evaporated and the resulting residue was recrystallized from hexane, to yield 0.331 g. (19%) of II (R = Ph), m.p. 190–192°.

Reaction of 2-Ethylamino-3,1,4-benzoxazone (II, R = Et) with Ethyl Isocyanate.—A solution of 0.050 g. of 2-ethylamino-3,1,4-benzoxazone and 2 ml. of ethyl isocyanate in 25 ml. of benzene was heated under reflux for 2 hr. The solvent was removed and the residue was recrystallized from hexane to yield 0.062 g. (95%) of V (R = Et), m.p. 93–94°.

Reaction of N-(Ethylcarbamyl)anthranilic Acid (I, R = Et) with Ethyl Isocyanate.—A mixture of 1 g. of N-(ethylcarbamyl)-anthranilic acid[3] and 5 ml. of ethyl isocyanate in 25 ml. of benzene was heated under reflux for 2 hr. The mixture was cooled and unreacted starting material, 0.47 g., m.p. 187–189°, was collected by filtration. The filtrate was evaporated and the residue was recrystallized from hexane to yield 0.190 g. (39% based on consumed starting material) of II (R = Et), m.p. 172–174°.

Reaction of Ethyl Anthranilate with Ethyl Isocyanate.—A mixture of 1.65 g. of ethyl anthranilate and 0.71 g. of ethyl isocyanate in 25 ml. of benzene was heated under reflux for 3 hr. An infrared spectrum of the reaction mixture showed no band near 1760 cm.$^{-1}$ attributable to benzoxazone carbonyl. The solvent was evaporated and the residue was dissolved in a minimum volume of ether. Petroleum ether was added to the cloud point and the product which crystallized was collected to yield 1.23 g. (53%) of ethyl N-(ethylcarbamyl)anthranilate as long needles, m.p. 98–99°.

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.0; H, 6.78; N, 11.9. Found: C, 61.4; H, 6.82; N, 12.0.

Reaction of 2-(Ethylcarbamyl)ethylamino-3,1,4-benzoxazone (V, R = Et) with Acid.—A mixture of 0.20 g. of 2-(ethylcarbamyl)ethylamino-3,1,4-benzoxazone and 30 ml. of 10% sulfuric acid containing a few drops of ethanol was heated until solution was complete. The solution was filtered and allowed to cool. The product which crystallized was collected and recrystallized from a mixture of ethanol and hexane to yield 0.084 g. of 1-(o-carboxyphenyl)-3,5-bisethylbiuret (VII), m.p. 165–166°. The n.m.r. spectrum showed two nonequivalent ethyl substituents appearing as a triplet (3H) centered at δ 1.21, a triplet (3H) centered at 1.39, a multiplet (2H) at 3.38, and a quartet (2H) centered at 4.07.

Anal. Calcd. for $C_{13}H_{17}N_3O_4$: C, 56.0; H, 6.10; N, 15.0. Found: C, 56.0; H, 6.53; N, 14.9.

In similar experiments II (R = Et) and II (R = Ph) were treated with acid to produce 72% of IV[3] (R = Et) and 48% of IV[21] (R = Ph), respectively.

Oxidation Products of Vitamin E and Its Model, 6-Hydroxy-2,2,5,7,8-pentamethylchroman.
VII. Trimer Formed by Alkaline Ferricyanide Oxidation[1]

W. A. SKINNER AND R. M. PARKHURST

Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, California

Additional evidence is presented for the structure of the trimers (II) produced by the oxidation of α-tocopherol or 6-hydroxy-2,2,5,7,8-pentamethylchroman with alkaline ferricyanide. A structure analogous to that of trimers produced from other o-quinone methides is proposed. An improved procedure for the isolation of the trimer derived from 6-hydroxy-2,2,5,7,8-pentamethylchroman was developed.

The oxidation of α-tocopherol with alkaline ferricyanide has been studied by a number of workers.[2–9] In addition, some of these workers[5–8] studied the oxidation of the model chroman, 6-hydroxy-2,2,5,7,8-pentamethylchroman. A dimer (I), whose structure was proposed by Nelan and Robeson,[5] proven by Schudel, *et al.*,[6] and further studied by Skinner and Alaupovic[7,8] and McHale and Green,[9] was produced in this reaction. A trimer,[7,8] designated as compound A, also was produced and structure II was proposed for it.[8] We now wish to report additional evidence confirming this proposed structure for the trimer (II).

Evidence of structure II for the trimer, compound A, is as follows: elementary analysis indicated the same empirical formula as the dimer (I); a molecular weight determination indicated it to be a trimer; the infrared absorption spectrum showed the presence of an α,β-unsaturated ketone function and the absence of hydroxyl groups; the trimer could be prepared by heating monomer (α-tocopherol or the model chroman) with the corresponding dimer (I); and the ultraviolet absorption spectrum of the trimer compared with spectra of the dimer (I) and monomer indicated the presence of an α,β-unsaturated ketone function as in the dimer plus the addition of the aromatic absorption of the monomer. The spectrum of the trimer in isooctane, minus twice the absorption of the monomer, showed the typical absorption of an α,β-unsaturated ketone [235 mμ (ε 14,800) and 310 mμ (ε 800)]. When absolute ethanol was used as solvent, the 235-mμ band showed a bathochromic shift of 3 and the 310-mμ band showed a hypsochromic shift of 5.

It would appear from this data that a quinone methide (III) was formed from the monomer (α-tocopherol or model chroman) upon oxidation by either the alkaline ferricyanide or the dimer. The quinone methide then added to the dimer present to form the trimer (II). Two modes of addition of the quinone methide to the dimer are possible wherein an α,β-unsaturated ketone is left in the adduct. One of these structures (IV) would be acid labile owing to the presence of a ketal grouping and the other (II) would be acid resistant. The trimer reported here proved to be stable toward acid.

Reductive acetylation of trimer II from the model chroman using zinc, acetic anhydride, and sodium acetate under reflux for 3 hr. afforded interesting results. The product isolated from this reaction was shown to be the acetate (V) of the dihydroxy dimer. Its melting point was 217–218.5° and a mixture melting point with a sample of V prepared from the dimer (I) by the

(1) For the preceding paper of this series, see W. A. Skinner, *Biochem. Biophys. Res. Commun.*, **15**, 469 (1964).

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

(3) H. H. Draper, A. S. Csallany, and S. N. Shah, *Biochim. Biophys. Acta*, **59**, 527 (1962).

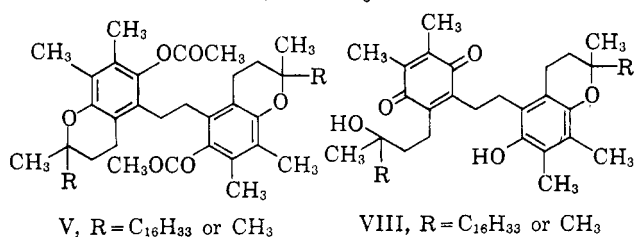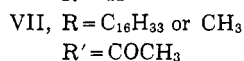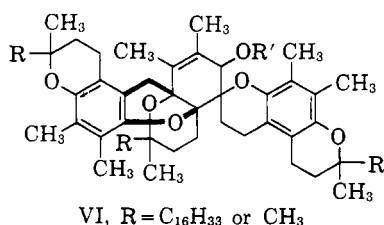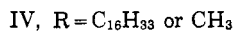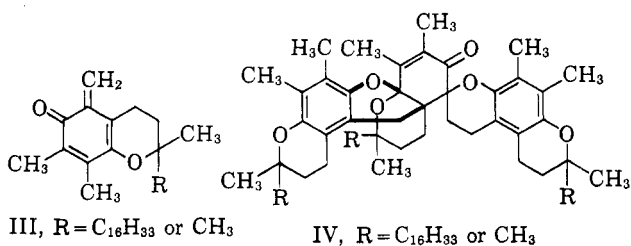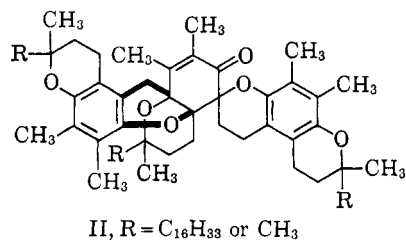(4) D. R. Nelan and C. D. Robeson, *Nature*, **193**, 477 (1962).

(5) D. R. Nelan and C. D. Robeson, *J. Am. Chem. Soc.*, **84**, 2963 (1962).

(6) P. Schudel, H. Mayer, R. Rüegg, and O. Isler, *Chimia* (Aarau), **16**, 368 (1962); P. Schudel, H. Mayer, I. Metzger, R. Rüegg, and O. Isler, *Helv. Chim. Acta*, **46**, 636 (1963).

(7) W. A. Skinner and P. Alaupovic, *Science*, **140**, 803 (1963).

(8) W. A. Skinner and P. Alaupovic, *J. Org. Chem.*, **28**, 2854 (1963).

(9) D. McHale and J. Green, *Chem. Ind.* (London), **29**, 366 (1964).

I, R = C₁₆H₃₃ or CH₃

II, R = C₁₆H₃₃ or CH₃

III, R = C₁₆H₃₃ or CH₃

IV, R = C₁₆H₃₃ or CH₃

VI, R = C₁₆H₃₃ or CH₃
R' = H
VII, R = C₁₆H₃₃ or CH₃
R' = COCH₃

V, R = C₁₆H₃₃ or CH₃

VIII, R = C₁₆H₃₃ or CH₃
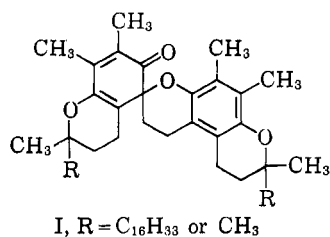
procedure of Schudel, et al.,[6] was not depressed. The infrared absorption spectra of the two were identical as were the n.m.r. curves and thin layer chromatographic data. A molecular weight determination of the product from trimer II also agreed with the structure of V.

Reduction of the trimer from the model chroman with lithium aluminum hydride in refluxing tetrahydrofuran for 6 hr. afforded a white solid, m.p. 229–230°. This product showed no carbonyl absorption in the infrared. Elementary analysis and molecular weight determination of this reduction product indicated an empirical formula of $C_{42}H_{56}O_6$. An active hydrogen determination showed the presence of one active hydrogen per mole although no strong absorption in the hydroxyl region of the infrared was present. It is proposed that this lithium aluminum hydride reduction product possesses structure VI. Acetylation of VI with acetic anhydride in pyridine afforded the monoacetate (VII), m.p. 242–244°.

The carbonyl function of the trimer (II) appeared to be unreactive towards carbonyl reagents, failing to react with hydroxylamine or 2,4-dinitrophenylhydrazine. This behavior is in agreement with that of other quinone methide trimers as reported by Merijan, et al.[10]

Catalytic hydrogenation of the trimer (II) with either platinum oxide or palladium–carbon in ethyl acetate at 25° and 780 mm. of hydrogen for 8 hr. also failed. Even at 90° and 1500 p.s.i.g. of hydrogen using platinum oxide in ethanol for 5 days only a small fraction of the trimer was changed. Purification of the mixture of products was not possible but their infrared absorption spectrum showed a hydroxyl band at 2.95 and a small carbonyl band at 5.84 μ indicating possible conversion to the saturated ketone and alcohol.

An excellent method of isolation of the pure trimer from the model chroman was developed. It involves the oxidation of the chroman with alkaline ferricyanide as previously described[6] followed by column chromatography of the products on Schlesinger silica gel to isolate a mixture of dimer and trimer leaving on the column the more polar materials. The eluted mixture is then crystallized once from ethanol–water and reduced with ascorbic acid to yield a mixture of the dihydroxy dimer and the trimer. Washing this mixture with petroleum ether (b.p. 30–60°) removes the insoluble dihydroxy dimer. From the petroleum ether solution the trimer is obtained by concentration and purified by recrystallization from ethanol–water–ether mixtures. This procedure circumvents the difficult separation of dimer I from trimer II.

The key features of the structural identification of trimer II are its resistance to acid hydrolysis and the presence of an α,β-unsaturated carbonyl grouping. Dimer I is quite susceptible to acid hydrolysis,[5] but the addition of the chroman to form trimer II prevents ring opening to the hydroxyquinone (VIII) oxidation product.

The interesting reductive acetylation of the trimer (II) to the dimer diacetate (V) deserves some comment. A concerted mechanism is proposed to account for the results, the driving force being aromatization of the product and production of quinone methide which dimerizes to form dimer I which is reductively acetylated.
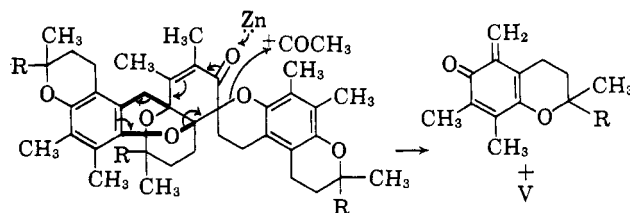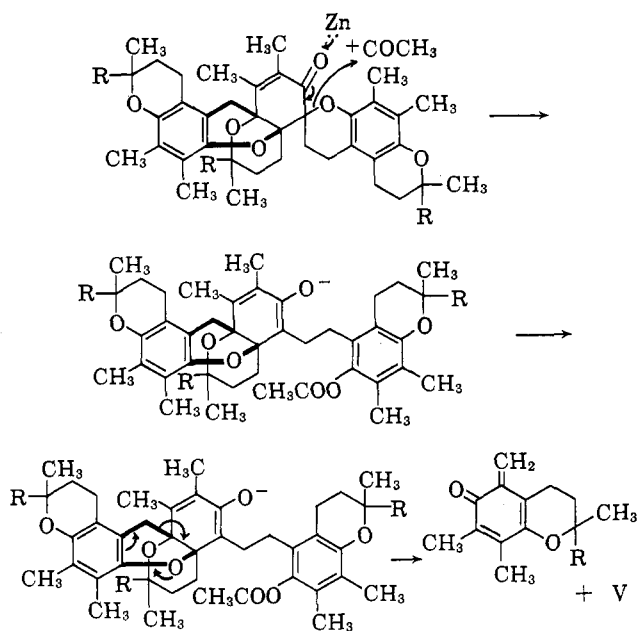

An alternative mechanism would be a two-step one, the first step of which is the same as for the production of the diacetate from dimer I.

Experimental[11]

Isolation of the Trimer from the Oxidation of 6-Hydroxy-2,2,5,-7,8-pentamethylchroman with Alkaline Ferricyanide.—6-Hydroxy-2,2,5,7,8-pentamethylchroman (10 g.) was oxidized by

(10) A. Merijan, B. A. Shoulders, and P. D. Gardner, J. Org. Chem., 28, 2148 (1963).

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

(Chemical reaction schemes with structures bearing CH₃, H₃C, R, CH₃CO, O⁻, +COCH₃, Zn, CH₂ labels, ending in "+ V")

the previously described procedure[3] and the yellow product was chromatographed on a column of Schlesinger silica gel. Elution of the column with benzene gave a mixture of dimer and trimer. When larger scale reactions were conducted, this chromatography step could be eliminated. Crystallization from ethanol–water afforded 5.3 g. (53%) of a yellow powder. Two grams of this material was added to 10 g. of ascorbic acid in 100 ml. of ethanol, 25 ml. of water, and a small amount of ether, and the mixture was stirred for several hours. Water was added and the mixture was cooled and filtered to give 1.93 g. of a colorless powder which was shown to be a mixture of the dihydroxy dimer and trimer. When this material was washed with petroleum ether (b.p. 30–60°) the insoluble dihydroxy dimer, 1.15 g., was isolated. Concentration of the petroleum ether solution afforded 780 mg. (20% from starting chroman) of crude trimer. Crystallization from alcohol–water–ether mixtures yielded analytically pure trimer (II): m.p. 227–228°; ultraviolet spectrum (isooctane), 220 mμ (ϵ 24,000) and 295 (5600), (absolute ethanol), 220 mμ (ϵ 24,200) and 295 (6000).

Attempted Acid Hydrolysis of Trimer II Derived from 6-Hydroxy-2,2,5,7,8-pentamethylchroman.—A solution of 20 mg. of the trimer in 2 ml. of 95% ethanol, 2 ml. of ethyl ether, and 2 ml. of 6 N HCl was allowed to stand for 3 hr. at room temperature followed by 3 hr. of heating on a steam bath. Upon cooling, 17.5 mg. (87.5%) of the crystalline trimer, m.p. 228–230°, deposited. The thin layer behavior and infrared absorption spectrum of this material were identical with that of the starting trimer. Thin layer chromatography of the mother liquor failed to show any new products.

When the trimer was heated on a steam bath under nitrogen in a sealed tube with ethanolic hydrochloric acid for 7 hr., it was recovered unchanged with the exception of trace quantities of decomposition products as detected by thin layer chromatography.

Attempted Acid Hydrolysis of α-Tocopherol Trimer (II).—A solution of 28.0 mg. of trimer, 3 ml. of ethanol, 6 ml. of diethyl ether, 15 drops of concentrated hydrochloric acid, and 5 drops of water was mixed and allowed to stand for 4 hr. The mixture was refluxed for 20 min., cooled to room temperature, diluted by the addition of 25 ml. of water, and extracted with diethyl ether. The ether extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The residual oil (27.0 mg., 96%) examined by silica gel thin layer chromatography (hexane–chloroform, 2:1) and by chromatog-

raphy on paraffin oil impregnated paper (95% n-PrOH) showed only one spot identical with that of the original trimer.

Reductive Acetylation of Trimer II Derived from 6-Hydroxy-2,2,5,7,8-pentamethylchroman.—A mixture of 252 mg. of trimer, 400 mg. of powdered zinc dust, and 400 mg. of sodium acetate in excess acetic anhydride was refluxed for 3 hr. After evaporation of the solvent in vacuo, the residue was extracted with warm tetrahydrofuran (THF). Evaporation of the THF yielded a solid that was recrystallized from ethanol–water and then from THF–petroleum ether (b.p. 30–60°) to yield 74 mg. of a solid: m.p. 213–214.5°; λ_{max}^{Nujol} (μ) 5.75 (C=O, acetate), absence of 5.95 (C=O, α,β-unsaturated ketone), and 9.15 (C—O—C chroman).

An analytical sample was prepared by recrystallization from THF–ethanol, m.p. 217–218.5°. Mixture melting point with the dihydroxy dimer diacetate prepared by reductive acetylation of the dimer (I) as previously described[6] was 217–218.5°. The infrared absorption spectra, n.m.r. curves, and thin layer chromatographic behavior of the two samples also were identical.

Lithium Aluminum Hydride Reduction of the Trimer Derived from 6-Hydroxy-2,2,5,7,8-pentamethylchroman.—A mixture of 3.18 g. of trimer (II) and 3.2 g. of lithium aluminum hydride was refluxed for 6 hr. in THF. Alcohol was added to the cooled mixture followed by a saturated solution of sodium sulfate, and the thick mass was filtered and washed with diethyl ether and THF. Concentration in vacuo afforded 3.77 g. of a gummy residue which showed no carbonyl absorption in the infrared. Thin layer chromatography on silica gel G gave 2.53 g. of a light yellow gum. A second chromatographic separation (CHCl₃ developed) afforded 1.78 g. of a material which upon crystallization from ethanol–water yielded 1.0 g. of a white solid. A second recrystallization from ethanol–water gave 770 mg., m.p. 221–229°. Two further recrystallizations from absolute ethanol afforded an analytical sample: 580 mg.; m.p. 229–230°; ultraviolet (isooctane), 219 mμ (ϵ 26,200) and 300 (6800); λ_{max}^{Nujol} (μ) 2.90 (OH), absence of C=O (5.95), 7.95, 8.60, 8.95, and 9.20 (C—O—C, chroman).

Anal. Calcd. for $C_{42}H_{56}O_6$: C, 76.8; H, 8.59; active H, 0.152; mol. wt., 657. Found: C, 76.8; H, 8.75; active H, 0.127; mol. wt., 632.

Acetylation of Trimer II Lithium Aluminum Hydride Reduction Product.—The above-described reduction product, 20 mg., was added to 1 ml. of acetic anhydride and 1 ml. of pyridine and allowed to stand for 2 days at room temperature. Concentration of the solvent in vacuo yielded a colorless powder which was crystallized from ethanol–water: m.p. 242–244°; λ_{max}^{Nujol} (μ) 5.75 (C=O, acetate), 8.20 (C—O—C, acetate), 9.15 (C—O—C, chroman); ultraviolet (isooctane), 217 mμ (ϵ 24,600) and 300 (6200).

Anal. Calcd. for $C_{44}H_{58}O_7$: C, 75.6; H, 8.36; mol. wt., 699. Found: C, 75.2; H, 8.50; mol. wt., 687.

Hydrogenation of Trimer II Derived from 6-Hydroxy-2,2,5,7,8-pentamethylchroman.—To a solution of 1 g. of trimer II in 20 ml. of ethanol in a stainless steel bomb was added 0.2 g. of platinum oxide catalyst. Hydrogen was added to 1500 p.s.i.g. and the bomb was shaken at 90° for 5 days. Thin layer chromatography on silica gel G of the products showed a mixture of unchanged trimer and two other components. Fractionation of the mixture on a thick silica gel plate gave the trimer fraction (308 mg.) and the two other products together (112 mg.). The latter mixture possessed absorption peaks at 2.95 (OH) and 5.84 μ (C=O, α,β-saturated). These two products could not be separated.