Spectroabsorptiometric Investigations of Complexing Reactions of Polyhydroxylic Flavylium Compounds

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The anhydrobasic form of delphinidin forms with Al(III) at pH 5.85 a complex, Dp:Al = 3:1, with the relative equilibrium constant of 14.38 ± 0.07, at room temperature and at ionic strength I = 0.2 mol/L. Complexation in acetate buffer has a positive effect on delphinidin stabilization, the decomposition rate constant of which is k = 0.052 min^{-1}, at pH 5.85 and room temperature, and which decreases to k = 0.0025 min^{-1} in the presence of Al(III) because of complexation.

INTRODUCTION

It is well-known from Bayer's published papers (Bayer, 1958, 1959; Bayer et al., 1960) that hydroxylated flavylium compounds, with OH groups in ortho positions in the B-ring, form complex compounds with some metals. Complexes thusly formed in vitro were expected to provide explanation for coloration of some fruits and flower petals in vivo. Within Bayer's investigation of complexation possibilities of different anthocyanidines and anthocyans (glycosidified anthocyanidines), he also paid close attention to the reaction of delphinidin (hexahydroxyflavylium compound) with aluminum. His investigation resulted in the formation of a ligand to the Al(III) complex of 3:1 for which he also proposed a possible structure but without calculating its stability constant. For this reason, by using electronic absorption spectra we made an attempt in our work to define the first transformations of delphinidin molecules in solutions with different pH values and then to investigate the complexation reaction with aluminum.

EXPERIMENTAL PROCEDURES

The reagents used were Britton–Robinson (Britton, 1952) (I = 0.2 mol/L) and acetate (Dobos, 1978) buffer solutions. Other chemicals used were delphinidin chloride (Fluka), $Al_3Cl_3 \cdot 6H_2O$ p.a., "Zorka". Delphinidin chloride stock solutions ($C = 10^{-3}$ mol/L) were prepared by dissolving precisely weighed quantities of substance. $AlCl_3$ solutions were also $C = 10^{-3}$ mol/L. Solutions were prepared by diluting 0.5 mL of delphinidin stock solution with different buffers up to 10 mL. Delphinidin concentration in all buffer solutions was constant, $C_{Dp} = 5 \times 10^{-5}$ mol/L. Complex formation was investigated by using 0.5 mL of delphinidin and different volumes of $AlCl_3$ stock solutions and by filling up with buffer solution up to 10 mL. Both stock solution concentrations were $C = 10^{-3}$ mol/L.

Absorption spectra were recorded on a Pye Unicam SP8-100 spectrophotometer, with 1-cm quartz cells, 1 min after preparation of the solution, at room temperature using the corresponding solution as a reference.

RESULTS

As shown by delphinidin spectra (Figure 1) in Britton–Robinson buffer solutions at pH 2.0–10.0 and at ionic strength of 0.2 mol/L, an increasing pH value results in the change of both the position and the intensity of the basic absorption maximum (Table I). The maximum of the cationic form (A^+) of delphinidin at pH 2.0 (Figure 1, curve 1) bathochromically shifts with increasing pH, pointing to the change of cationic structure to anhydrobasic (A) during the deprotonization process and finally to the

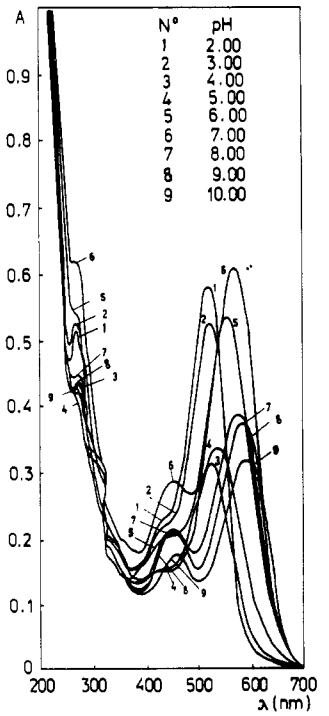

Figure 1. Absorption spectra of delphinidin in Britton–Robinson buffer solutions. $C_{Dp} = 10^{-5}$ mol/L.

formation of anhydrobase (A) at pH 7.0, as illustrated by the given scheme. At further increase, up to pH 10.0, a new bathochromic shift indicates further deprotonization and formation of the anionic form of this chromophore (A^-) (Figure 1, curve 9). The transformational sequence of delphinidin structure with changing pH made it possible first to determine the range of pH values characteristic of the formation of the anhydrobase form of this chromophore (pH 5.0–6.0) and to determine, in accordance with the literature (Bayer, 1959), the optimum values for the formation of complex compounds. To be able to choose the optimum buffer system for complexation, measurements were also performed with delphinidin in acetate buffer solutions in the same pH region (pH 5.0–6.0). It is seen in Figure 2 that the percentage of the anhydrobase form is highest at pH 5.85 (the most intensive maximum), and this appears to be the most suitable medium for complexation reaction. Figure 3 shows the spectra of delphinidin and the aluminum complex in acetate buffer solutions at different pH values. The highest bathochro-

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Table I. Characteristics of Delphinidin Absorption Spectra in Britton-Robinson Buffers, pH 2.00–10.00

		nm					
no.	pH	λ_1	λ_2	λ_3	A_1	A_2	A_3
1	2.00	524	440i	275	0.580	0.235	0.525
2	2.50	524	440i	275	0.585	0.240	0.560
3	3.00	524	440i	275	0.525	0.225	0.535
4	3.50	525	440i	275	0.360	0.160	0.442
5	4.00	527	430i	270	0.315	0.153	0.430
6	4.50	533	430i	275i	0.270	0.130	0.390
7	5.00	540	425i	275i	0.335	0.150	0.420
8	5.50	555	430i	270i	0.375	0.150	0.430
9	6.00	560	440i	270i	0.535	0.205	0.540
10	6.50	565	445i	270i	0.528	0.223	0.560
11	7.00	571	450	270	0.605	0.285	0.620
12	7.50	579	455	275	0.600	0.310	0.640
13	8.00	585	460	280	0.382	0.215	0.440
14	8.50	588	460	280	0.380	0.215	0.440
15	9.00	595	460	280	0.370	0.210	0.430
16	9.50	595	460	280	0.360	0.205	0.442
17	10.00	599	460	280	0.370	0.175	0.420

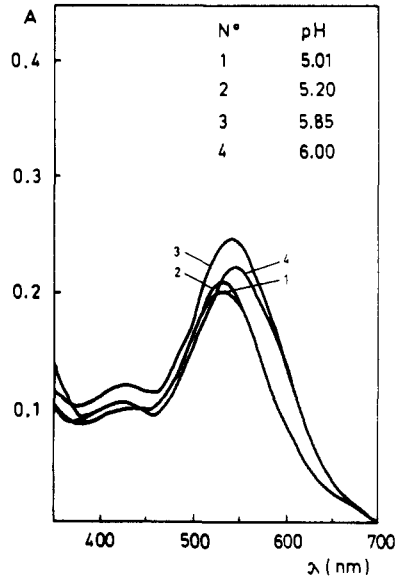


Figure 2. Absorption spectra of delphinidin in acetate buffers. $C_{Dp} = 10^{-5}$ mol/L.

mic and hyperchromic effects are also observed at pH 5.85 (Figure 3, curve 3). Delphinidin and delphinidin–aluminum complex spectra in acetate buffer solution at pH 5.85 are shown in Figure 4, curves 1 and 2. The absorption band of the complex is bathochromically shifted to 565 nm, followed by a strong hyperchromic effect. Figure 4, curve 3, shows the spectrum of a $AlCl_3$ solution in the same buffer solution.

The metal to ligand ratio in the formed complex was determined by the molal ratios method (Šlefer, 1964). The composition of the formed complex was determined from the graphical presentation of the complex absorbency dependence on metal concentration at constant ligand concentration (5×10^{-5} mol/L) (Figure 5). The concentration Al^{3+} in the complex, determined from the segment of the straight line drawn normal to the abscissa at the tangents intersection point, is 1.8×10^{-5} mol/L (Figure 5). The number of delphinidin molecules bonded to one Al(III) ion in the complex was calculated from the obtained delphinidin and Al^{3+} concentration ratio, $n_{Dp} = (5 \times 10^{-5})/(1.8 \times 10^{-5}) = 2.78 \approx 3$. The corresponding formula is therefore $AlDp_3$. The presence of the maximum concentration of the complex, $(C_{AlDp_3})_{max}$, equal to Al(III) concentration, is evident from the part of the curve in Figure 5 which does not show absorbency change with

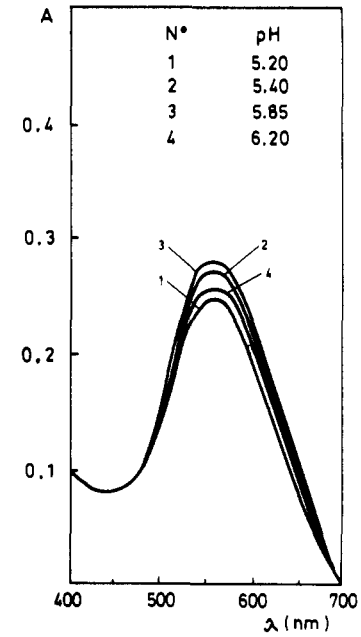


Figure 3. Absorption spectra of $AlDp_3$ complexes in acetate buffers.

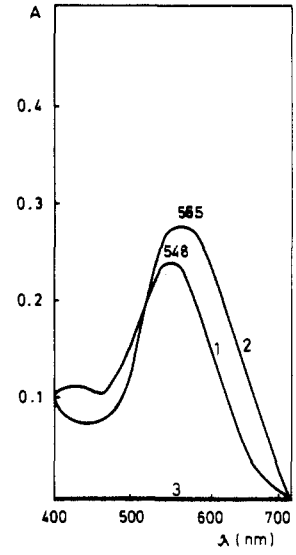


Figure 4. Absorption spectra of delphinidin solution (1) and $AlDp_3$ complexes (2) in acetate buffer, pH 5.85.

increasing metal concentration. Absorptivity of the complex has been calculated from these values. The complex absorbency was obtained as the difference between delphinidin (A_{Dp}) and delphinidin and aluminum solution (A_1) absorbencies. Delphinidin (C_{Dp}) concentrations are identical in both solutions. Concentration of the complex was calculated according to Beer's law:

$$C_{AlDp_3} = (A_1 - A_{Dp})/a_{AlDp_3}b$$

Metal and delphinidin equilibrium concentrations are

$$C_{Al^{3+}} = C^0_{Al^{3+}} - C_{AlDp_3}$$

and

$$C_{Dp} = C^0_{Dp} - 3C_{Al^{3+}}$$

respectively, where C^0_{Al} and C^0_{Dp} are total stoichiometric concentrations.

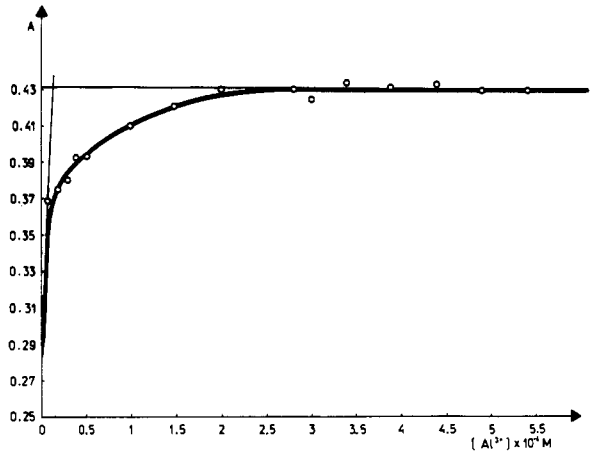


Figure 5. Absorbency dependence of delphinidin solution Dp solution on Al^{3+} concentration.

Table II. Time-Dependent Absorbency Change of Delphinidin (Dp) and $AlDp_3$ Complex Basic Maxima at pH 5.85

	Dp				$AlDp_3$			
no.	t, min	A	ln A	k, min^{-1}	t, min	A	ln A	k, min^{-1}
1	1	0.243	−1.42	0.052	1	0.272	−1.30	0.0025
2	4	0.205	−1.59		25	0.256	−1.36	
3	7	0.177	−1.73		60	0.232	−1.46	
4	10	0.165	−1.80		120	0.202	−1.60	
5	15	0.150	−1.89		240	0.165	−1.80	

The concentration stability constant

$$\beta'_3 = C_{AlDp_3}/C_{Al^{3+}}C^{3-}_{Dp}$$

is obtained from the complexation reaction:

$$Al^{3+} + 3Dp \leftrightarrows AlDp_3$$

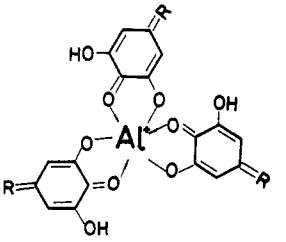
The obtained stability constant value is only relative, being calculated from the total concentration Dp instead of that of the respective ionic form. Stability constant values of the complex, the mean value of which, including standard deviation, is $\log \beta'_3 = (14.38 \pm 7) \times 10^{-2}$, have been calculated according to the above-described procedure using the molal ratios method. The complexation effect on molecular stability of delphinidin was investigated using kinetic measurements at pH 5.85. For this reason, spectra were recorded for Dp solutions, with and without Al(III), of the same concentration, as well as of absorbency change of the same solutions as a function of time, $A = f(t)$ (Table II).

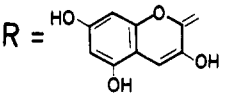
Decomposition rate constants were calculated from the slopes of the straight lines obtained by graphical presentation of the reaction rate equation (the first-order reaction): $\ln A = \ln A_0 - kt$. The obtained numerical values for delphinidin solution without Al(III) and delphinidin with Al(III) complex solution are given also in Table II.

DISCUSSION AND CONCLUSION

In the first phase our investigations have shown a transformational process of delphinidin molecules in dependence on pH value of solutions, as can be seen in Figure 1 and Table I. The transformational process developing according to Scheme I does not show any change of the π-electronic system of the flavylium chromophore of the investigated molecule in solutions having different pH values. This enabled clear defining of the regions of cationic (A^+), anhydrobasic (A), and anionic (A^-) forms of this chromophore as well as the second phase of our study, owing to the known anhydrobasic form of delphinidin, i.e., optimum pH value, for the formation of its solution. In the second phase of our study we have found formation of the complex of the anhydrobasic form of delphinidin, as a ligand, and Al(III) ion in acetate buffer solutions.

The stoichiometric ratio of the formed complex was found to be 1:3 = Al:ligand, and the assumed formula is $AlDp_3$. Since we have obtained the same metal to ligand ratio as in the Bayer complex, we also accepted Bayer's explanation of the complex structure, assuming complexation to involve orthohydroxylated flavylium molecules including delphinidin.

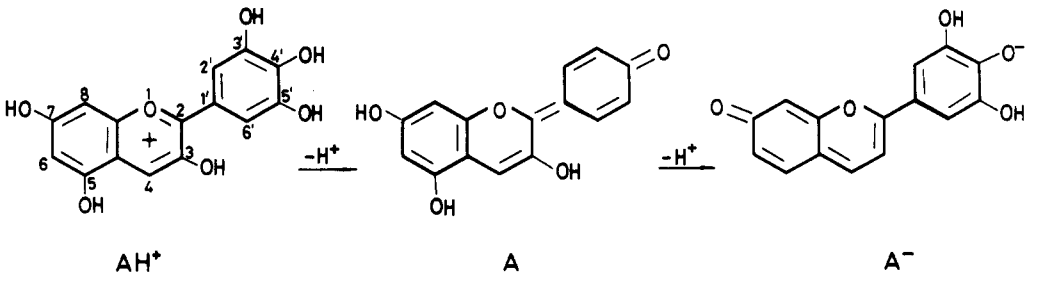




According to this assumption, oxygen in position 4′ of delphinidin anhydrobase is coordinationally bonded with Al(III), while oxygen from the hydroxyl group in position 3′, which loses hydrogen in the presence of metal, is bonded chemically.

The same types of bonding are assumed for the remaining two delphinidin molecules participating in the formation of the Al(III):Dp = 1:3 complex. Our results cannot be compared with the literature data (Harborne, 1958) since the complex, although the same, was formed in acidified methanol and ethanol solutions. The curve obtained according to the molal ratio method points to the predominant presence of the $AlDp_3$ complex. This conclusion is also supported by fairly good agreement between different β'_3 constant values. However, the

Scheme I



obtained constant value is only relative because of its being calculated by using the total stoichiometric concentration of anhydrobasic delphinidin instead of the stoichiometric concentration of ions participating in the complexation reaction (because of unknown dissociation constants).

Comparison of the constant values (Table II) shows anhydrobasic delphinidin decomposition to be a considerably slower process when occurring in the complex compared to its rate in free form. This can doubtless be ascribed to the presence of Al(III) in the complex and its inhibiting effect on Dp transformation. Proof of the existence of the anhydrobasic region, the formation of the complex, and its positive effect on the stabilization of anhydrobasic structure can contribute to presently very interesting investigations of stability of flavylium compounds, especially from the point of view of their industrial application.

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