# Effect of Substitution on the Stability of 3-Deoxyanthocyanidins in Aqueous Solutions

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The effects of ring substitution on the color stability of a group of 14 synthetic 3-deoxyanthocyanidins have been studied. In acid solution (pH 2.8), none of the synthetic analogues prepared show significant advantages in overall performance over the naturally occurring apigeninidin (2). At pH 7.0, however, the presence of a carboxylate group at C4 results in unusually stable colorants.

The anthocyanins are naturally occurring red pigments having the basic structure 1 (Timberlake and Bridle, 1975,

1. R_1 = H, OH, OCH_3
   R_2 = H, OH, OCH_3
   R_3 = H, Sugar

2. R_1 = R_2 = H

3. R_1 = H, R_2 = OH
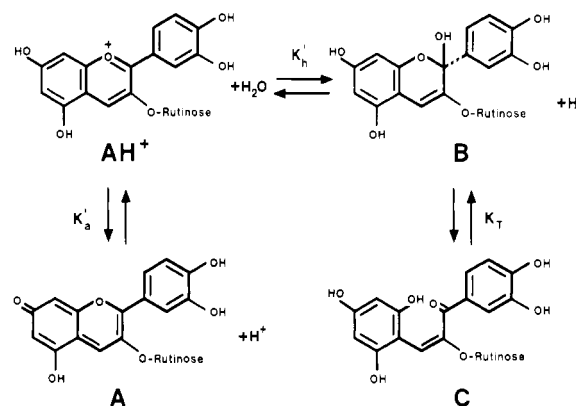
4. R_1 = R_2 = OH
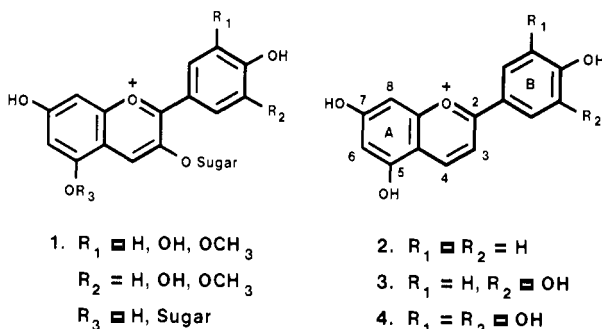
1980). These are found widely distributed in nature, mainly in flowers and the more common fruits and vegetables. Extracts of several anthocyanin-containing fruits, especially grapes, have long been used as food colorants. This use of anthocyanins, however, is entirely restricted to those foods or beverages having a pH below 4; above this pH, anthocyanins are rapidly decolorized.

In addition to the red anthocyanins, there are also three yellow anthocyanidins—apigeninidin (2), luteolinidin (3), and tricetinidin (4)—occurring in foods. These 3-deoxyanthocyanidins, although present primarily in ferns and mosses (Timberlake and Bridle, 1975, 1980), also occur in hybrids of sorghum (Nip and Burns, 1971) and purple corn (Nakatani et al., 1979). Tricetinidin is a pigment found exclusively in black tea, where it is formed during the fermentation stage by the enzymatic oxidation of epigallocatechin gallate (Coggon et al., 1973). Their use as food colorants has been suggested previously (Timberlake, 1969; Jurd, 1964, 1966, 1967a,b), as many analogues are readily prepared by synthesis. Although some information is available on the stability of these compounds in beverage systems (Jurd, 1964, 1966, 1967a,b; Ohta et al., 1980), a comparison of the numerous examples synthesized to date has not appeared. In particular, we felt that an evaluation of the 4-substituted anthocyanidins introduced by Bülow and Wagner, 1901, 1903) and Timberlake (Timberlake, 1969), and found by the latter to be resistant to SO_2 and ascorbic acid bleaching, would be of value.

The transformations anthocyanins undergo in aqueous solutions are well understood today (Brouillard and Delaporte, 1977; Brouillard and Dubois, 1977). Upon dissolution, a complex equilibrium is quickly established between two colored species (flavylium cation AH+ and quinone methide conjugated base A) and two colorless ones (the chromenol B and the chalcone C) resulting from the
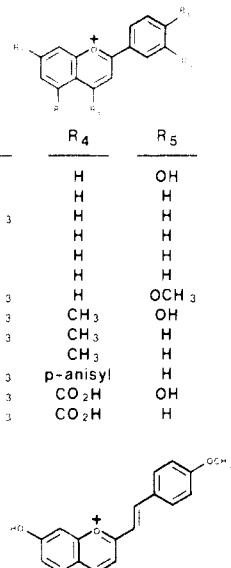
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Scheme I

hydration of AH+. Thus, the final color of the solution at equilibrium is the direct consequence of the rate constants K'_a, K'_h, and K_T, controlling the ionization, hydration, and tautomerization reactions, respectively, as illustrated in Scheme I for cyanidin 3-rutinoside. The values of these constants are variable among the several anthocyanins, anthocyanidins, and synthetic flavylium cations studied and found to depend on structural factors like the patterns of aromatic hydroxylation and the chemical substitution at C4 (Brouillard, 1982; Brouillard et al., 1982).

Judgment on the performance of synthetic flavylium salts as color additives for carbonated beverages and other liquid food products can be passed simply by following spectrophotometrically the changes of absorbance at the λ_{max} of buffered solutions. The resistance of the color to fading is a measure of its performance in solution, herein defined as its stability. Clearly, such stability is the overall reflection of the changes illustrated in Scheme I for solutions stored at room temperature under laboratory lights. Under the influence of additional factors like ascorbic acid/O_2 (King et al., 1980) and sunlight irradiation (Sweeny et al., 1981b), the stability of anthocyanins in solution is further affected. These effects are important for understanding the fate of anthocyanin colors in complex food formulations like fruit juices, which could be exposed to unfavorable conditions during storage and distribution in the marketplace.

EXPERIMENTAL SECTION

The 3-deoxyanthocyanidins used in this study were synthesized by known procedures (Timberlake, 1969; Jurd, 1966, 1967a,b; Bülow and Wagner, 1901, 1903; Sweeny and Iacobucci, 1977a,b, 1981). The purity of each sample (>95%) was checked by reverse-phase HPLC on C_{18} μBondapak using mixtures of methanol, acetic acid, and water as the eluant (Wilkinson et al., 1977). All of the anthocyanidins had molar extinction coefficients at the

	R$_3'$	R$_4'$	R$_4$	R$_5$	R$_7$	λ_{max} (pH 2.8)
2	H	OH	H	OH	OH	470
5	H	H	H	H	OCH$_3$	427
6	H	OCH$_3$	H	H	H	437
7	H	OH	H	H	H	436
8	H	H	H	H	OH	428
9	H	OH	H	H	OH	458
10	OCH$_3$	OCH$_3$	H	OCH$_3$	OCH$_3$	480
11	H	OCH$_3$	CH$_3$	OH	OH	448
12	H	OCH$_3$	CH$_3$	H	OH	445
13	H	H	CH$_3$	H	OH	418
14	H	OCH$_3$	p-anisyl	H	OH	460
15	H	OCH$_3$	CO$_2$H	OH	OH	458
16	H	OCH$_3$	CO$_2$H	H	OH	458
17						506

Figure 1. Structures of the 3-deoxyanthocyanidins tested.

Table I. Stability of 3-Deoxyanthocyanidins in 0.01 M Citric Acid Solution, pH 2.8, at Room Temperature[a]

	anthocyanidins							
days	2	5	6	7	8	9	10	17
0	100	100	100	100	100	100	100	100
4	100	40	87	98	100	100	96	93
11	99		75	95	99	98	95	84
25	100		62	94	97	97	91	67
55	99		35	92	90	93	78	42
85	98		17	91	87	89	61	23
146	92			86	74	80	37	5
225	93			86	61	76	9	

[a] Values are percent color remaining = $A_t/A_0 \times 100$.

λ_{max} between 30 000 and 40 000 as noted in the synthetic literature. With the exception of compounds **11** and **14** all the anthocyanidins had solubilities greater than 100 ppm. These two materials were only soluble (at pH 2.8) to the extent of 25 ppm. Stock solutions were prepared by dissolving the anthocyanidin in 0.01 M citric acid solution (pH 2.8) containing 200 ppm of sodium benzoate as a preservative. The stock solution was then filtered (when necessary) and diluted to the appropriate concentration (20 ppm) by measuring the absorbance at the λ_{max}. Samples were stored in glass-stoppered Pyrex flasks at room temperature and laboratory lighting, under air. Aliquots were then removed periodically and their absorbance was measured at the λ_{max}.

The samples run at neutral pH were prepared in 0.01 M phosphate buffer, pH 7.0, instead of citric acid. The λ_{max} for the samples run at pH 7.0 were as follows: for **2**, 510 nm; for **9**, 490 nm; for **11**, 480 nm; for **12**, 475 nm; for **15**, 485 nm; for **16**, 490 nm. Absorbance values were determined on a Beckmann 25 UV–vis spectrometer. Sunlight exposures were measured on an Eppley black and white pyranometer, Model 8-48A, sensitive to UV, vis, and IR radiation and expressed in langleys = cal/cm².

RESULTS AND DISCUSSION

The rate of fading for the 3-deoxyanthocyanidins listed in Figure 1 was studied at ambient temperature during prolonged storage (8 months). Table I presents the results for the eight anthocyanidins not containing substitution at C4. From the data it appears that the addition of hydroxyl substituents on the A or B rings increases stability while methylation of these hydroxyls has the opposite effect. As noted by Jurd (1964), insertion of an ethylene

Table II. Stability of 3-Deoxyanthocyanidins Bearing Substitution at C4, in 0.01 M Citric Acid Solution, pH 2.8, at Room Temperature[a]

	anthocyanidins				
days	2	11	12	13	14
0	100	100	100	100	100
28	99	95	98	99	97
59	98	88[b]	94	95	95
87	95	77	92	93	94
150	92	44	82	83	89
210	88	18	71	72	82

[a] Values are percent color remaining = $A_t/A_0 \times 100$.
[b] Precipitation of anthocyanidin was evident at this time.

Table III. Effect of Ascorbic Acid (600 ppm) on 3-Deoxyanthocyanidins (20 ppm), in 0.01 M Citric Acid Solution (pH 2.8), under Air at Room Temperature[a]

	anthocyanidins				
days	2	11	12	13	14
0	100	100	100	100	100
1	83	93	91	91	85
2	69	89	81	84	73
3	57	82	69	75	63
4	43	76	58	65	53
6	26	66	44	51	42
8	16	58	35	41	34
10	10	53	29	34	30
13	7	47	24	27	17

[a] Values are percent color remaining = $A_t/A_0 \times 100$.

Table IV. Sunlight Bleaching of 3-Deoxyanthocyanidins in 0.01 M Citric Acid Solution (pH 2.8), under Air[a]

	anthocyanidins						
langleys	2	7	9	11	12	13	14
0	100	100	100	100	100	100	100
521	96	88	82	93	93	94	92
1044	91	70	69	71	88	89	78
1639	81	47	49	32	79	74	52
2122	66	19	33	5	69	54	28
2746	48	3	19		53	33	8

[a] Values are percent color remaining = $A_t/A_0 \times 100$.

function between carbon 2 and the B ring (as in **17**) to extent the conjugation results in a compound of reduced stability.

Apigeninidin chloride (**2**), the most stable of the simple anthocyanidins tested, was then compared in a repeat experiment with three representative 4-methyl-substituted anthocyanidins and one 4-phenyl-substituted anthocyanidin. As is shown in Table II, under ambient storage conditions, the 4-substituted anthocyanidins offer no advantages over apigeninidin chloride, as they have similar stabilities.

In the presence of ascorbic acid (600 ppm) and air, the 4-methyl-substituted and 4-phenyl-substituted anthocyanidins (20 ppm) show increased fading resistance when compared to apigeninidin chloride. The data listed in Table III, therefore, confirm the previous findings of Timberlake (Timberlake, 1969; and Timberlake and Bridle, 1968).

Poor resistance to fading under sunlight exposure is also a common problem with anthocyanins (Sweeny et al., 1981b). When a representative sample of the anthocyanidins listed in Figure 1 were placed under direct sunlight, the colors faded as shown in Table IV. From these data it is apparent that compounds **12** and **13**, both bearing CH$_3$ groups at C4 and no hydroxyls at C5, showed the highest resistance to photooxidative bleaching, com-

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Table V. Stability of 3-Deoxyanthocyanidins in 0.01 M Phosphate Buffer, pH 7.0, at Room Temperature[a]

days	anthocyanidins					
	9	2	11	12	15	16
0	100	100	100	100	100	100
1	11	89	93	100	100	100
2		77	91	100	100	100
4		58	83	97	100	100
7		42	79	96	100	100
11		30	65	87	100	100
18		23	21	75	95	98
28		19	7	62	93	99
39		16		44	89	98
49		13		32	88	98

[a] Values are percent color remaining = $A_t/A_0 \times 100$.

parable in extent to that of apigeninidin (2).

Anthocyanidins containing a 4-carboxy group have been known for 80 years, but no indication of their stability has appeared. We have found them to be exceptionally stable in acidic and neutral solutions.

As demonstrated in Table V, 3-deoxyanthocyanidins in general fade rapidly at pH 7. Substitution of a methyl group at carbon 4 improves the stability slightly, but the presence of a 4-carboxy group provides an unusually stable molecule. Unike all the natural anthocyanins, then, a 4-carboxy-3-deoxyanthocyanidin could be of advantage to color foods and beverages of neutral pH.

For acidic systems, the anthocyanidin of choice would be apigeninidin (2), although in those cases where ascorbic acid is present at high levels (600 ppm) the C4-substituted flavylium salts seem to perform much better. The mutagenicity found associated to the quinone methide forms of 12 and 13 (Sweeny et al., 1981a), however, certainly exclude them from any further consideration.

Previous studies on the stability of flavylium salts in acid solutions (Ohta et al., 1980) are in general agreement with the conclusions of this work, although they were conducted on synthetic flavyliums having no substitution at C4 and C5. It was shown previously (Brouillard et al., 1982) that the presence of a hydroxy group at C5 (as is the case in all natural flavylium cations) and in particular substitution at C4 both stabilize the colored forms (A and AH+) through the arresting of the normal hydration reactions which lead to the formation of the two colorless species (chromenol B and chalcone C, Scheme I). As shown by the results presented here, such structural stabilization is reflected particularly well in the exceptional performance of the color of compounds 15 and 16 in neutral aqueous solutions.

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