[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIF., AND BASIC COTTON RESEARCH LABORATORY, AND DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AUSTIN, TEX.]

α-Tocopurple, an Oxidation Product of α-Tocopherol[1]

BY VERNON L. FRAMPTON, W. A. SKINNER,[2] PARIS CAMBOUR AND PHILIP S. BAILEY

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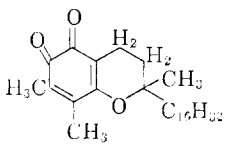
α-Tocopurple, an oxidation product of α-tocopherol (vitamin E), has been isolated and its structure proved to be 2,7-dimethyl-6-hydroxy-2-(4,8,12-trimethyltridecyl)-5,8-chromanquinone (III). The three isomeric tetramethyl-6 hydroxychromans (VII, IX, X) have been synthesized and obtained as analytically pure solids. Oxidation of 2,2,7,8-tetramethyl-6-hydroxychroman (VII) and of 2,2,5,7-tetramethyl-6-hydroxychroman (IX) with ferric chloride in methanol yielded the same hydroxyquinone (XII), thereby proving its structure.

Interest in the oxidation products of α-tocopherol (vitamin E) exists due to several reasons: (1) all the chemical methods of analysis of α-tocopherol depend on oxidation, (2) the role of the tocopherols in muscle metabolism and as antioxidants in vegetable oils is undoubtedly tied in with their oxidation.

Frampton, *et al.*,[3] have reported isolation of five products produced upon oxidation of a methanolic solution of *dl*-α-tocopherol with ferric chloride at 50°. Chromatographic separation on zinc carbonate yielded four colored oils and a colorless wax. Two of these oils have been identified. The red oil was identified as α-tocored[4] (I) and the yellow oil as α-tocopherylquinone[3] (II).

Three of the oxidation products remained unidentified, a purple band material, a blue-gray band material, and the colorless wax. This paper reports identification of the purple band material as a salt of 2,7-dimethyl-6-hydroxy-2-(4,8,12-trimethyltridecyl)-5,8-chromanquinone (III).

The purple band material, α-tocopurple, when purified by repeated chromatography on zinc carbonate, is an orange-colored, low-melting wax in the acid form. The alkaline salts of α-tocopurple are purple in alcohol. The chromatographic behavior (strong adsorption on basic columns) as well as the acidity and indicator properties suggest that α-tocopurple is a hydroxyquinone. The ultraviolet absorption spectrum in methanol (maximum at 296 mμ, ε 14,400) and infrared absportion spectrum [peaks at 2.95 μ (OH), 6.1, 6.2 μ (C=O)] of α-tocopurple are compatible with this formulation.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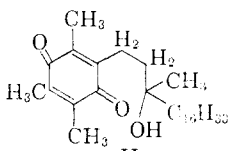
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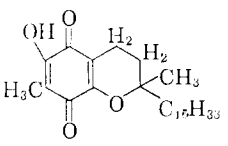
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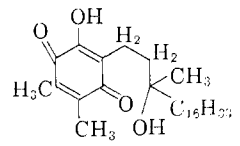
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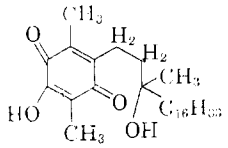
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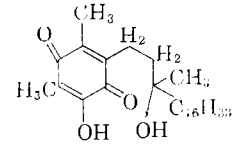
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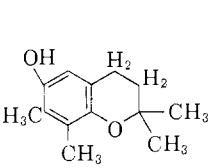
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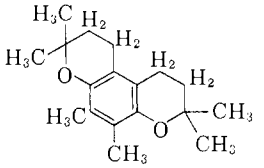
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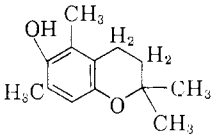
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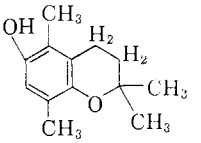
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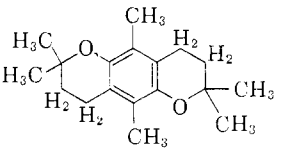
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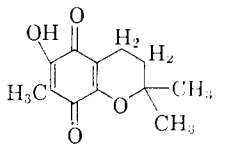
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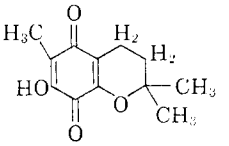
XIII

The elementary formula of α-tocopurple could be either $C_{27}H_{44}O_4$ (III) or $C_{28}H_{48}O_4$ (IV, V or VI). The major differences between the possible hydroxyquinones are the presence or absence of a tertiary alcohol group formed by opening of the chroman ring and the presence of either one or two methyl groups on the aromatic ring.

The nuclear magnetic resonance spectrum of α-tocopurple *unequivocally proved* the structure to be III rather than IV, V or VI. The spectrum was run at a frequency of 60 mc. in $CDCl_3$ as a solvent (8 mg./ml.). Tetramethylsilane was used as an internal reference standard. A peak at 435 c.p.s. is due to the phenolic proton. A peak at 114

(1) Taken in part from a dissertation presented by W. A. Skinner to the Graduate School of the University of Texas in partial fulfillment of the requirements for the Ph.D. degree, June, 1952.

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(3) V. L. Frampton, W. A. Skinner and P. S. Bailey, *Science*, **116**, 34 (1952).

(4) V. L. Frampton, W. A. Skinner and P. S. Bailey, THIS JOURNAL, **76**, 282 (1954).

c.p.s. is characteristic of a CH_3 on a quinone ring and is definitely not large enough to represent two methyls. Furthermore, it is quite sharp and it is very unlikely that two structurally non-equivalent methyls would give a single sharp line. It also compares in size with the sharp line just to the left of the peak at 73 c.p.s. This is probably the CH_3 group on the carbon next to the chroman ring oxygen. A peak at 73 c.p.s. arises from the CH_2 groups in the side chain, while the double peak at 49 represents the methyl groups attached to CH. No hydroxyls other than the phenolic hydroxyl are present. Of the possible structures for α-tocopurple, only III could account for such a spectrum.

Attempts to form a solid derivative by reduction of α-tocopurple with zinc and acetic acid yielded only a colorless waxy material which was probably the trihydroxy derivative. The extreme ease of air oxidation of this derivative to yield α-tocopurple is reminiscent of the behavior of α-tocopherylhydroquinone.

Acetylation of α-tocopurple with acetic anhydride in pyridine gave an oily yellow acetate, as evidenced by its infrared absorption spectrum, which showed the appearance of an O–acetyl band and the disappearance of the hydroxyl band. Reductive acetylation with zinc and acetic anhydride in pyridine also gave an impure, oily acetate that showed the absence of both hydroxyl bands and carbonyl bands along with the presence of strong O–acetyl absorption, 5.6 μ.

Failure to obtain crystalline derivatives of α-tocopurple led to synthesis of model compounds, tetramethyl - 6 - hydroxychromans, for oxidation studies. The three isomeric dimethylhydroquinones were condensed with isoprene, using zinc chloride as the catalyst.[5] By sublimation, 2,2,7,8-tetramethyl-6-hydroxychroman (VII) was obtained as a pure crystalline solid, m.p. 86–87° (lit.[5] 84.5–85.5°), yield 29%. In addition to VII the condensation with isoprene also yielded a double chroman (VIII), m.p. 101–102° (lit.[5] 102.5–103.5°), in 16% yield. The infrared absorption spectrum of VII is very similar to that of α-tocopherol.

The condensation of 2,6-dimethyl-1,4-hydroquinone with isoprene yielded 2,2,5,7-tetramethyl-6-hydroxychroman (IX) in 9% yield. Compound IX was also obtained as an analytically pure solid, m.p. 92.5–93.5°, when purified by sublimation followed by recrystallization from petroleum ether (30–60° b.p.).

Condensation of 2,5-dimethyl-1,4-hydroquinone with isoprene was made difficult by the very low solubility of the hydroquinone in the condensing solvent, acetic acid. An analytically pure sample of 2,2,5,8-tetramethyl-6-hydroxychroman (X), m.p. 77–78°, was obtained by sublimation of the crude chroman followed by recrystallization from petroleum ether (30–60° b.p.). The double chroman (XI) formed from this condensation was isolated in an analytically pure form, m.p. 193–196°.

Oxidation of VII and IX with a 20:1 weight excess of ferric chloride hexahydrate in refluxing methanol for three hours yielded several oxidation products which could be separated by column chromatography. When the oily oxidation products were chromatographed on a zinc carbonate column and eluted with petroleum ether (30–60° b.p.), a purple band remained at the top of the column while the yellow and orange bands moved off the column. The purple band was removed from the column and extracted with 0.25 wt. % methanolic potassium hydroxide solution, water was added, and the solution acidified with 5% hydrochloric acid until a yellow color appeared. Ether extraction removed XII, which crystallized from petroleum ether (30–60° b.p.) as an orange solid (m.p. 142–143.5°). The same compound (XII) was obtained by oxidation of either IX or VII, as shown by the identity of their infrared absorption spectra, ultraviolet absorption spectra, melting points, mixed melting points and elementary analyses.

Compound XII was previously obtained by John[6] from the nitric acid oxidation of 2,2,5,7,8 pentamethyl-6-hydroxychroman and identified as either XII or XIII by elementary analysis, which showed the loss of two methyl groups and appearance of two oxygens and allowed John to eliminate the open chroman structures analogous to those of IV, V or VI. Although John did not isolate α-tocopurple, he did observe the purple color produced in basic solutions by one of the nitric acid oxidation products of α-tocopherol and postulated a structure analogous to XII or XIII.

Since the same compound, XII, was obtained in this Laboratory by oxidation of either VII or IX, the structure is definitely proved to be XII rather than XIII. Compound IX could not lead to XIII by elimination of one methyl group upon oxidation, although conceivably VII could lead to XIII *via* ring opening and reclosure at the 5-position prior to final oxidation of one of the two methyl groups remaining at 7- and 8-positions.

The similarity between the model compounds (VII, IX, X) and α-tocopherol strongly suggests that the hydroxyquinone obtained upon oxidation of α-tocopherol has structure III. The ultraviolet absorption spectra and infrared absorption spectra of III and XII are very similar as would be expected if III were the correct structure for α-tocopurple.

Experimental

Preparation of α-tocopurple; 2,7-Dimethyl-6-hydroxy-2-(4,8,12-trimethyltridecyl)-5,8-chromanquinone (III).—*dl*-α-Tocopherol (1 g.), absorption maximum in methanol 292 mμ $E^{1\%}_{1cm}$ 74.2, was oxidized with methanolic ferric chloride and the products chromatographed on zinc carbonate mixed with Celite as previously described.[3,1] The purple band portion of the column was removed and eluted with a methanolic solution, 0.25 wt. % in potassium hydroxide. After acidification of the purple methanol solution to a yellow color with 5% hydrochloric acid followed by addition of 100 ml. of water, the α-tocopurple could be ether extracted. Concentration of the dried ether extracts *in vacuo* yielded an orange-colored oil. Repeated chromatographing on zinc carbonate yielded an orange wax which was homogeneous on paper chromatography. α-Tocopurple moved as one spot on Whatman No. 1 paper in benzene–methanol–Skellysolve C–water (3:8:6:2), benzene–water–methanol (2:1:6) butanol–water (1:1). The infrared and ultraviolet absorp-

(5) L. I. Smith, H. E. Ungnade, H. H. Hoehn and S. Wawzonek, *J. Org. Chem.*, **4**, 311 (1939); L. I. Smith and R. W. H. Tess, THIS JOURNAL, **66**, 1523 (1944).

(6) W. John and W. Emte, *Z. physiol. Chem.*, **268**, 85 (1941).

tion spectra showed no change on further chromatographing; $\lambda_{max(\mu)}^{film}$ 2.95 (OH), 3.45 (CH), 6.10, 6.20 (C=O, C=C), 7.25, 7.40 (CH_3), 7.85 (=C—O—C), 8.70 (=C—OH).

Anal. Calcd. for $C_{23}H_{44}O_4$ (chroman ring closed): mol. wt., 432; C, 75.0; H, 10.3. For $C_{28}H_{48}O_4$ (chroman ring open): mol. wt., 448: C, 75.0; H, 10.8. Found: C, 74.6; H, 10.4; mol. wt., Rast, 967 (dimer); equiv. wt. from potentiometric titration, 477–510.

Synthesis of Tetramethyl-6-hydroxychromans.—The synthetic scheme of Smith[5] was followed for the synthesis of the model 6-hydroxychromans. 2,3-Dimethyl-1,4-hydroquinone was synthesized by the method of Emerson[7] *via* dichromate oxidation of *o*-xylidine sulfate followed by reduction of the quinone with zinc and acetic acid.

2,2,5,7-Tetramethyl-6-hydroxychroman (IX).—Condensation of 2,6-dimethyl-1,4-hydroquinone with isoprene by refluxing for 2 hours in glacial acetic acid with zinc chloride[5] as catalyst yielded an oil after addition of water and extraction with petroleum ether (b.p. 30–60°). Unreacted hydroquinone was removed by extraction of the petroleum ether solution with 5% aqueous sodium hydroxide. Extraction with Claisen alkali removed the desired chroman (IX) from the petroleum ether solution.

The crude oily chroman was sublimed at 60° and 1 mm. to yield a colorless oil which crystallized upon addition of petroleum ether (b.p. 30–60°) to yield IX in 9% yield, m.p. 92.5–93.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.80, 2.90 (OH), 7.25, 7.40 (CH_3), 8.12 (=C—O—C), 8.55 (=C—OH).

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.7; H, 8.80. Found: C, 75.7; H, 9.00.

2,2,5,8-Tetramethyl-6-hydroxychroman (X).—Condensation of 2,5-dimethyl-1,4-hydroquinone with isoprene in acetic acid, using zinc chloride as catalyst, gave a very low (<5%) yield of X, m.p. 77–78° from petroleum ether (b.p. 30–60°). (An equal amount of the double chroman XI, m.p. 193–196°, was also produced.) The insolubility of the hydroquinone in acetic acid hampers the reaction, most of it being recovered unchanged; (X) $\lambda_{max(\mu)}^{Nujol}$ 3.05 (OH), 6.21, 6.68 (aryl), 7.20, 7.30 (CH_3), 8.00 (=C—O—C), 8.55 (=C—OH); (XI) $\lambda_{max(\mu)}^{Nujol}$ 7.25, 7.30 (CH_3), 7.90, 8.20 (=C—O—C).

Anal. Calcd. for $C_{13}H_{18}O_2$ (X): C, 75.7; H, 8.80. Found: C, 75.7; H, 8.73. Calcd. for $C_{18}H_{26}O_2$ (XI): C, 78.8; H, 9.55. Found: C, 78.5; H, 9.32.

Oxidation of VII and IX with Ferric Chloride.—Ferric chloride hexahydrate (16 g.) was added to a 25-ml. volume of methanol to which had been added 800 mg. of VII or IX. The solution was refluxed for 3 hours, cold water was added, and the oxidation products were ether extracted; the ether extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The brown-red oil was chromatographed on a zinc carbonate–Celite column with the purple band remaining at the top of the column and an orange band moving ahead upon petroleum ether elution. The purple band was removed from the column with methanol containing 0.25 wt. % potassium hydroxide and acidified to a yellow color with 5% hydrochloric acid. The hydroxyquinone XII was extracted with diethyl ether after adding water to the methanol solution. Concentration of the ether extracts after drying yielded crude XII. Recrystallization from petroleum ether (b.p. 30–60°) yielded an analytical sample of XII, m.p. 142–143.5°, orange crystals; John[6] reported m.p. 140° (same compound obtained from oxidation of either VII or IX); $\lambda_{max(\mu)}^{Nujol}$ 3.00 (OH), 6.05, 6.20 (C=O, C=C), 7.25, 7.40 (CH_3), 7.75 (=C—O—C), 8.66 (=C—OH). The ultraviolet absorption maximum in methanol was at 296 mμ with ε 18,200; John[6] reported a maximum at 298 mμ with ε 20,000.

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.8; H, 6.35. Found: C, 64.7; H, 6.36.

Acknowledgments.—The authors wish to thank Dr. J. Shoolery of Varian Associates for interpretation of the nuclear magnetic resonance spectra and to thank Dr. Peter Lim of the Institute's Biological Sciences Department for interpretation of the infrared absorption spectra. We are also indebted to the Institute's Physical Sciences Divisional Research Committee for partial support of this work.

(7) O. H. Emerson and L. I. Smith, THIS JOURNAL, **62**, 141 (1940).

[CONTRIBUTION FROM AVERY LABORATORY, UNIVERSITY OF NEBRASKA]

Cinnolines. VII. The Neber–Bossel Synthesis[1,2]

BY HENRY E. BAUMGARTEN AND PAUL L. CREGER[3]

RECEIVED FEBRUARY 11, 1960

Although the cyclization of *o*-hydrazinomandelic acid is known to yield 3-cinnolinol (3-hydroxycinnoline) (the Neber-Bossel synthesis), the cyclization of α-substituted *o*-hydrazinomandelic acids may yield either the corresponding 4-substituted 3-cinnolinol or the 3-substituted 1-aminodioxindole.

At present 3-cinnolinol (3-hydroxycinnoline, Va) is best synthesized by a sequence consisting of the diazotization of *o*-aminomandelic acid (IIIa), reduction of the diazonium salt with stannous chloride and hydrochloric acid and cyclization of the resultant *o*-hydrazinomandelic acid (IVa) by heating in aqueous acid. The original work on this synthesis was published only in dissertation form,[4] and all of the available knowledge of this method is due to a study by Alford and Schofield,[5] who have labeled the procedure the Neber–Bossel synthesis.

(1) Paper VI. THIS JOURNAL, **82**, 3977 (1960).

(2) This work was initiated with the support of National Science Foundation grant, G-1090, and completed with the support of U. S. Public Health Service grant, CY-3090, and a grant from the University of Nebraska Research Council. This communication is abstracted from the Ph.D. thesis (June, 1957) of P.L.C.

(3) Eastman Kodak Co. Fellow, 1955–1956.

(4) G. Bossel, Inaug. Diss. Tubingen, 1925; *Chem. Zentr.*, **100, II**, 3015 (1929).

(5) E. J. Alford and K. Schofield, *J. Chem. Soc.*, 2012 (1952).

In their experiments Alford and Schofield prepared IIIa by converting *o*-nitrobenzaldehyde into the corresponding cyanohydrin, *o*-nitromandelonitrile, followed by hydrolysis of the nitrile function and reduction of the nitro group. Although they were able to realize a 59% yield of Va (based on *o*-nitrobenzaldehyde), the attempted application of their multi-step version of the Neber–Bossel procedure to the synthesis of 6-chloro- and 6-methoxy-3-cinnolinol gave only low yields of the expected products. The present communication describes an extension of this synthesis to 3-cinnolinols having a substituent other than hydrogen in the 4-position.

Although *o*-nitroaryl ketones might be the logical starting materials for the objective at hand, the difficulty with which such materials are prepared caused us to direct our attention to the use of isatin (I) for this purpose. The reaction sequence is