separation of these reducing compounds several samples of 5 mg. each were dissolved in hexane (2 ml.) and applied on paraffin-oil coated papers (12 cm. wide). Papers were developed with 95% *n*-PrOH and a narrow longitudinal end of each paper cut off and sprayed with the Emmerie–Engel reagent. The areas corresponding to the R_f values of 0.68–0.72 and 0.85–0.90 were marked on the remaining papers and cut out separately. Papers were eluted with ethanol, the extracts filtered, and the solvent evaporated *in vacuo*. The oily residues containing paraffin oil and reaction products were dissolved in petroleum ether and placed on the neutral alumina columns (10 × 1 cm.). Both columns were eluted first with 100 ml. of petroleum ether–diethyl ether (8:2) to remove the paraffin oil and then with 100 ml. of diethyl ether–methanol (8:2) to obtain from the first column the reducing compound, R_f 0.68, and from the second column the reducing compound, R_f 0.88. The former compound showed an ultraviolet absorption spectrum, $E_{1cm}^{1\%}$ 126 (299 mμ), and on acetylation yielded, after several crystallizations from ethanol, a crystalline derivative, m.p. 88°. A mixture melting point with an analytical sample of diacetate of dihydroxy dimer XII prepared by lithium aluminum hydride reduction of dimer III showed no depression. The oil with the R_f value of 0.88 showed an ultraviolet absorption spectrum, $E_{1cm}^{1\%}$ 72 (292–295 mμ), and cochromatographed with an authentic sample of *dl*-α-tocopherol in several paper and thin-layer chromatographic systems (see Table I).

TABLE I

R_f VALUES OF THE OXIDATION PRODUCTS AND METABOLITES OF *dl*-α-TOCOPHEROL ON PARAFFIN-IMPREGNATED PAPERS[a]

Compounds	75% EtOH	95% *n*-PrOH	99% 1-PrOH
dl-α-Tocopherol (I)	0.25–0.28	0.88	0.92
dl-α-Tocopherylquinone	0.50–0.55	0.95	0.97
dl-α-Tocopherol dimer (III)	0.00	0.30–0.35	...
dl-α-Tocopherol trimer (XIV)	0.00	0.02–0.05	0.10–0.15
Compound B	0.00	0.02–0.05	0.10–0.15
dl-α-Tocopherol dihydroxy dimer (XII)	0.00	0.64–0.68	...
dl-α-Tocopheronolactone (urinary metabolite)	0.88	S.F.	...
Compound O (liver metabolite)	0.00	0.02	0.10
Compound F (liver metabolite)	0.65–0.68	0.90	...

[a] Whatman 3 MM papers were impregnated with a 3% (w/v) petroleum ether solution of liquid paraffin heavy oil (Stanolind) for 10 min. according to the procedure by Eggitt and Ward,[17] and the papers developed by descending chromatography.

Oxidation of 6-Hydroxy-2,2,5,7,8-pentamethylchroman (II) with Alkaline Ferricyanide.—The procedure used was essentially the same as that employed for the oxidation of *dl*-α-tocopherol with the exception that a 10-min. reaction period was used. Silica gel thin-layer chromatography of the crude oxidation products showed the presence of six separate products when chloroform was used as the developing solvent.[15]

Isolation of Compound A. Trimer XV.—When the crude oxidation product was dissolved in petroleum ether (b.p. 30–60°) and chromatographed on a Schlesinger silica gel column, elution with petroleum ether yielded a colorless oil that could be crystallized to a colorless solid with methanol–water. Repeated recrystallization from ethanol–ether (1:1) by addition of water afforded an analytical sample, m.p. 228–230°; R_f silica gel thin-

(15) Silica gel thin-layer chromatographs were sprayed with 30% H_2SO_4 and heated at 100° to show the products.

layer chromatography, 0.60 ($CHCl_3$); λ_{max}^{Nujol} 5.95 (α,β-unsaturated C=O), 6.10 (aryl), 9.15 μ (chroman C—O—C), ultraviolet (isooctane), $E_{1cm}^{1\%}$ 85 (294 mμ); n.m.r. ($CDCl_3$, τ, 60 Mc.), 9.20, 9.10, 9.00 (unknown); 8.70, 8.67 ($6CH_3$ on chroman ring); 8.48, 8.30, 8.23, 8.13, 8.00 (unknown); 7.87, 7.83, 7.77, 7.70 ($4CH_3$ on benzene ring), 7.57, 7.47, 7.23, 7.15 (unknown).

Anal. Calcd. for $C_{42}H_{54}O_6$: C, 77.0; H, 8.25: mol. wt., 654. Found: C, 77.1; H, 8.50; mol. wt., 659.

Isolation of Keto Ether. Dimer IV.—Elution of the chromatographic column with benzene–petroleum ether (1:1) after initial elution with petroleum ether alone yielded a yellow oil that crystallized to a solid. Recrystallization from methanol–water afforded a yellow solid, m.p. 84–87°. Thin-layer chromatography showed two spots when developed with chloroform R_f 0.60 (trimer) and R_f 0.52 (dimer). Recrystallization of the impure product from methanol–water, removing the first crystal crop which was rich in trimer, yielded a more pure sample of dimer, m.p. 80–85°; R_f 0.52 with a trace spot, 0.60. Crystallization from petroleum ether (b.p. 30–60°), drying at 30° (1 mm.) overnight yielded an analytical sample, m.p. 77–79°; R_f 0.52 ($CHCl_3$); λ_{max}^{Nujol} 5.98 (α,β-unsaturated C=O); 6.05 (aryl); 6.28 (C=C); 9.15 μ (chroman C—O—C); ultraviolet(is ooctane), $E_{1cm}^{1\%}$ 85.6 300 mμ); n.m.r. ($CDCl_3$, τ, 60 Mc.), 8.69 ($4CH_3$ on chroman ring), 8.30, 8.20 ($3CH_2$ β to unsaturated system), 8.14, 8.02 ($2CH_3$ on dienone ring), 7.83 ($2CH_3$ on aromatic ring), 7.46 ($3CH_2$ α to unsaturated system).

Anal. Calcd. for $C_{28}H_{36}O_4$: C, 77.0; H, 8.25: mol. wt., 436. Found: C, 77.6; H, 8.82: mol. wt., 433.

Preparation of Dihydroxy Dimer XIII.—To a solution of 1 g. of ascorbic acid in 100 ml. of ethanol and 10 ml. of water was added 150 mg. of the dimer IV. The solution was allowed to stand overnight at room temperature during which time the yellow color disappeared. Water was added, the solution extracted with ether, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The crude product was crystallized from methanol; 110 mg.; m.p. 186–188°; R_f 0.30 ($CHCl_3$); λ_{max}^{Nujol} 3.00 (OH), absence of C=O (5.8—6.3), 8.58 (=C—O), 9.05 μ (chroman C—O—C).

Anal. Calcd. for $C_{28}H_{38}O_4$: C, 76.5; H, 8.67: mol. wt., 438. Found: C, 76.4; H, 8.72: mol. wt., 457.

Isolation of Dihydroxy Dimer XIII from $FeCN_6^{-3}$ Oxidation.—A white solid, m.p. 184–187°, was isolated from crude $FeCN_6^{-3}$ oxidation product of II by virtue of its low solubility in petroleum ether (b.p. 30–60°). Crystallization from methanol led to a sample that agreed in melting point, infrared absorption spectrum, and thin-layer chromatographic behavior with that of an authentic sample of XIII prepared by ascorbic acid reduction of IV.

Preparation of Trimer XV and Dihydroxy Dimer XIII from Dimer IV and 6-Hydroxy-2,2,5,7,8-pentamethylchroman (II).—A solution of 220 mg. (0.5 mmole) of IV and 110 mg. (0.5 mmole) of II in 40 ml. of benzene was mixed and allowed to stand at room temperature overnight. The benzene solution was refluxed and samples removed after 1, 5, and 8 hr. for silica gel thin-layer chromatography. The benzene was distilled and replaced with toluene. The toluene solution was then refluxed for 1 hr. and a sample taken for thin-layer chromatography using chloroform as solvent.

The thin-layer chromatography data showed the presence of two new spots after the solution had stood overnight at room temperature. One of these spots, R_f 0.58, corresponds to trimer XV and the other to dihydroxy dimer XIII, R_f 0.30. The size of these two spots increased upon heating in benzene as did a trace spot near the origin. Chromatography ($CHCl_3$) of the crude product after removal of toluene on a thick silica gel plate allowed separation of the two major products produced by heating. The trimer isolated had a m.p. of 135–140°, 11.5 mg., and an infrared absorption spectrum identical to that of analytically pure XV. The isolated dihydroxy dimer XIII melted at 185–188° after crystallization from methanol–water and tetrahydrofuran–petroleum ether (b.p. 30–60°) several times. A mixture melting point with an analytical sample of XIII prepared by ascorbic acid reduction of dimer IV showed no depression. The infrared absorption spectrum was also identical to that of analytically pure IV.

Preparation of Maleic Anhydride Adduct of Dimer IV.—A solution of 450 mg. of IV and 158 mg. of sublimed maleic anhydride in 20 ml. of toluene was refluxed for 2 hr. during which time the color changed from yellow to orange. The solvent was removed *in vacuo* yielding pale yellow crystals. Two crystallizations from tetrahydrofuran–petroleum ether (b.p. 30–60°) gave an analytically pure colorless solid (yield, 20%), m.p. 143–145°, λ_{max}^{Nujol} absence of OH (3.0 μ); 5.43, 5.63 (C=O) of anhydride, 5.80 (C=O of keto); 6.10 (aryl); 9.15 μ (chroman C—O—C); ultraviolet (isooctane), $E_{1cm}^{1\%}$ 71.4; n.m.r. ($CDCl_3$, τ, 60 Mc.), 8.70 ($4CH_3$ on chroman ring), 8.47 (CH_3 adjacent to C=O), 8.38 (CH_3 on isolated C=C), 8.20 ($3CH_2$ β to unsaturated system), 7.90 ($2CH_3$ on aromatic ring), 7.43 ($3CH_2$ α to unsaturated system), 6.86, 6.00 (2H on maleic anhydride).

Anal. Calcd. for $C_{32}H_{38}O_7$: C, 71.9; H, 7.16: mol. wt., 535. Found: C, 71.9; H, 7.41: mol. wt., 567.

Oxidation of Dihydroxy Dimer to Dimer IV.—A mixture of 15 mg. of XIII in 25 ml. of petroleum ether (b.p. 30–60°) and 90. mg. of potassium ferricyanide in 10 ml. of 0.2 *N* sodium hydroxide was shaken at room temperature for 10 min. The yellow petroleum ether layer was separated, dried, and concentrated *in vacuo* to yield 14.5 mg. of a yellow sirup. This product moved as a single spot with the same R_f value as IV on silica gel chromatography in chloroform and benzene. The infrared absorption spectrum was identical to that of IV and showed no OH bands characteristic of XIII.

Oxidation of 6-Hydroxy-2,2,5,7,8-pentamethylchroman (II) with Azobisisobutyronitrile.—A solution of 1.0 g. of II and 460 mg. of azobisisobutyronitrile in 200 ml. of benzene was heated for 0.5 hr. at 60°, 0.5 hr. at 70°, and refluxed for 3 hr. After removal of the benzene *in vacuo* a white solid was obtained, 1.3 g. On thin-layer chromatography ($CHCl_3$) this mixture showed four spots after spraying with 30% sulfuric acid. The largest spot was due to starting chroman. One of the spots corresponded to that of the dihydroxy dimer XIII, another moved slightly faster than trimer XV, while the other remained close to the origin. The infrared absorption spectrum of the crude product showed no carbonyl bands.

When dioxane was used as the solvent for this reaction and three times the amount of azobisisobutyronitrile added in portions over a period of 18 hr. of refluxing, the infrared absorption spectrum of the crude solid product showed no carbonyl absorption. Petroleum ether addition allowed the separation of solid, aliphatic products insoluble in petroleum ether from the azonitrile decomposition. Concentration of the petroleum ether solution yielded a solid, m.p. 175–176°, which was recrystallized from methanol to yield XIII, m.p. 186–187°; mixture melting point with analytical sample from ascorbic acid reduction of IV showed no depression.

Upon chromatography of the crude oxidation products on a column of Schlesinger silica gel and elution with petroleum ether (b.p. 30–60°), petroleum ether–benzene (1:1), benzene, benzene–ether (1:1), ether, and finally methanol, several different products were obtained in addition to a considerable amount of the original chroman. These products have not yet been identified; however, the ether eluate yielded a yellow oil which had carbonyl absorption (5.8, 5.9 μ) in the infrared. This material moves only slightly from the origin on silica gel thin-layer chromatography ($CHCl_3$). These various products are now under investigation.

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