

THE FORMATION OF XANTHYLIUM SALTS FROM PROANTHOCYANIDINS

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Abstract—Acid treatment of monomeric and condensed natural leucoanthocyanidins yields red anthocyanidins and hitherto unidentified yellow pigments. Model leucocyanidin-polyphenol condensation products also yield yellow pigments with acids, and spectral evidence is now presented which indicates that these yellow compounds are xanthylum salts. The possible significance of these observations in relation to the structures and acid degradation of condensed tannins and phlobaphenes is discussed.

THE DETAILED structures of condensed tannins are still largely obscure. However, as a result of the elucidation of the structures of co-occurring dimeric proanthocyanidins, e.g. of types I and II, it is now generally believed that repeating units of type III, in which C₄ of one flavan unit is linked to C₈ or C₆ of a second nucleus, form a substantial part of those natural tannin molecules derived from polymerized phenolic flavan-3,4-diols.¹⁻⁵

Flavan-3,4-diols and condensation products of types I-III yield monomeric anthocyanidins when heated with alcoholic mineral acids. This property is characteristic of natural proanthocyanidins and forms the basis of the widely used method for detecting these compounds in plant extracts.^{6,7} Highest yields of anthocyanidin pigments have been reported for monomeric flavan-3,4-diols and dimers,⁸ the yield of anthocyanidin markedly decreasing with increasing degree of polymerization, e.g. natural monomeric, trimeric and pentameric leucosisetinidin yield 23.5 per cent, 6.75 per cent and 4.78 per cent of fisetinidin chloride respectively.⁹ The structural or reaction factors responsible for these very low anthocyanidin yields have not been adequately explained, particularly since C₄-C₈ (or C₆) carbon-carbon linkages in structures such as I-III are quite labile and known^{1,2} to be readily hydrolyzed by acids.

The red anthocyanidin pigments formed by the action of acids on natural proanthocyanidins are always accompanied by other unidentified pigments.¹ Thus, Swain and Hillis¹⁰ noted that the spectra of anthocyanidins (λ_{\max} 550 nm) formed from monomeric and polymeric leucoanthocyanins by hot butanol-HCl showed a pronounced shoulder at 450 nm.

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¹ T. A. GEISSMAN and H. F. K. DITTMAR, *Phytochem.* **4**, 359 (1965).

² K. FREUDENBERG and K. WEINGES, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN) p. 197, Pergamon Press, Oxford (1962); K. WEINGES and K. FREUDENBERG, *Chem. Commun.* 220 (1965).

³ K. WEINGES, *Chem. Ber.* **94**, 3032 (1961).

⁴ S. E. DREWES, D. G. ROUX, H. M. SAAYMAN, S. H. EGGERS and J. FEENEY, *J. Chem. Soc. (C)*, 1302 (1967).

⁵ E. HASLAM, *Chemistry of Vegetable Tannins*, p. 14, Academic Press, London (1966).

⁶ E. C. BATE-SMITH and T. SWAIN, *Chem. Ind.* 377 (1953).

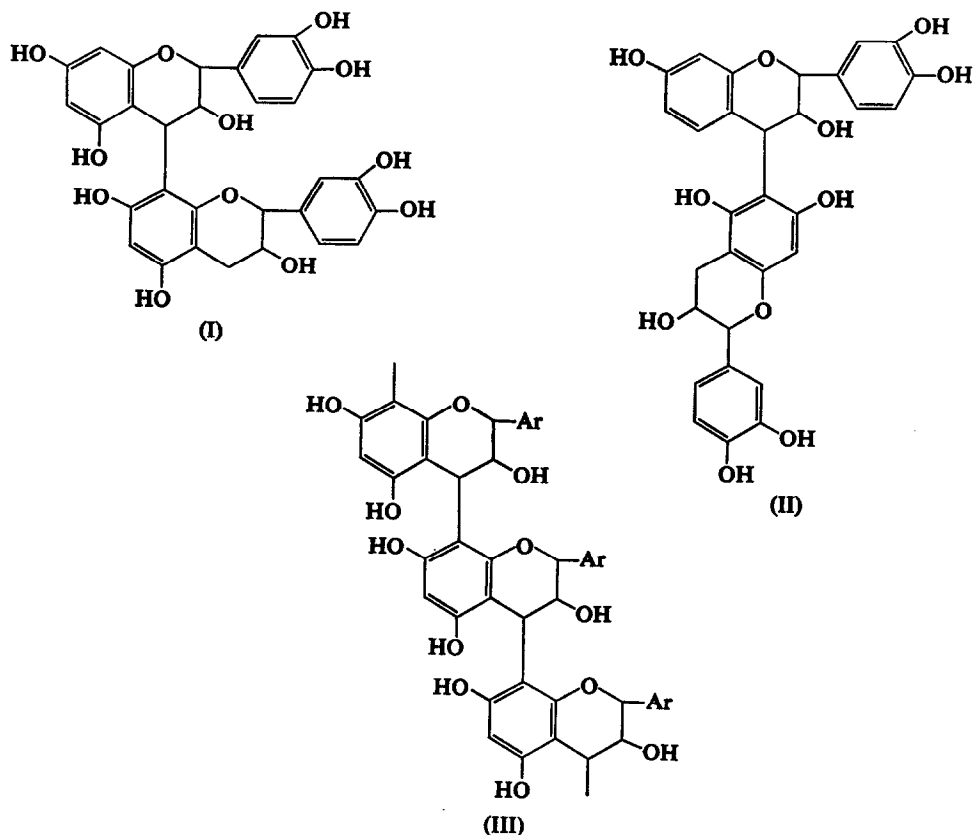
⁷ D. G. ROUX, *Nature* **180**, 973 (1957).

⁸ L. L. CREASY and T. SWAIN, *Nature* **208**, 151 (1965).

⁹ D. G. ROUX and E. PAULUS, *Biochem. J.* **82**, 320 (1962).

¹⁰ T. SWAIN and W. E. HILLIS, *J. Sci. Food Agr.* **10**, 63 (1959); see also W. E. HILLIS, *Australian J. Biol. Sci.* **9**, 263 (1956).

With hot aqueous- or acetic acid-HCl the absorbance at the λ_{\max} of the anthocyanidin substantially decreased and the shoulder at 450 nm developed into a definite peak. It was suggested that this peak is due to the formation of phlobaphene-like polymers. Similar ill-defined yellow products with $\lambda_{\max} \sim 450$ nm (and a minor peak at 365 nm) were formed by acid treatment of apple¹¹ and grape-seed¹² proanthocyanidins, and the additional significant observation was made that, in contrast to anthocyanidin formation, production of the yellow pigment from grape proanthocyanidins is not dependent on the presence of oxygen. The formation of discrete, mobile, yellow pigments on acid hydrolysis of some



tannins has also been reported. For example, Hillis¹³ found that methylated kino, a polymeric leucodelphinidin exuded from injured eucalyptus cambial tissue, gives delphinidin derivatives (λ_{\max} 521–527 nm) and appreciable amounts of an orange-colored compound with butanol-HCl. Roux and his associates^{7, 14, 15} reported that black wattle tannin gives relatively high yields of a yellow pigment with acids. The tannin was separated into five polymeric fractions, three of which yield mainly anthocyanidins (robinidin and fisetinidin)

¹¹ S. ITO and M. A. JOSLYN, *J. Food Sci.* **30**, 44 (1965).

¹² C. PERI, *Am. J. Enol.* **18**, 168 (1967).

¹³ W. E. HILLIS, *Biochem. J.* **92**, 516 (1964).

¹⁴ D. G. ROUX and S. R. EVELYN, *Biochem. J.* **69**, 530 (1958).

¹⁵ D. G. ROUX, *Chem. Ind.* 278 (1962).

and two of which give primarily the unidentified, yellow, mobile (R_f 0.66 in formic acid-3 N HCl), crystalline compound, λ_{\max} 470 nm. In accord with Roux's observations, Hillis and Urbach¹⁶ have made the reasonable suggestion that the very low yield of anthocyanidins from wattle tannin may indicate the existence in this tannin of linkages (unspecified) different in type from those, e.g. as in III, commonly associated with tannins from other sources.

RESULTS AND DISCUSSION

The crystalline leucocyanidin-phloroglucinol condensation product (IV) has been prepared^{17, 18} as a model carbon-carbon linked proanthocyanidin. As with natural pro-

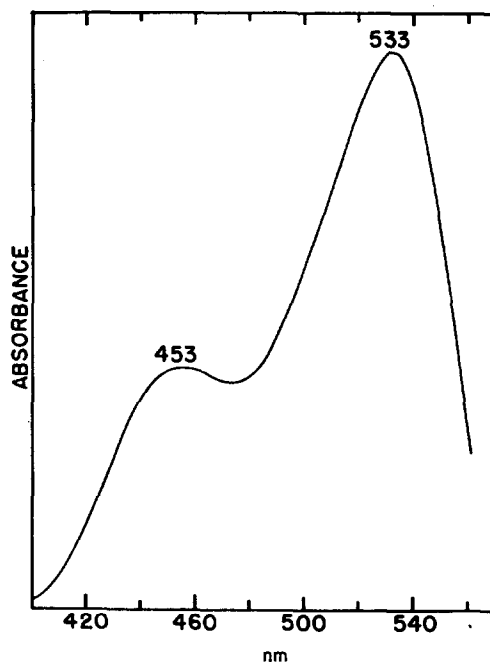


FIG. 1. SPECTRUM OF IV AFTER TREATMENT WITH HOT BUTANOL-HCl.

anthocyanidins, it has now been determined that, when treated with butanol-HCl, IV yields 5,7,3',4'-tetramethyl-cyanidin chloride, λ_{\max} 533 nm, and a yellow pigment, λ_{\max} 453 nm. The spectrum of the crude reaction mixture (Fig. 1) is, in fact, virtually identical in detailed shape to those reported by Swain and Hillis¹⁰ and Peri¹² for butanol-HCl hydrolyses of natural leucoanthocyanins. Since IV and natural condensed proanthocyanidins are considered to be structurally related, this spectral correlation indicates that the color of the yellow products formed from IV and from natural monomeric and polymeric flavan-3,4-diols may well be due to the presence of essentially similar chromophoric nuclei.

When IV is heated in aqueous acetic acid without mineral acid, anthocyanidin formation

¹⁶ W. E. HILLIS and G. URBACH, *Nature* **182**, 657 (1958).

¹⁷ T. A. GEISSMAN and N. N. YOSHIMURA, *Tetrahedron Letters* **24**, 2669 (1966).

¹⁸ L. JURD and R. LUNDIN, *Tetrahedron* **24**, 2653 (1968).

does not occur and the yellow compound(s) is the only pigment formed.* Chromatograms of an aqueous acid solution of this product, freed of colorless contaminants by exhaustive liquid-liquid extraction with ether and ethyl acetate, showed the presence only of a mobile yellow compound, which migrates as a discrete spot, and traces of a second, similar yellow compound with slightly lower R_f . The spectrum of the acidified yellow aqueous solution

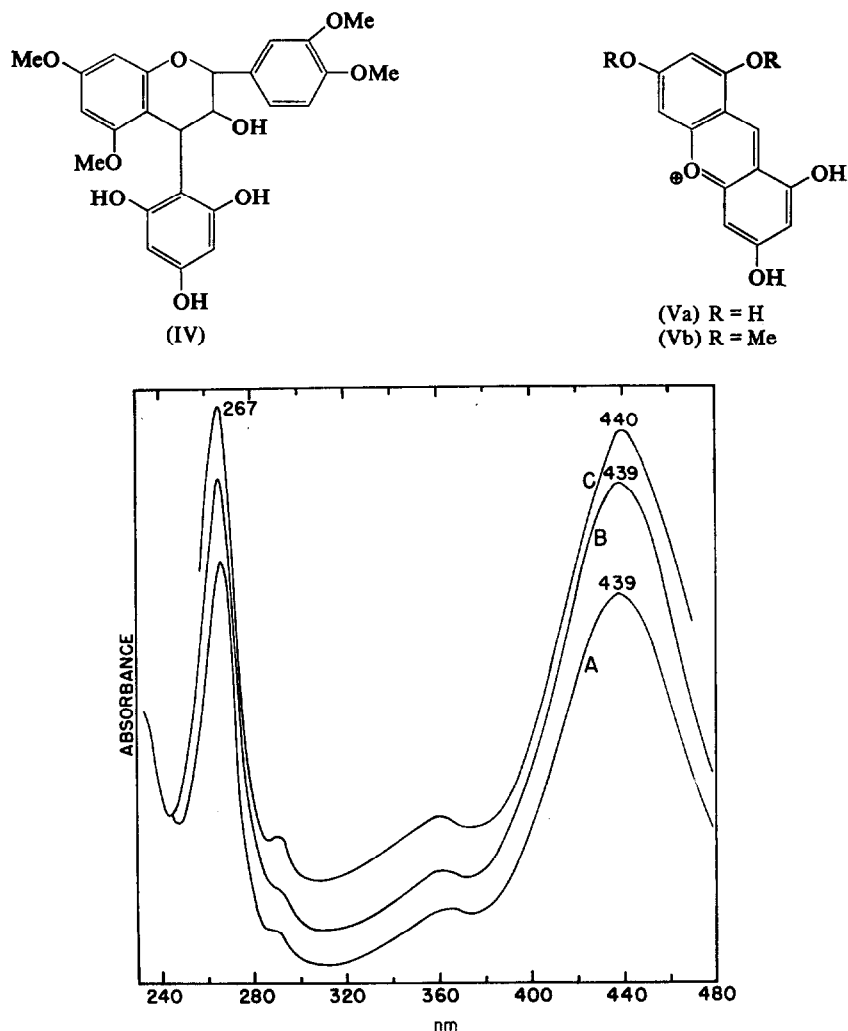


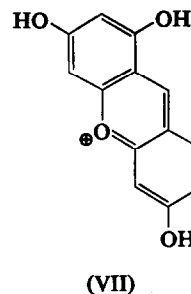
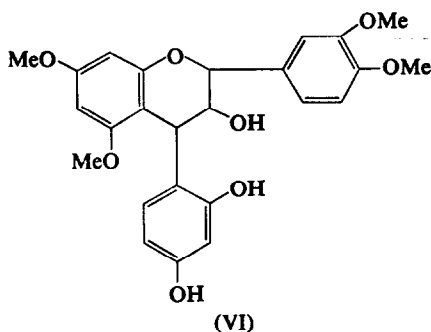
FIG. 2. SPECTRUM IN 1% aq. HCl OF (A) YELLOW PRODUCT FROM IV, (B) Va AND (C) Vb.

(Fig. 2) shows sharp, well-defined maxima at 439 and 267 nm, and minor peaks at 363 and 291 nm. The ratio of the absorbance at λ_{\max} 267 nm to the absorbance at λ_{\max} 439 nm = 1.09. The low visible λ_{\max} and the high absorbance ratio are not compatible with a flavylum structure. However, in *all* of the above details, including positions of minima, the spectrum

* Professor Joslyn, private communication, has found that apple proanthocyanidins behave similarly, giving a yellow product (λ_{\max} 450 nm) only in aqueous acetic acid solutions.

of the yellow product is essentially identical with the spectra of the synthetic tetrahydroxy-xanthylium salt (Va) and its dimethyl derivative (Vb) in aqueous HCl (Fig. 2). For Va and Vb absorbance ratios at λ_{\max} 267 and 439 nm are 1.02 and 1.06 respectively. On extraction into butanol-HCl the λ_{\max} of the yellow product shift to 453, 370, 297, 269 nm; in butanol-HCl Va has λ_{\max} 456, 369, 297, 269 nm. Addition of alkali to the aqueous solution of the yellow product results in a pronounced bathochromic shift of λ_{\max} to 478, ~375, 273 nm. The xanthylium salt (Va) behaves similarly in alkaline solution, its λ_{\max} shifting to 482, 382, 275 nm.* Chromatographically the yellow product from IV migrates similarly to the xanthylium salts, its R_f in aqueous acid solvents being intermediate between those of Va and the more mobile Vb.

Additional evidence for a xanthylium salt structure was obtained by aqueous acetic acid decomposition of the crystalline leucocyanidin-resorcinol¹⁸ condensation product (VI). The yellow product formed in this instance had λ_{\max} 418, 264 nm in aqueous HCl, and an absorbance ratio of 1.0. In alkali the long wavelength band shifted to give a characteristic double λ_{\max} at 483, 433 nm. The synthetic trihydroxy-xanthylium salt (VII) had λ_{\max} 412, 263 nm (absorbance ratio 1.0) and with alkali it gave a similar double λ_{\max} at 483, 444 nm.

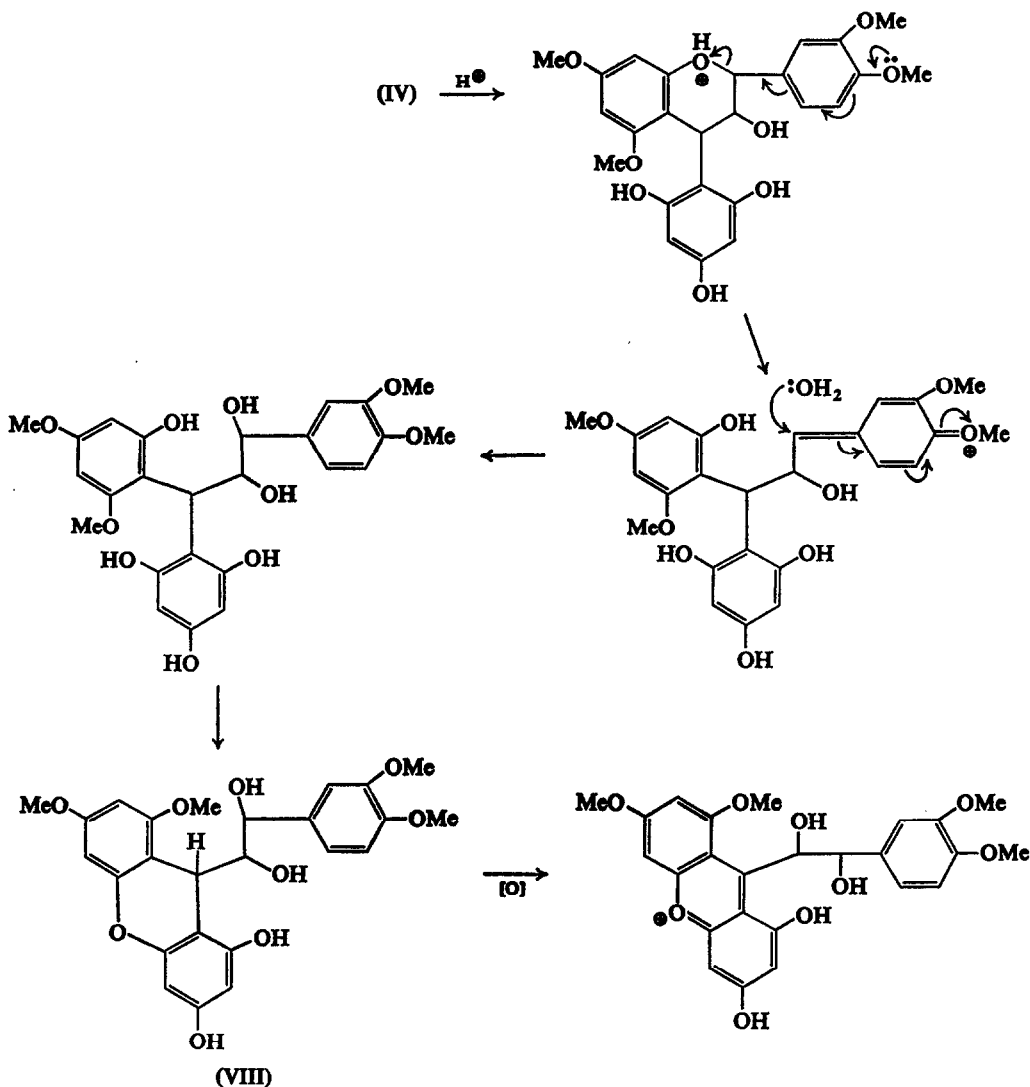


In view of the extensive coincidence of these spectral data it seems almost certain that the yellow products from the leucocyanidin-phloroglucinol and -resorcinol condensation compounds are xanthylium derivatives of Va and VII respectively. Because of the relatively small amounts of these yellow substances formed from IV and VI, however, their isolation in the quantities necessary for detailed structural analysis is difficult and has not yet been attempted. Thus their mode of formation and possible significance in relation to tannin structures and degradation products is necessarily quite speculative at this time. However, since the trimethyl ether of the phloroglucinol condensation product (VI) does not form a yellow pigment on long heating in aqueous acetic acid, a reasonable mechanism for xanthylium salt formation involves initial protonation of the benzylic oxygen atom, opening of the flavan heterocyclic ring, and recyclization to an easily oxidized xanthan derivative† (Scheme 1).

* For reasons which are not clear, the initial alkali spectrum of Vb is unstable, the λ_{\max} at 480 nm rapidly decreasing with the formation of peaks at 425 and 405 nm.

† Phloroglucinol has been detected chromatographically among the other, unidentified products formed by aqueous acetic acid with IV. In the presence of excess of resorcinol acid decomposition of IV still yields the same yellow product, λ_{\max} 439 nm. Thus, it is unlikely that this yellow compound results from a intermolecular condensation of phloroglucinol with other fragmentation products.

Well established analogies exist for each of these proposed steps. Thus, benzylic C-O linkages are protonated and subsequently ruptured much more readily than benzylic C-C linkages, e.g. addition of a drop of concentrated HCl to a solution of 5,7,3',4'-tetramethoxyflavan-3,4-diol in warm butanol instantly gives the red color of the anthocyanidin. With IV,



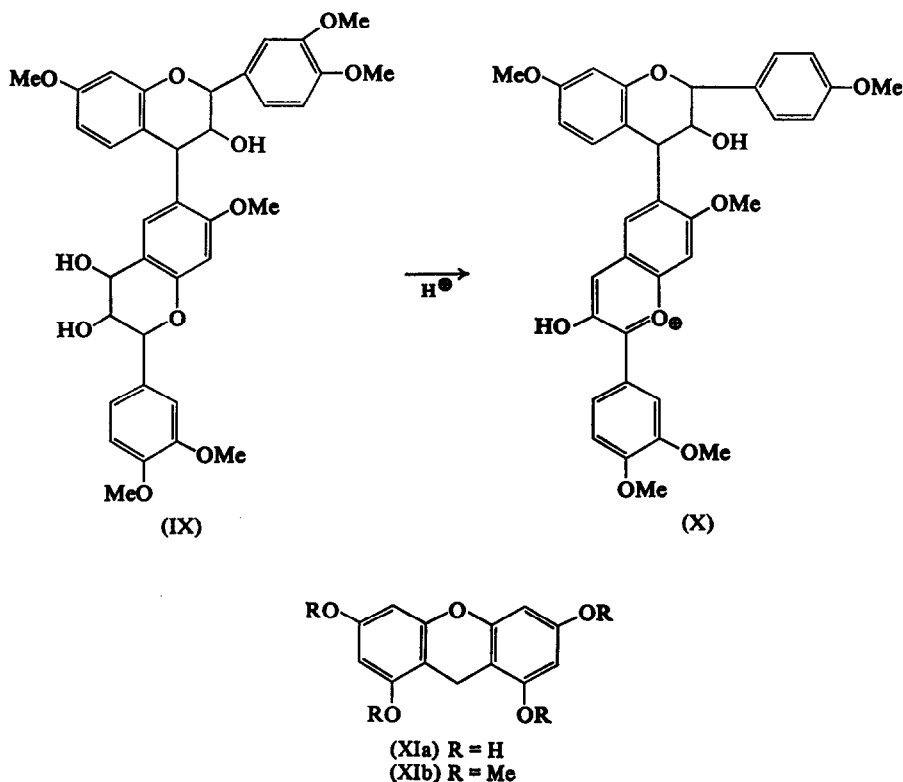
SCHEME 1. FORMATION OF XANTHENES FROM LEUCOCYANIDIN-PHLOROGLUCINOL DIMER.

on the other hand, several minutes boiling is necessary before discernible formation of anthocyanidin occurs. In further accord with this it has been shown¹⁹ that methylated dimeric leucofisetinidin (IX) yields the substituted fisetinidin (X) with acids. Facile opening of the flavan heterocyclic ring is supported by the demonstrated ease with which mollisca-

¹⁹ S. E. DREWES, D. G. ROUX, S. H. EGGERS and J. FEENEY, *J. Chem. Soc. (c)*, 1217 (1967).

cidin and related flavan-3,4-diols epimerize at C₂, as well as at C₄, in boiling water,²⁰ and by the formation of C₂-C₆ linked dimers^{2,5} from catechin in dilute acid solutions. Like flavenes, xanthenes are readily oxidized to xanthylium salts, and in the present investigation model experiments have shown that the synthetic xanthenes XIa and XIb are oxidized by air, or better, by benzoquinone,¹⁹ in aqueous acetic acid to the xanthylium salt (Va) and its tetramethyl derivative respectively. The structure of the veratryl-C₂ fragment (represented in VIII) is unknown. Variations in its structure, however, could reasonably account for the formation of two yellow products with acid.

If dimeric and polymeric flavan-3,4-diols can undergo similar ring fission and rearrangement reactions under mildly acidic conditions, then xanthene nuclei might be expected to



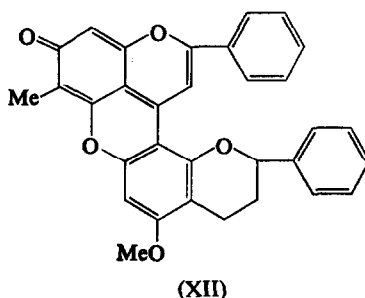
occur in some condensed tannins or in the simple polyphenols with which they are associated. It may be significant in this connection that dracorubin, an anhydro base present²² in the resin of the palm *Dracaena draco*, is a xanthylium derivative (XII). Furthermore, Roux's observation^{7,14,15} that black wattle tannins give mainly either anthocyanidins or a yellow compound with acids is explicable on the basis that the second group contains a significant number of xanthene nuclei. Rearrangement with xanthene formation may account, similarly, for Hillis' observation¹³ that the leucodelphinidin polymer from eucalyptus kino changes in aqueous solution to a product which gives appreciably lower yields of delphinidin. Finally,

²⁰ S. E. DREWES and D. G. ROUX, *Chem. Commun.* 282 (1965).

²¹ L. JURD, *Chem. Ind.* 1683 (1966).

²² A. ROBERTSON, W. B. WHALLEY and J. YATES, *J. Chem. Soc.* 3117 (1950).

the formation of yellow "phlobaphenes" on treatment of leucoanthocyanins with acids could involve (a) initial polymerization to substances of type III and (b) subsequent rearrangement and oxidation of some of the flavan-flavan units to substituted xanthylum salts. In connection with the reduced solubility of phlobaphenes, it is noteworthy that ring opening as in VIII would yield a reactive benzylic alcohol (at C₂) (Scheme 1), and further condensation at this center could lead to cross-linking of the polymer chains and reduced solubility.



EXPERIMENTAL

Spectra were determined on a Cary 15 recording spectrophotometer with 1 cm silica cells. In paper chromatography the following solvent systems were used, viz. (A) water/acetic acid/conc. HCl, 80:20:5 v/v; (B) formic acid:3 N HCl, 1:1 v/v; (C) water/acetic acid/conc. HCl, 80:40:5 v/v.

Formation of Yellow Pigments From IV

A solution of IV (0.50 g)* in 50% aq. acetic acid was heated on a steam-bath for 17 hr, cooled, and diluted with H₂O (200 ml) and 10% aq. HCl (10.0 ml). The flocculent, off-white precipitate was filtered, the filtrate was extracted continuously with Et₂O for 10 hr, and finally washed with ethyl acetate (2 × 50 ml). For determination of spectra (Fig. 2A), 2.0 ml of the residual, brightly yellow, aqueous acid solution were diluted with 12.0 ml of H₂O to give λ_{max} 439 nm (O.D. 0.742), 267 nm (A, 0.830). Chromatography of the solution showed a major yellow compound (orange with ammonia vapor), *R_f* 0.39, 0.59, 0.56, and traces of a second yellow compound (fluorescing yellow with u.v./NH₃), *R_f* 0.32, 0.45, 0.45, in solvent systems A, B and C respectively.

1,3,6,8-Tetrahydroxanthylum Chloride (Va)

Va was synthesized by reaction of phloroglucinol and ethyl formate in the presence of HCl gas, as described by Pratt and Robinson.²³ It migrates as a yellow spot, becoming orange and fluorescing with ammonia vapor, *R_f* 0.35 (A), 0.47 (B), 0.47 (C).

1,3-Dihydroxy-6,8-Dimethoxyxanthylum Perchlorate (Vb)

A solution of 2,4-di-*O*-methyl phloroglucinaldehyde (3.64 g) and phloroglucinol (3.24 g) in EtOH (20 ml) and conc. HCl (2.0 ml) was heated to boiling for 5 min and allowed to cool. The crystalline, orange xanthylum chloride (4.0 g) was collected and recrystallized from a mixture of acetic acid and 10% HClO₄. Vb perchlorate separated as yellow-orange needles, m.p. 278–279°, *R_f* 0.56 (A), 0.80 (B), 0.71 (C). (Found: C, 48.4; H, 3.61. Calc. for C₁₅H₁₃O₉Cl: C, 48.3; H, 3.52%.)

1,3,6,8-Tetrahydroxyxanthene (XIa)

NaBH₄ (0.70 g) was added in portions during 3 min to a suspension of the xanthylum salt (Va) (2.0 g) in methanol (10.0 ml). Water (50 ml) was added and the solution acidified with glacial acetic acid. The cream-colored crystals (1.8 g) were recrystallized from aq. methanol. XIa separated as slightly yellow, glistening needles, m.p. 277–279° (dec.). (Found: C, 63.2; H, 4.12. Calc. for C₁₃H₁₀O₅: C, 63.4; H, 4.09%.)

Warmed briefly with acetic anhydride and pyridine XIa formed a tetraacetate. This crystallized from acetone-methanol as colorless felted needles, m.p. 156°. (Found: C, 61.0; H, 4.41; CH₃CO—, 41.1. Calc. for C₂₁H₁₈O₉: C, 60.9; H, 4.38; CH₃CO—, 41.5%.)

²³ D. D. PRATT and R. ROBINSON, *J. Chem. Soc.* 739 (1923).

1,3-Dihydroxy-6,8-Dimethoxyxanthene

The xanthylum salt (Vb) (0.60 g), suspended in methanol (3.0 ml), was reduced with NaBH_4 (0.30 g). The crystalline product (0.50 g), obtained on adding water, separated from aqueous methanol as slightly yellow prisms, m.p. 219–220°. (Found: C, 65.7; H, 5.16. Calc. for $\text{C}_{15}\text{H}_{14}\text{O}_5$: C, 65.7; H, 5.15%.)

The *diacetate* of the above product, prepared by brief warming with acetic anhydride and pyridine, crystallized from acetone–methanol as colorless prisms, m.p. 159–160°. (Found: C, 63.6; H, 5.02. Calc. for $\text{C}_{19}\text{H}_{18}\text{O}_7$: C, 63.7; H, 5.06%.)

1,3,6,8-Tetramethoxyxanthene (XIb)

The tetrahydroxyxanthene (XIa) (0.8 g) was heated under reflux with Me_2SO_4 (4.0 ml), K_2CO_3 (5.0 g) and acetone (100 ml) for 1 hr. The mixture was evaporated and treated with H_2O . XIb crystallized from acetone–methanol as colorless needles, m.p. 165° (0.70 g). (Found: C, 67.7; H, 6.02; MeO—, 40.5. Calc. for $\text{C}_{17}\text{H}_{18}\text{O}_5$: C, 67.5; H, 6.00; 4 MeO—, 41.0%.)

1,3,6,8-Tetramethoxyxanthylum Perchlorate

A mixture of the tetramethoxyxanthene (XIb) (0.20 g) and benzoquinone (0.20 g) in acetic acid (3.0 ml) and water (1.0 ml) was warmed at 100° for 3 min. 10% aq. HClO_4 was added and the crystalline product collected. After washing with Et_2O , the product was recrystallized from acetic acid. 1,3,6,8-Tetramethoxyxanthylum perchlorate separated as orange needles, m.p. 258–259° (0.21 g); $\lambda_{\text{max}}^{\text{EtOH}-0.5\% \text{HCl}}$ 452, 365, 296, 267 nm. (Found: C, 50.9; H, 4.26. Calc. for $\text{C}_{17}\text{H}_{17}\text{O}_9\text{Cl}$: C, 50.9; H, 4.28%.)

Similar oxidation of the tetrahydroxyxanthene (XIa) in the presence of Na_2SO_4 gave an almost quantitative yield of the highly insoluble sulfate of Va.

1,3,6-Trihydroxyxanthylum Chloride (VII)

A solution of phloroglucinol (1.62 g) and 2,4-dihydroxy-benzaldehyde (1.38 g) in glacial acetic acid (10.0 ml) and conc. HCl (2.0 ml) was heated at 100° for 1 hr. Orange crystals separated. 10% aq. HCl (10 ml) was added and the crystalline xanthylum chloride was collected, washed with Et_2O and dried (1.90 g). Recrystallized from acetic acid–aq. HCl the chloride separated as orange needles, $\lambda_{\text{max}}^{1\% \text{aq. HCl}}$ 413, 263 nm. The chloride becomes dark at about 255° but does not melt below 340°.

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