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SYNTHESIS OF ANTHOCYANIDINS—I

THE OXIDATIVE GENERATION OF FLAVYLIUM CATIONS USING BENZOQUINONES

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[Dedicated to Prof. ROBERT B. WOODWARD on the occasion of his 60th birthday]

Abstract—The addition of benzoquinone and especially chloranil has been found to greatly enhance the yields of 3-deoxyanthocyanidins produced on oxidation of flavan-4-ols. In contrast, only slight improvements are noted when the same oxidants are used in the oxidation of the related flavan-3,4-diols.

Since the early contributions of Willstätter to the total synthesis of anthocyanidins and the more definitive work of Robinson that followed,[1] several alternative approaches have been considered to facilitate the synthesis of the highly oxygenated, phenolic natural pigments.

Convenient transformations of related compounds like flavonols, flavanones and dihydroflavonols to yield flavylium salts have been sought, sometimes motivated by chemurgic interests, in other cases in search of plausible sequences of biosynthetic significance for this group of natural products.

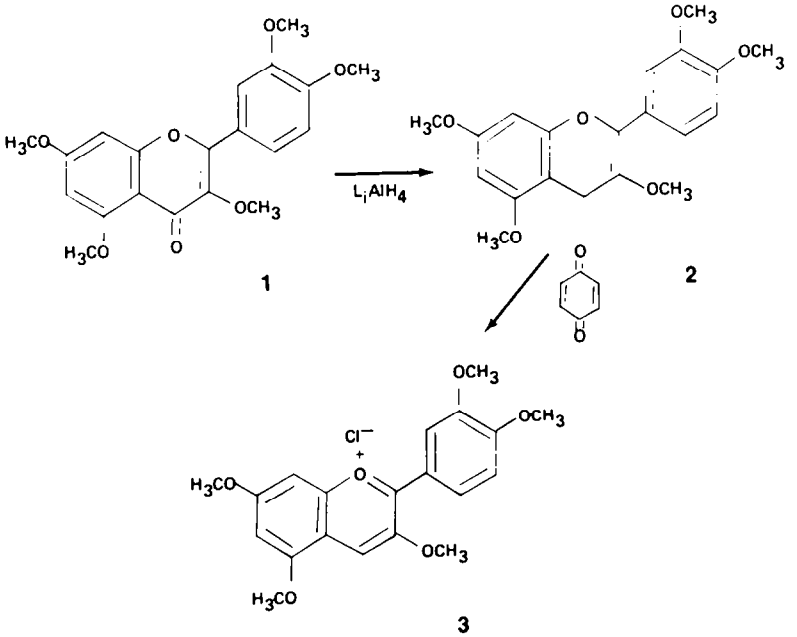
Of particular synthetic interest is the report by Jurd[2] that the flav-2-ene **2** is readily oxidized to pentamethylcyanidin **3** in high yield, on heating with benzoquinone in acetic acid at 100°, thus facilitating the overall conversion of the flavonol **1** to the flavylium salt **3**.

As a part of our own studies, we have explored in further detail the scope of this oxidation, by extending it to a series of flavan-4-ols and flavan-3,4-diols of varied structure.

The flavan-4-ols shown in Table 1 were prepared by $NaBH_4$ reduction of the corresponding flavanones and yielded exclusively the 2,4-*cis*-conformers having the C_4-OH equatorial. As expected, inversion of C_4 occurred during the synthesis of **6** from **4** using EtOH/HOAc. The results shown in Table 1 confirm the effect of quinone addition upon anthocyanidin yield[2] and are best rationalized by assuming the intermediate formation of flav-3-enes, which make possible the subsequent abstraction of the C_2 allylic hydrogen by the quinone.

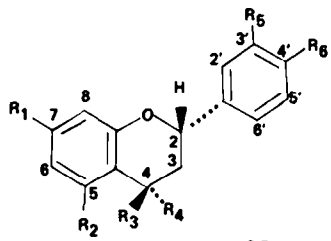
Compounds **7** and **10** failed to react under these conditions, suggesting that oxygen substitution at C_7 facilitates the formation of the intermediate flavene.

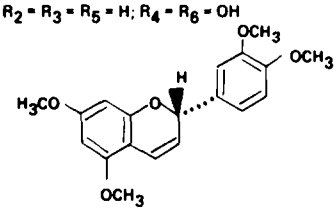
From a preparative point of view, the oxidation of a flavan-4-ol is preferred over that of a flavene, mainly because the former is directly available from the flavanone in one step, while the preparation of the latter



Scheme 1.

Table 1. Effect of benzoquinone and chloranil addition on the oxidation of flavan-4-ols to 3-deoxyanthocyanidins



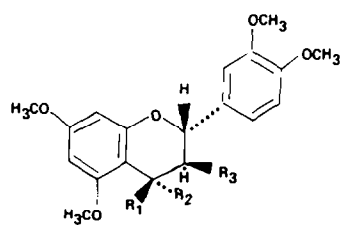
	Compound	3-Deoxyanthocyanidin Yield (%) [a] None	Benzoquinone	Chloranil
4	$R_1 = R_2 = R_5 = R_6 = OCH_3$; $R_3 = H$; $R_4 = OH$	3	25	66
5	$R_1 = R_2 = R_6 = OCH_3$; $R_3 = R_5 = H$; $R_4 = OH$	0	31	59
6	$R_1 = R_2 = R_5 = R_6 = OCH_3$; $R_3 = OC_2H_5$; $R_4 = H$	6	22	69
7	$R_1 = R_2 = R_3 = R_5 = R_6 = H$; $R_4 = OH$	0	0	0
8	$R_2 = R_3 = R_5 = R_6 = H$; $R_1 = R_4 = OH$	1	25	49
10	$R_1 = R_2 = R_3 = R_5 = H$; $R_4 = R_6 = OH$	0	0	0
9		2	45	63

[a] *Reaction mixture consisted of 10 mg flavanol, 10 mg oxidant and 1 ml acetic acid, heated at 100°C for 30 minutes. Yields of 3-deoxyanthocyanidin estimated spectrophotometrically after chromatography on acid-treated polyclar, with reference to the appropriate pure standards in 0.01 N HCl: tetramethylluteolinidin chloride ($\varepsilon_{476\,nm}$ = 38,000) for compounds* **4**, **6**, *and* **9**; *trimethylapigeninidin chloride ($\varepsilon_{460\,nm}$ = 38,000) for compound* **5**, *7-hydroxyflavylium chloride ($\varepsilon_{440\,nm}$ = 32,700) for compound* **8**.

requires first a flavanone-to-chalcone conversion, followed by borohydride reduction and acid catalyzed cyclization.[3] The flavene route is, of course, the method of choice for flavanones lacking oxygen functionality at C_7.

The flavan-3,4-diols and their methyl ethers listed in Table 2 were prepared by borohydride reduction of tetramethyldihydroquercetin, separation of the isomeric

Table 2. Effect of benzoquinone and chloranil addition on the oxidation of flavan-3,4-diols and their ethers to anthocyanidins



	Compound	Anthocyanidin Yield (%) [a] None	Benzoquinone	Chloranil
11	$R_1 = R_3 = OH$; $R_2 = H$	13	17	21
12	$R_1 = R_3 = OAc$; $R_2 = H$	12	20	14
13	$R_1 = H$, $R_2 = OH$; $R_3 = OCH_3$	9	24	27
14	$R_1 = OH$; $R_2 = H$; $R_3 = OCH_3$	12	25	27
15	$R_1 = OCH_3$; $R_2 = H$; $R_3 = OCH_3$	6	24	28

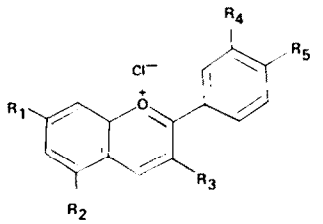
[a] *Reaction mixture 10 mg flavanol, 10 mg oxidant, 0.5 ml acetic acid and 0.5 ml 6 N HCl 2, heated at 100° for 30 minutes. Anthocyanidin yields were estimated spectrophotometrically in 0.01 N HCl in MeOH, as indicated in Table 1, using $\varepsilon_{540\,nm}$ = 34,700*[2]

C_4 alcohols, and methylation with MeI/Ag_2O (Experimental). In this series, the oxidations did not proceed unless HCl was added to the reaction mixture. Contrary to a previous observation,[2] the addition of benzoquinone did not improve the yield of anthocyanidin formed from the diol **11** (Table 2). Moreover, the situation did not improve when chloranil was used instead. When the stronger oxidant DDQ was reacted with **11** in acetic acid at room temperature, the parent 3-hydroxyflavanone was regenerated, but no formation of flavylium cation was noted. Table 2 shows that the anthocyanidin yields are neither dependent on the stereochemistry of the oxygen at C_4 nor are affected by the nature of its substitution. Also, the nature of the oxygen function at C_3 seems unimportant. This is especially surprising in the case of the 3-methoxyderivatives **13**, **14** and **15**, which yield the more stable pentamethylcyanidin **3**. In parallel experiments, this anthocyanidin was recovered unchanged (95% yield) after heating at 100°C for 30 min in acetic acid, acetic acid-6N HCl (5:1), and acetic acid-6N HCl (5:1) containing 2 equivalents of chloranil. As shown in Table 3, the stability of **3** in aqueous acid is two orders of magnitude higher than that of its tetramethyl analogue **16**.

Comparison of Tables 1 and 2 indicates that the addition to the flavan-4-ols of an oxygen function at C_3 results in lower yields of anthocyanidins upon quinone oxidation, with no advantage of chloranil over benzoquinone.

The known tendency of flavan-3,4-diols to condense with themselves or other flavans in the presence of strong acids,[4] may be a factor in determining the differences in reactivity noted above.

Table 3. Stability of anthocyanidins in 0.01 N HCl solution



	Anthocyanidin	Half-Life[a] (days)
16	$R_1 = R_2 = R_4 = R_5 = OCH_3$; $R_3 = OH$	0.04
17	$R_1 = R_2 = R_3 = R_4 = R_5 = OH$	0.5
3	$R_1 = R_2 = R_3 = R_4 = R_5 = OCH_3$	6
18	$R_1 = R_2 = R_4 = R_5 = OCH_3$; $R_3 = H$	> 100

[a] *Measured spectrophotometrically at 540 nm* (**3**, **16** *and* **17**) *and 476 nm* (**18**).

EXPERIMENTAL

M.ps are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ unless otherwise stated. Chemical shifts are given in ppm downfield from TMS. Coupling constants (J) are in c/s. Abbreviations: s, singlet; b.s., broad singlet; d, doublet; t, triplet; m, multiplet. Chloranil (Eastman) was recrystallized from toluene before use. All other reagents were used as received from the supplier and were reagent grade. Microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, Tenn.

Quinone oxidation of flavan-4-ols [Table 1]. The mixture consisted of 10 mg flavan, 10 mg oxidant and 1 ml HOAc. After heating for 30 min at 100° in air, the soln was cooled and diluted with 0.01 N HCl in MeOH to a volume of 10 ml. A one ml aliquot was then chromatographed on acid-treated polyclar[5] (15 × 3 cm column) using 0.01 N HCl as eluant. The anthocyanin band was collected, diluted to 100 ml with 0.01 N HCl, and its absorption measured at 476 nm (ϵ = 38,000) for tetramethylluteolinidin chloride and 460 nm (ϵ = 38,000) for trimethylapigeninidin chloride. 7-OH flavylium chloride was eluted from the column with 0.01 N HCl in MeOH–H_2O (1:1) and had λ_{max} 440 nm (ϵ = 32,700).[6] It showed the reported shift to λ_{max} 370 nm at higher pH due to hydrolysis to the chalcone.

Quinone oxidation of flavan-3,4-diols [Table 2]. The mixture consisted of 10 mg flavanol, 10 mg oxidant, 0.5 ml HOAc and 0.1 ml 6 N HCl.[2] After heating at 100° for 30 min, the soln was diluted to 10 ml with 0.01 N HCl in MeOH. A one ml aliquot of this soln was then chromatographed on acid-treated polyclar[5] (15 × 3 cm column) using 0.01 N HCl in MeOH as eluant. The red anthocyanin band was diluted to 100 ml with 0.01 N HCl in MeOH and the absorbance read at 540 mμ. The pentamethylcyanidin chloride (**3**) used as standard was synthesized via quinone oxidation of **2**.[2,7]. Two recrystallizations from 6 N HCl gave m.p. 145–8° and λ_{max} = 512 nm (0.01 N HCl). (*lit.*[8] 152°, λ_{max} = 512).

3',4',5,7 - *Tetramethoxy* - 2,3 - trans - 3,4 - cis - *flavan* - 3,4 - *diol* (**11**) was prepared via the peracid oxidation of **9** using one equivalent of *m*-chloroperbenzoic acid in methylene chloride, instead of peracetic acid as described by Clark-Lewis.[3] The hydrolysis was then conducted as reported to give **11** (36%) m.p. 166–7° (lit.[3] 165–7°). Acetylation (18 hr, Ac_2O/py) afforded the 2,3-trans-3,4-cis-*diacetate* (**12**) m.p. 159–60° (lit.[3] 155–7°).

Methylation of 3',4',5,7-tetramethyldihydroquercetin. 3',4',5,7-Tetramethyldihydroquercetin[9] (900 mg), Ag_2O (750 mg), MeI (0.33 ml) and DMF (6 ml) were shaken vigorously in a 25 ml stoppered-flask in the dark for 18 hrs, as described.[10] 6 ml $CHCl_3$ was then added and the soln filtered through celite. After washing the celite with an additional 10 ml $CHCl_3$, the combined filtrates were washed with 2 × 20 ml H_2O, dried (Na_2SO_4) and evaporated. TLC (silica gel) using EtOAc-hexane (1:1) as moving phase showed two spots, which were separated by column chromatography on SilicAR CC-7. The first compound to elute was obtained as light yellow crystals from MeOH, 128 mg, m.p. 138–9°. It was identified as 2',3,4,4',6',α-hexamethoxychalcone (lit.[9] 132–3°), by analysis of the MS (*m/e* 388), NMR (6-Me groups), UV ($\lambda_{max\ 343}^{EtOH}$, ϵ = 22,400) and particularly the IR, which was identical to the published one.[9] The second material to elute was identified as 2,3 - trans - 3,3',4',5,7 - pentamethyldihydroquercetin, 331 mg, m.p. 151–2° (MeOH). Clark-Lewis and Korytnyk[11] report m.p. 169–70° for the completely racemic material prepared by a different route. Although our compound was only slightly optically active [α_D] < 1°, the m.p. difference may be due to this factor. IR (KBr)μ: 5.92, 6.20, 6.34, 6.58, 6.80, 7.00, 7.89, 8.02, 8.21, 8.63, 9.00, 9.72. NMR: 3 H m at 6.93 (ArH), 2 H b.s. at 6.06 (ArH), 1 H d at 5.17 (J = 9, C_2H), 6 H s at 3.88 (OCH_3), 3 H s at 3.78 (OCH_3), 3 H s at 3.44 (OCH_3) and 1 H m at 3.95 (C_3H obscured by the OCH_3 absorptions). (Calc. for $C_{20}H_{22}O_7$: C, 64.17; H, 5.88. Found: C, 64.44; H, 6.13%).

Sodium borohydride reduction of 2,3 - trans - 3,3',4',5,7 - *pentamethyldihydroquercetin.* While stirring at room temp, 100 mg $NaBH_4$ was added to a soln of 400 mg pentamethyldihydroquercetin in 50 ml of THF/EtOH (1:1). After 30 min an additional 100 mg was added and stirring continued for a total of 1 hr. The soln was then poured into 150 ml of cold 0.5% HOAc and extracted with 2 × 50 ml $CHCl_3$. Drying (Na_2SO_4) and evaporating the $CHCl_3$ layers gave an oil which showed two spots on TLC analysis [silica gel, EtOAc/hexane (1:1) elution]. The mixture was separated by column chromatography on silicAR CC-7 to give: 2, 3 - *trans* - 3,4 - *trans* - 3,3',4',5,7 - *pentamethoxyflavan* - 4 - ol (**13**), 93 mg (23%), m.p. 157–8° (MeOH) (lit.[3] 155°), MS, IR, and NMR in complete agreement with those reported,[3] and 2,3 - trans - 3,4 - cis - 3,3',4',5,7 - *pentamethoxyflavan* - 4 - *ol* (**14**), 280 mg (70%), m.p. 119–20° (EtOAc-hexane). IR (KBr)μ: 2.87, 3.40, 6.18, 6.28, 6.59, 6.81, 7.89, 8.32, 8.70, 9.10, 9.71. MS *m/e* (rel. intensity): no molecular ion, 358 (87), 343 (37), 327 (100), 221 (85), 194 (67), 179 (43) and 151 (39). NMR: 3 H m at 6.90 (ArH), 2 H b.s. at 6.01 ($C_6 + C_8H$), 1 H d at 5.05 (J = 4, C_4H), 1 H d at 4.95 (J = 10, C_2H), 6 H s at 3.84 (OCH_3), 3 H s at 3.78 (OCH_3), 3 H s at 3.66 (OCH_3), 1 H q at 3.44 (J = 4 and 10, C_3H) and 3 H s at 3.24 (OCH_3). (Calc. for $C_{20}H_{24}O_7$: C, 63.83; H, 6.38. Found: C, 64.02; H, 6.55%).

2,3 - trans - 3,4 - cis - 3,4,3',4',5,7 - *Hexamethoxyflavan* (**15**). 3',4',5,7 - Tetramethoxyflavan - 3,4 - diol (m.p. 172–8°, mixture of 3,4-*cis* and 3,4-*trans* isomers from $NaBH_4$ reduction of tetramethyldihydroquercetin[12]), 450 mg, was methylated with MeI (0.17 ml) and Ag_2O (375 mg) in DMF (3 ml) as described above for tetramethyldihydroquercetin. The product mixture showed three spots on TLC which were separated by column chromatography on silicAR CC-7 (20% EtOAc-hexane elution). The first material to elute was 2,3 - trans - 3,4 - cis - 3,4,3',4',5,7 - *hexamethoxyflavan* (**15**) 145 mg (30%), m.p. 120–3° (MeOH) raised to 126–7° after 2 recrystallizations. IR (KBr)μ: 6.19, 6.28, 6.58, 6.82, 7.01, 7.88, 8.31, 8.70 and 9.08; MS *m/e* (rel. intensity): 388 (6.5), 358 (100), 327 (83), 194 (79), 57 (94), 55 (60) and 43 (93). NMR: 3 H m at 6.90 (ArH), 2 H b.s. at 6.02 (C_6 and C_8H), 1 H d at 5.12 (J = 10, C_2H), 1 H d at 4.78 (J = 4, C_4H), 6 H s at 3.84 (OCH_3), 3 H s at 3.79 (OCH_3), 3 H s at 3.70 (OCH_3), 3 H s at 3.48 (OCH_3), 1 H m at ~3.40 (C_3H) and 3 H s at 3.14 (OCH_3). (Calc. for $C_{21}H_{26}O_7$: C, 64.61; H, 6.67. Found: C, 64.69; H, 6.72%) In addition, there was obtained 179 mg (38%) of **13** and 115 mg (25%) of **14**.

2,4 - cis - 4 - *Hydroxy* - 3',4',5,7 - *tetramethoxyflavan* (**4**), m.p. 112.5–113.5° (lit.[13] 114°); 2,4 - cis - 4 - *hydroxy* - 4',5,7 - *trimethoxyflavan* (**5**), m.p. 156–8° (lit.[13] 159°); 2,4 - cis - 4 - *hydroxyflavan* (**7**), m.p. 147–8° (lit.[14] 148–9.5°); and 2,4 - cis - 4,4' - *dihydroxyflavan* (**10**), m.p. 182–4° (lit.[15] 181°) were prepared by $NaBH_4$ reduction of the corresponding flavanones in EtOH. The stereochemistry is based on the assignments of the original authors and comes from analysis of the NMR spectra.[16]

2,4 - trans - 4 - *Ethoxy* - 3',4',5,7 - *tetramethoxyflavan* (**6**), oil, was prepared from **4** by refluxing with HOAc in EtOH.[3]

2,4 - cis - 4,7 - *Dihydroxyflavan* (**8**). 7-hydroxyflavanone[15]

(200 mg) was reduced with $NaBH_4$ (100 mg) in EtOH at room temp. The BH_4^- was added over 1 hr, then the mixture allowed to stir at room temp. for 18 hr. Pouring the mixture into H_2O, neutralizing with HOAc, extracting with $CHCl_3$ and evaporation of the $CHCl_3$ gave 2,4-cis-4,7-*dihydroxyflavan* as an amorphous solid. After recrystallization from EtOAc-hexane, there was obtained 61 mg (30%), m.p. 138–9° (lit.[15] 42–6°). IR (KBr)μ: 2.90, 6.12, 6.78, 8.66, 8.91, 9.42, 9.71. NMR: 1 H b.s. at 8.2 (OH), 6 H b.s. at 7.2–7.5 (ArH), 1 H m at 6.5 (C_6H), 1 H m at 6.4 (C_8H), 2 H m at 5.0 (C_2H and C_4H) and 2 H m at 2.2–2.4 (C_3H). MS *m/e* (rel. intensity): 242 (4), 224 (100), 223 (97), 165 (10), 152 (7.4), 147 (39) and 115 (4.7). (Calc. for $C_{15}H_{14}O_3$: C, 74.38; H, 5.78. Found: C, 74.25; H, 5.60%).

4',5,7-Trimethoxyflavylium chloride (*trimethylapigeninidin chloride*). A mixture of 200 mg **5**, 160 mg chloranil and 5 ml HOAc was heated at 100° with stirring for 30 min. After cooling in ice, the soln was diluted with 5 ml of 0.01 N HCl in MeOH and added to the top of an 18 × 2.5 cm polyclar column (acid treated).[5] The anthocyanin band was eluted with 0.01 N HCl and the eluant freeze-dried. After washing with 2 × 10 ml EtOAc to remove unreacted chloranil, there was obtained 104 mg (39%) of *4',5,7-trimethoxyflavylium chloride pentahydrate*.[17] $UV_{(0.01\ N\ HCl\ in\ MeOH)}$: λ_{max} 242 (4.07), 277 (4.33), 324 (3.74) and 473 nm (4.60). Reported:[17] 242 (3.96), 277 (4.29), 325 (3.72) and 474 (4.59). A portion of the product was converted to the $FeCl_4^-$ salt and recrystallized from AcOH. m.p. 186–7° (lit.[18] 187°).

3',4',5,7-Tetramethoxyflavylium chloride (*Tetramethylluteolininidin chloride*). A mixture of 200 mg **4**, 160 mg chloranil, and 5 ml HOAc were reacted and chromatographed as above. After freeze-drying and washing with EtOAc, there was obtained 131 mg (50%) of *3',4',5,7-tetramethoxyflavylium chloride* as a red-orange solid. $UV_{(0.01\ N\ HCl\ in\ MeOH)}$: λ_{max} 241 (4.19), 279 (4.28), 3.20 (3.61) and 488 nm (4.57). Lit.[17] λ_{max} = 488 (4.62). A portion of the product converted to the $FeCl_4^-$ salt and recrystallized from AcOH had m.p. 206–7.5° (lit.[19] 205–6°).

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