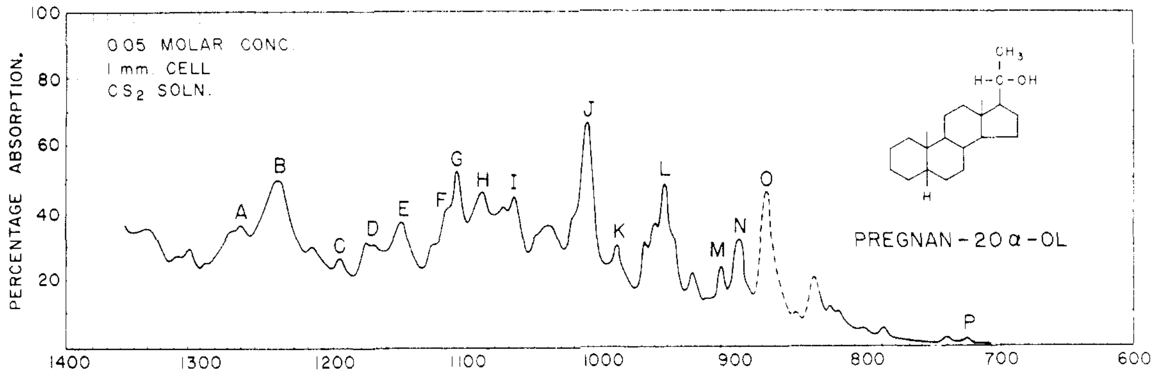


Fig. 10.

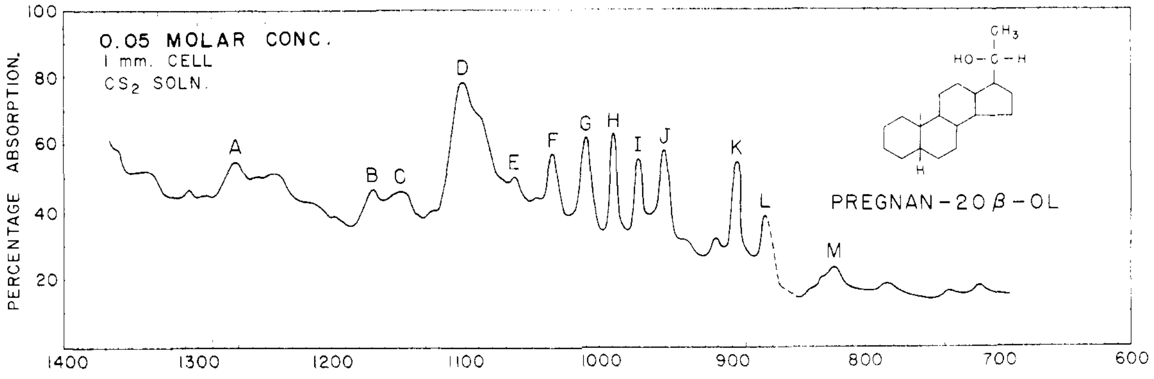


Fig. 11.

Acknowledgments.—We wish to thank Dr. E. M. Augdahl and Dr. A. Nickon who synthesized a number of the compounds of the C(21) series. We also wish to acknowledge the technical assistance of Mrs. M. A. MacKenzie, Miss Beate Spiegel and Miss Doris Leskin who measured a number of the spectra supplementing those published in the two volumes of the atlas of steroid spectra.[5,6] This investigation has been supported in part by a grant from the American Cancer Society, and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

NEW YORK, N. Y.
OTTAWA, CANADA

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, R. J. REYNOLDS TOBACCO CO.]

Flue-cured Tobacco. III. Solanachromene and α-Tocopherol

BY R. L. ROWLAND

RECEIVED APRIL 7, 1958

A high molecular weight phenol, solanachromene, has been isolated from aged flue-cured tobacco leaf in 0.05% of the dry weight of the leaf. Structural studies indicate that solanachromene is 2,7,8-trimethyl-6-hydroxy-2(4',8',12',16',20',24',28',32',36'-nonamethyl-3',7',11',15',19',23',27',31',35'-heptatriacontanonenyl)-1,2-benzopyran (V, $R_1 = R_2 = CH_3$, $R_3 = H$). α-Tocopherol was isolated from aged tobacco leaf in an amount 0.01–0.02% of the dry weight of the leaf.

In a study of the ether-soluble compounds extracted from aged flue-cured tobacco, we have recently reported the isolation of solanesol[1] and neophytadiene.[2] We now wish to report the isolation of two phenol fractions, α-tocopherol and a high molecular weight unsaturated phenol to which we have given the name solanachromene. Isolation of these two compounds was accomplished by repeated chromatography using silicic acid.

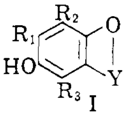
Solanachromene, which constituted about 0.05% of the dry weight of the tobacco leaf, is a colorless oil which, after solidification at reduced temperature, melted at 16-19°. The infrared absorption is shown in Fig. 1. Absorption at 3 μ indicated the presence of a hydroxyl group, absorption at 6 μ indicated unconjugated double bonds of the type present in solanesol and absorption at 6.3 μ sug-

(1) R. L. Rowland, P. H. Latimer and J. A. Giles, THIS JOURNAL, **78**, 4680 (1956).

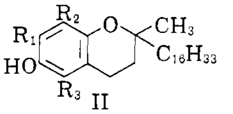
(2) R. L. Rowland, *ibid.*, **79**, 5007 (1957).

gested aromatic or conjugated double bonds. With the exception of the absorption bands due to double bonds, the spectrum of solanachromene was similar to that of γ-tocopherol below 10 μ.[3,4]

Other properties suggestive of a structure similar to the tocopherols were noted. In titration with tetrabutylammonium hydroxide in pyridine, solanachromene exhibited an end-point at 1020 mv. with a neutral equivalent of approximately 830. The elemental analyses of solanachromene were consistent with a formula in the range $C_{50}H_{76}O_2$ to $C_{65}H_{102}O_2$ but, limiting the formula on the basis of the neutral equivalent, a formula of $C_{57}H_{88}O_2$ to $C_{60}H_{94}O_2$ was indicated. Active hydrogen determination showed only one active hydrogen, indicating that the phenolic group is the only hydroxyl present and that the second oxygen must be present in an ether linkage. This was in agreement with the analyses of the acetate and 3,5-dinitrobenzoate esters which were monoesters of a molecular weight in agreement with the neutral equivalent determined for solanachromene. These results indicate a structure (I) similar to the tocopherols (II), where R_1, R_2 and R_3 = CH_3 or H.

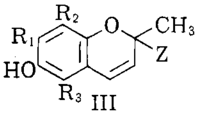


I



II

The ultraviolet absorption of solanachromene, however, is not compatible with a 6-hydroxychroman structure. The ultraviolet absorption of solanachromene showed maxima at 233 mμ, log ε 4.37; 267 mμ, log ε 3.76; 332 mμ, log ε 3.63 with a shoulder at 275 mμ, log ε 3.74, indicating additional unsaturation conjugated with the aromatic ring as in structure III.



III

An absorption spectrum similar to that of solanachromene has been reported for 5″-hydroxy-2,2-dimethyl-3,4,5,6-tetrahydrodibenzopyran: λ_{max} 264 mμ, log ε 3.74; λ_{max} 328 mμ, log ε 3.71.[5] Maximum absorption at 334 mμ, log ε 3.57, has been reported for 2,2,4-trimethyl-6-hydroxychromene.[6] We have observed two maxima for this compound: 330 mμ, log ε 3.56; 260 mμ, log ε 3.47. To note the effect upon the ultraviolet absorption of additional methyl groups in the aromatic ring, 2,2,4,7,8-pentamethyl-6-hydroxychromene was prepared and showed the maxima: 223 mμ, log ε 4.29; 264 mμ, log ε 3.76; 330 mμ, log ε 3.65 with a shoulder at 272 mμ, log ε 3.68.

It is apparent that Y (formula I) must be about $C_{50}H_{82}$. This value suggests that Y is derived from the allylic alcohol, solanesol, $C_{50}H_{82}O$ (IV), which is present in the tobacco leaf in the relatively large amount of 0.4% of the weight of the dry leaf, and that the structure of solanachromene can be represented by V.

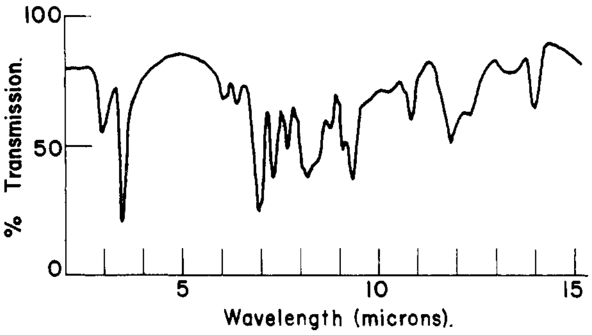
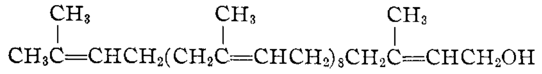
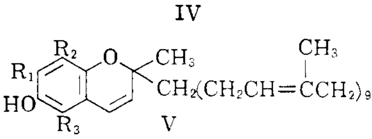


Fig. 1.—Infrared spectrum of solanachromene, oil.



$$CH_3\overset{\displaystyle CH_3}{\overset{|}{C}}{=}CHCH_2(CH_2\overset{\displaystyle CH_3}{\overset{|}{C}}{=}CHCH_2)_8CH_2\overset{\displaystyle CH_3}{\overset{|}{C}}{=}CHCH_2OH$$

IV



V

A structure of type V for solanachromene was indicated by catalytic reduction. Quantitative catalytic hydrogenation showed equivalent weights of 88.0 and 88.8, indicating 9.3 double bonds in a molecular weight of 817. The product from the catalytic reduction showed an ultraviolet absorption maximum at 296 mμ, log ε 3.47, as expected for a 6-hydroxychroman. The infrared absorption of the reduction product was identical with that of γ-tocopherol.[3,4] This similarity of infrared absorptions is reasonable since the only difference in structure is that the side chain of γ-tocopherol contains three repeating saturated isoprene units while the side chain of hydrogenated solanachromene is considered to contain nine repeating saturated isoprene units.

In an attempt to effect a partial synthesis of compounds similar to solanachromene, the condensation of solanesol with substituted hydroquinones was effected at 180° in the presence of phosphorus pentoxide, even though we realized that the conditions might effect cyclization of the unsaturated solanesol chain. The products from the condensation of trimethylhydroquinone and toluhydroquinone with solanesol showed infrared spectra differing significantly from the spectra of solanachromene and its reduction product while the condensation product from 2,3-dimethylhydroquinone and solanesol showed an infrared spectrum similar to the spectra of solanachromene and its reduction product. Catalytic reduction of the condensation product from solanesol and 2,3-dimethylhydroquinone consumed 8 to 9 moles of hydrogen for a molecular weight of 819. The infrared absorption of the resulting viscous oil was indistinguishable from the infrared absorption of reduced solanachromene.

From the pyrolysis of solanachromene at 360°, a small yield of 2,3-dimethylhydroquinone was isolated. If solanachromene had pyrolyzed in a

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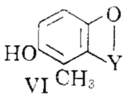
(3) M. H. Stern, C. D. Robeson, L. Weisler and J. G. Baxter, THIS JOURNAL, **69**, 869 (1947).

(4) H. Rosenkrantz and A. T. Milhorat, *J. Biol. Chem.*, **187**, 83 (1950).

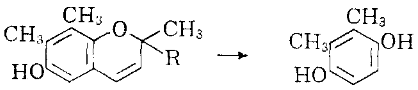
(5) R. Ghosh, A. R. Todd and S. Wilkinson, *J. Chem. Soc.*, 1121 (1940).

(6) F. Bergel, A. Jacob, A. R. Todd and T. S. Work, *ibid.*, 1375 (1938).

manner analogous to the tocopherols, one would propose a structure (VI) for solanachromene similar


VI

to the structure of ϵ-tocopherol.[7] However, the great similarity of the infrared absorptions of solanachromene and its reduction product to the infrared absorption of γ-tocopherol suggests that cleavage of the chromene occurs in the fashion



Accordingly, the pyrolysis of hydrogenated solanachromene was studied since this should pyrolyze in the same manner as the tocopherols. From the pyrolysis of hydrogenated solanachromene, we have obtained a mixture of 2,3-dimethylhydroquinone and trimethylhydroquinone. The pyrolysis of the hydrogenated condensation product from solanesol and 2,3-dimethylhydroquinone also yielded a mixture of 2,3-dimethylhydroquinone and trimethylhydroquinone. Finally, the pyrolysis of γ-tocopherol, reported[8] to give a mixture of trimethylhydroquinone with a little durohydroquinone, was repeated. The infrared absorption of the sublimate obtained from pyrolysis of d-γ-tocopherol showed it to be a mixture of trimethylhydroquinone and 2,3-dimethylhydroquinone, with no evidence for the presence of durohydroquinone.

On the basis of the pyrolysis results and the similarity of hydrogenated solanachromene to the hydrogenated condensation product from solanesol and 2,3-dimethylhydroquinone, hydrogenated solanachromene is postulated to be a 2,7,8-trimethyl-6-hydroxychroman. On the basis of the ultraviolet absorption, solanachromene is then a 2,7,8-trimethyl-6-hydroxychromene. The assignment of the position of the nine double bonds in the long aliphatic side chain is not possible from the available evidence but, on the assumption of a relationship to solanesol, it is reasonable to expect that the side chain is that shown in structure V.

Considering the multiple nature of the tocopherols, one would expect the existence of a similar family of chromenes, varying in the nature of R_1, R_2 and R_3. It is possible that tobacco contains similar chromenes with variations in the nature of R, but, of the material which we have isolated, the major compound must be that in which $R_1 = R_2 = CH_3$, $R_3 = H$.

In the process of isolating solanachromene by chromatography, a fraction eluted before solanachromene was found to give an infrared absorption identical with that reported for α-tocopherol,[3,4] with the exception that the material from tobacco consistently showed a weak absorption band at 5.85 μ. This we considered due to contamination by a small amount of neutral carbonyl compound. An impurity was indicated also by the facts that the intensity of absorption at 293 mμ was slightly low [E (1%, 1 cm.) 69 compared with a reported value of 73.7][9] and the value of the neutral equivalent was high. The p-nitrophenylurethan of the crude material from tobacco was prepared and was found to be identical in melting point and infrared absorption with the p-nitrophenylurethan prepared from authentic d-α-tocopherol.

Acknowledgments.—We are indebted to Mr. John J. Whalen for our infrared spectra, to Messrs. R. H. Cundiff, A. J. Sensabaugh, J. R. Martin and Dr. P. C. Markunas for analytical data, to Mr. T. C. James for extraction of the tobacco, and to Mr. J. F. Luper for chromatographic separations.

Experimental[10]

Isolation of Solanachromene.—The extraction and partial separation of the hexane-soluble materials from aged flue-cured tobacco was accomplished by the procedures reported previously.[1,2] From chromatography of 140 g. of hexane-soluble material on silicic acid, 152 cm. (diam.) × 508 cm., 21.8 g. of a dark oil was eluted by 4 l. of benzene after previously eluting the column with 8 l. of hexane and 4 l. of carbon tetrachloride. The fraction eluted by benzene was purified further by repeated chromatography using silicic acid to yield 2.0 g. of solanachromene (0.055% of the dry weight of the tobacco leaf) eluted just prior to solanesol. Solanachromene was eluted from alumina by 9:1 ether–methanol and from Florisil by benzene. Chromatography using charcoal resulted in extensive decomposition. Crystallization was accomplished from acetone at −27°, but the solid melted on warming to room temperature.

Solanachromene.—Solanachromene is a viscous oil, n^{26}_{D} 1.5298. The solid which formed at 0° melted at about 16–19°. Optical activity is not detectable. The infrared absorption is shown in Fig. 1. The ultraviolet absorption shows the following maxima: λ_{max} 233 mμ, log ϵ 4.37; λ_{max} 268 mμ, log ϵ 3.76; λ_{max} 275 mμ, log ϵ 3.74; λ_{max} 332 mμ, log ϵ 3.63. *Anal.* Calcd. for $C_{58}H_{88}O_2$: C, 85.23; H, 10.85; O, 3.92; active H, 0.12; neut. equiv., 817. Found: C, 85.04, 85.06; H, 10.07, 10.90; O, 3.86; active H, 0.11; neut. equiv., 840, 822; mol. wt. (ebull.), 864.

Solanachromene Acetate.—A mixture of 0.84 g. of solanachromene, 40 ml. of toluene, 0.47 g. of acetic anhydride and 4 drops of anhyd. pyridine was heated under reflux for 5 hours. After purification by standard procedures, the acetate was chromatographed on silicic acid from which it was eluted by carbon tetrachloride–benzene mixtures; yield 0.87 g. After crystallization from acetone at −27°, the acetate melted at 32–34°. The ultraviolet absorption showed the maxima: 317 mμ, log ϵ 3.56; 276 mμ, log ϵ 3.77; 267 mμ, log ϵ 3.82; 236 mμ, log ϵ 4.45; 228 mμ, log ϵ 4.50. *Anal.* Calcd. for $C_{60}H_{90}O_3$: C, 83.86; H, 10.56. Found: C, 83.64, 83.66; H, 10.04, 10.48.

Solanachromene 3,5-Dinitrobenzoate.—The dinitrobenzoate ester was prepared by heating a mixture of 1.4 g. of solanachromene, 1.3 g. of dinitrobenzoyl chloride, 40 ml. of toluene and 0.5 ml. of pyridine under reflux for two hours. The mixture was diluted with 100 ml. of ether and was extracted successively with two 50-ml. portions of 10% sulfuric acid, two 50-ml. portions of 10% sodium carbonate solution and two 50-ml. portions of water. The residue from concentration of the ethereal solution was purified by chromatography using silicic acid. The ester, 1.05 g., was crystallized from acetone at −27°. On warming to room temperature, the solid melted and resolidified, m.p. 46–48°. *Anal.* Calcd. for $C_{65}H_{90}N_2O_7$: C, 77.19; H, 8.98; N, 2.77. Found: C, 77.32, 77.04; H, 8.59, 8.63; N, 3.06, 2.75.

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(7) P. W. R. Eggitt and F. W. Norris, *J. Sci. Food and Agric.*, **7**, 493 (1956).

(8) O. H. Emerson, THIS JOURNAL, **60**, 1741 (1938).

(9) J. G. Baxter, C. D. Robeson, J. D. Taylor and R. W. Lehman, *ibid.*, **65**, 918 (1943).

(10) All melting points were determined using a Fisher–Johns melting point apparatus. All chromatographic separations were made by the use of flowing chromatograms, in which the separated materials were obtained by use of eluents in a graded series. Elemental analyses were by Micro-Tech Laboratories, Skokie, Ill., the Clark Microanalytical Laboratory, Urbana, Ill., and the Huffman Microanalytical Laboratories, Wheatridge, Colo. The neutral equivalents were determined by the procedure of R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792 (1956).

Pyrolysis of Solanachromene.—Solanachromene, 400 mg., was heated under nitrogen at 350–365° for 1.2 hours. The hexane-insoluble portion of the sublimate, 24 mg., was crystallized from benzene, giving 4 mg. of solid. The melting point appeared to be *ca.* 215°, but could not be determined satisfactorily because of sublimation. The infrared absorption was identical with that of 2,3-dimethylhydroquinone.[11]

Pyrolysis of γ-Tocopherol.—γ-Tocopherol, 446 mg., was heated under nitrogen at 345–360° for one hour. The pentane-insoluble portion of the sublimate (30 mg.) showed the infrared absorption expected for a mixture of trimethylhydroquinone and 2,3-dimethylhydroquinone, with the latter present in the smaller amount. Crystallization from benzene gave 8 mg. of solid, subliming from 195–220°, which showed the infrared absorption of 2,3-dimethylhydroquinone containing a slight amount of trimethylhydroquinone. The 2,3-dimethylhydroquinone shows sharp absorption bands at 12.48 and 13.45 μ, while trimethylhydroquinone shows pronounced absorption at 8.3 μ.

Catalytic Hydrogenation of Solanachromene.—Quantitative hydrogenation was accomplished in ethyl alcohol using palladium-on-charcoal catalyst at atmospheric pressure. Values of 88.8 and 88.0 were obtained for the equivalent weight, indicating 9.3 double bonds in a molecular weight of 817. The product of the reduction was purified by chromatography on silicic acid giving a 90% yield of an oil, $n^{27}\text{D}$ 1.4827, with an absorption maximum at 296 mμ, log ε 3.47. The infrared absorption was identical with that reported for γ-tocopherol[3,4] and differed distinctly from the infrared absorption of ε-tocopherol, kindly furnished us by Dr. J. Green and by Dr. P. W. R. Eggitt. *Anal.* Calcd. for $C_{58}H_{108}O_2$: C, 83.18; H, 13.00; neut. equiv., 837. Found: C, 82.54; H, 12.75; neut. equiv., 783, 873.

Hydrogenated solanachromene, 0.4 g., was heated under nitrogen at 335–365° for one hour. The hexane-insoluble portion of the sublimate, 14 mg., showed an infrared spectrum indicating it to be a mixture of 2,3-dimethylhydroquinone and trimethylhydroquinone. This material became transparent at 170–175° and sublimed below 193°.

From the reaction of hydrogenated solanachromene, 87 mg., with formaldehyde and stannous chloride–hydrochloric acid,[7] was obtained a 60% yield of an oil showing infrared absorption essentially the same as that of α-tocopherol.

Reaction of 2,3-Dimethylhydroquinone and Solanesol.[12]—A mixture of 1 g. of solanesol, 1 g. of 2,3-dimethylhydroquinone and 0.02 g. of phosphorus pentoxide was heated at 180–190° under nitrogen for 10 minutes. The reaction mixture was extracted with four 10-ml. portions of hexane and the hexane extract was chromatographed twice using silicic acid, giving 0.14 g. of unsaturated hydrocarbon (solanesene) and 0.3 g. of a very viscous oil showing an infrared spectrum similar to that of γ-tocopherol. Maximum absorption in the ultraviolet appeared at 295 mμ, log ε 3.54. *Anal.* Calcd. for $C_{58}H_{90}O_2$: C, 85.02; H, 11.07; neut. equiv., 819. Found: C, 84.44; H, 11.17; neut. equiv., 831, 833.

The condensations of trimethylhydroquinone and of toluhydroquinone with solanesol were accomplished in a manner similar to that of 2,3-dimethylhydroquinone and solanesol. The infrared absorptions of the products were observed after purification by chromatography using silicic acid.

Reduction of the Condensation Product of 2,3-Dimethylhydroquinone and Solanesol.—Catalytic reduction of 110 mg. of the condensation product was accomplished using Adams catalyst at atmospheric pressure in ethyl alcohol. From chromatographic separation using silicic acid, 85 mg. of oil showing infrared absorption identical with that of reduced solanachromene were obtained. *Anal.* Calcd. for $C_{58}H_{108}O_2$: C, 83.18; H, 13.00; neut. equiv., 837. Found: C, 82.97; H, 13.32; neut. equiv., 781, 779.

The reduction product (130 mg.) was heated under nitrogen at 360–370° for 1.3 hours. The sublimate, 18 mg., transparent *ca.* 175°, sublimed by 195°, showed the infrared absorption of a mixture of 2,3-dimethylhydroquinone and trimethylhydroquinone.

4,7,8-Trimethyl-6-hydroxycoumarin.—A mixture of 0.5 g. of 2,3-dimethylhydroquinone, 1 g. of ethyl acetoacetate and 6 g. of sulfuric acid, after standing at room temperature for two days, was poured into 30 ml. of water. The precipitate was crystallized from ethanol–water giving 0.25 g. of yellow solid, m.p. 228–230°.

4,7,8-Trimethylcoumarin[13] (2 g.) was dissolved by warming gently with 80 ml. of 20% sodium hydroxide solution. To the cooled solution was added a solution of 3.5 g. of potassium persulfate in 100 ml. water. After 20 hours at room temperature, the mixture was acidified with concentrated hydrochloric acid and was extracted with two 100-ml. portions of ether. To the aqueous layer was added 150 ml. of concd. hydrochloric acid and 1 g. of sodium dithionite. The mixture was heated under reflux for one hour. The precipitate from the cooled solution was crystallized from ethanol–water giving 0.5 g. of yellow solid, m.p. 229–230°, with infrared absorption identical with that of the material obtained by the reaction of 2,3-dimethylhydroquinone with ethyl acetoacetate. *Anal.* Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.10; H, 5.96.

2,2,4,7,8-Pentamethyl-6-hydroxychromene.—To a cold solution of methylmagnesium iodide prepared from 2 g. of magnesium, 6 g. of methyl iodide and 70 ml. of ether was added a suspension of 0.13 g. of 4,7,8-trimethyl-6-hydroxycoumarin in 100 ml. of ether. After the addition of 200 ml. of benzene, the ether was removed by distillation and the reaction mixture was heated under reflux for one hour. The cooled mixture was poured into ice–hydrochloric acid. The benzene layer was concentrated and the residue was combined with the residue from extraction of the aqueous layer with 200 ml. of ether. The combined residues were extracted with three 30-ml. portions of hot hexane. The hexane-insoluble material (80 mg.) appeared from its infrared absorption to be 4,7,8-trimethyl-6-hydroxycoumarin. By crystallization of the hexane-soluble material from pentane at −27° were obtained 27 mg. of crystals, m.p. 99–100°. *Anal.* Calcd. for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 76.98; H, 8.35. The infrared spectrum showed a distinct absorption band at 6.3 μ similar to the absorption of solanachromene, which band is considered to be due to the conjugation of the double bond with the aromatic ring.

Isolation of α-Tocopherol from Tobacco.—α-Tocopherol was isolated from aged flue-cured tobacco in the process of isolating solanachromene. The α-tocopherol was eluted from silicic acid by 9:1 hexane–benzene shortly before the elution of solanachromene. The colorless oil, $n^{29.5}\text{D}$ 1.5025, was obtained in an amount 0.02% of the dry weight of the tobacco leaf. The infrared absorption was identical with that of α-tocopherol[3,4] with the exception that the material from tobacco showed a weak band at 5.85 μ. *Anal.* Calcd. for $C_{29}H_{50}O_2$: neut. equiv., 431. Found: neut. equiv., 495.

α-Tocopherol *p*-Nitrophenylurethan.—A mixture of 0.24 g. of α-tocopherol from tobacco, 0.5 g. of *p*-nitrophenyl isocyanate, 5 ml. of benzene and 1 drop of pyridine was allowed to stand at room temperature for 20 hours. A mixture of 5 ml. of acetone and 0.5 ml. of water was added and the mixture was shaken for 0.5 hour. The precipitate was removed by filtration and was washed with 10 ml. of hexane. The residue from concentration of the combined filtrate and washings was crystallized from methanol three times to give 105 mg. of colorless solid, m.p. 137.5–139° after sintering at 136°.[14] *Anal.* Calcd. for $C_{36}H_{54}N_2O_5$: C, 72.69; H, 9.15; N, 4.71. Found: C, 72.53, H, 9.14; N, 4.90. An additional 70 mg. of white solid showing the identical infrared absorption but a melting point of 130–132° was obtained from the recrystallization filtrates. The *p*-nitrophenylurethan obtained from *d*-α-tocopherol (Eastman) by this procedure melted at 137.5–139° and there was no depression of m.p. of a mixture of the two urethans. The infrared absorptions of the two urethans were identical.

WINSTON-SALEM, N. C.

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(12) F. von Werder, U. S. Patent 2,230,659 (1941); *C. A.*, **35**, 3270 (1941).

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(14) H. M. Evans, O. H. Emerson and G. A. Emerson, *J. Biol. Chem.*, **113**, 319 (1936), report a m.p. of 129–131°.