Spectrophotometric Studies on the Acid Dissociation of Anthocyanins in Aqueous Solutions

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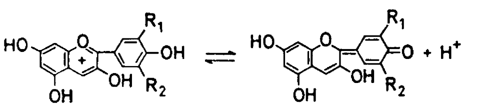
The acid dissociation reaction of an anthocyanin is reflected in absorption spectra of the compound in solutions where the pH is varied.[1-4] Although absorption spectra for solutions of different anthocyanins have been reported in several earlier studies, no precise measurements of the corresponding reaction equilibria seem to be available.

The absorption spectra of cyanidin-3-galactoside, delphinidin, pelargonidin, malvidin-3,5-diglucoside, and peonidin-3,5-diglucoside in aqueous solutions of various hydrogen ion concentration were recorded, and dissociation constants were evaluated by the common absorptiometric method.

The acid dissociation of anthocyanins can be represented as:



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Cyanidin: R_1 = OH, R_2 = H
Delphinidin: R_1 = R_2 = OH
Pelargonidin: R_1 = R_2 = H
Peonidin: R_1 = OCH_3, R_2 = H
Malvidin: R_1 = R_2 = OCH_3

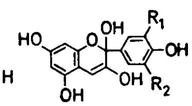

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Table 1 lists the absorptivities of the spectra of cyanidin-3-galactoside and shows the precision with which the equilibrium constant is determined. Table 2 presents the results obtained for the other anthocyanins. It was not possible to determine any precise K_a value for pelargonidin; but it could be roughly estimated as $K_a \simeq 1.1 \times 10^{-2}$. The absorption spectra are presented in Figs. 1 and 2.

The spectrophotometric determinations of 1×10^{-4} M solutions of cyanidin-3-galactoside were also carried out in solu-

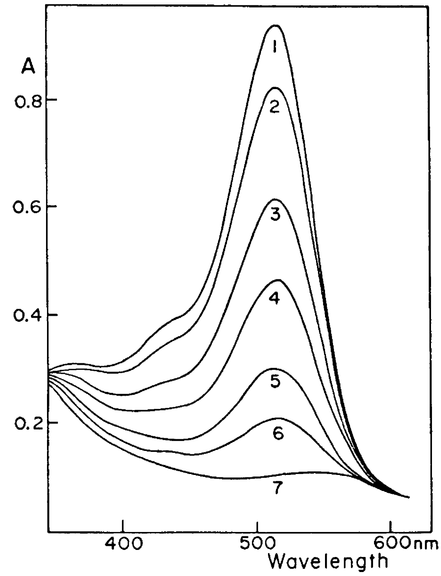

Fig. 1. Absorption spectra of cyanidin-3-galactoside in solutions of various hydrogen ion concentration ($c = 7 \times 10^{-5}$ M, 25°C). Curves: (1) $-\log$ [H^+] 1.0, (2) 2.2, (3) 2.8, (4) 3.1, (5) 3.4, (6) 3.7, and (7) 5.0.

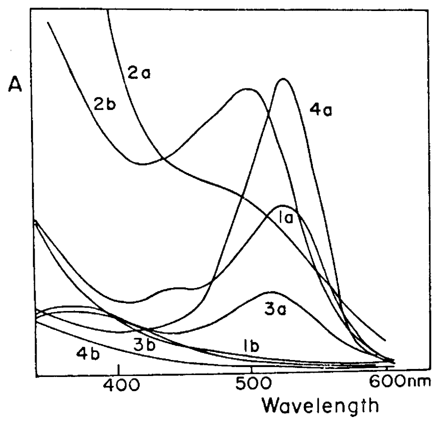

Fig. 2. Absorption spectra of aqueous solutions of anthocyanins at 25°C. Curves: (1) Delphinidin $c = 5 \times 10^{-4}$, $-\log [H^+] = 0$ (a) and 3.1 (b); (2) Pelargonidin $c = 3 \times 10^{-4}$, $-\log [H^+] = 0.3$ (a) and 3.9 (b); (3) Peonidin-3,5-diglucoside $c = 3 \times 10^{-4}$, $-\log [H^+] = 0$ (a) and 3.3 (b); (4) Malvidin-3,5-diglucoside $c = 2 \times 10^{-5}$ $-\log [H^+] = 0$ (a) and 3.5 (b).

Table 1. Determinations of pK_a values of cyanidin-3-galactoside in aqueous solution at 25°C. $c = 7 \times 10^{-5}$ M, $I = 0.005$.

$-\log [H^+]$	496 nm	506 nm	516 nm	526 nm	536 nm	Mean value
1.89	2.984	2.963	2.977	2.949	2.958	2.966
2.20	2.905	2.999	3.002	2.984	2.983	2.975
2.50	2.953	2.939	3.000	3.009	3.018	2.984
2.81	2.970	2.958	3.013	2.999	3.007	2.989
3.20	2.960	2.980	2.973	2.963	2.987	2.973
pK_a= (mean value)	2.954	2.967	2.993	2.981	2.991	2.977

Table 2. pK_a values obtained for different anthocyanins in aqueous solution at 25°C. $I = 0.005$.

	n	c, M	λ_{max}	pK_a
Cyanidin-3-galactoside	3	7×10^{-5}	516	2.98 ± 0.05
Delphinidin	4	$3-10 \times 10^{-4}$	524	1.56 ± 0.20
Peonidin-3,5-diglucoside	2	5×10^{-4}	512	2.09 ± 0.10
Malvidin-3,5-diglucoside	2	2×10^{-5}	524	1.75 ± 0.10
Pelargonidin	3	3×10^{-4}	500	—

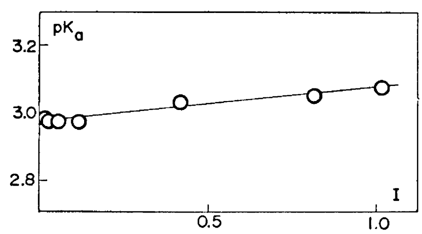

Fig. 3. Dependence of pK_a of cyanidin-3-galactoside on ionic strength of aqueous solutions at 25°C. $c = 1 \times 10^{-4}$ M, KCl as inert salt; pK_a° (extrapolated) $= 2.98 \pm 0.02$.

tions of various ionic strengths (potassium chloride was added as inert salt). The results are shown in Fig. 3. The dependence of the pK_a values on the ionic strength of the solutions is linear: p$K_a = [H^+][L]/[HL^+]$.

Anthocyanins are relatively unstable in aqueous solutions, and may form colourless pseudo-bases.[5] It appears that neither the acid dissociation reactions nor the disappearance of the red colour of anthocyanins proceed directly from forms I to II. Instead a colourless pseudo-base is formed as intermediate, the proposed structure of which is shown as form III.[6] In these experiments only pelargonidin proved unstable.

Experimental. Cyanidin-3-galactoside was isolated from the berries of mountain ash. The other anthocyanins were in the form of hydrochlorides obtained by the purification (paper chromatography) of commercial reagents from Fluka AG.

The absorption spectra were measured and recorded at 25°C with a Perkin-Elmer Model EPS-3T spectrophotometer.

The K_a values were obtained by the common absorptiometric method in which the absorptivities of solutions of various hydrogen ion concentration were measured at different wave lengths near the isoelectric point in the buffer range.

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