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CHIRAL EXCITON COUPLED STACKING OF ANTHOCYANINS: INTERPRETATION OF THE ORIGIN OF ANOMALOUS CD INDUCED BY ANTHOCYANIN ASSOCIATION

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Summary: Natural anthocyanidin 3,5-diglucosides show exciton coupling type of CD in neutral aqueous solutions, indicating the chiroptical stacking of anthocyanidin chromophores. A minor change of substituents on the anthocyanidin alters the chiral direction of stacking. Commelinin also consists of such chiroptically stacked molecules.

Anthocyanins are generally unstable in neutral or weakly acidic aqueous solutions; quinonoidal base of anthocyanin hydrates easily via flavilium ion to form the corresponding colorless pseudobase.[1] Nevertheless color of anthocyanins holds beautifully in flower petals, in which pH of cell saps is usually between 4 and 7. The factors affecting the stability and color variation of anthocyanins in flower petals have been explained in terms of co-pigmentation[2,3] with flavone, etc. Complexation of anthocyanins with metal ions has also been suggested.[4]

Recently we reported that naturally occurring anthocyanidin 3,5-diglucosides such as cyanin,[5] malvin,[6] etc. in neutral aqueous solutions form aggregates without any other components in the concentrations usually found in flower petals as evidenced by circular dichroism (CD). Six common natural anthocyanidin 3,5-diglucosides listed in Table 1 show the characteristic CD around their visible absorption region in neutral aqueous solutions.[7,8] For example, the CD curve of cyanin quinonoidal base splits into a pair of positive and negative Cotton bands (Fig. 1). Very interestingly delphin shows Cotton bands similar in shape but opposite in sign to that of cyanin, although the structure of delphin differs from that of cyanin by only one extra OH group at 5' position. These splitting CD curves belong to the typical exciton coupling type;[9] areas of the positive and the negative Cotton bands are nearly equal and both bands are found in the visible absorption band region of each of the anthocyanins. Thus, two or more anthocyanidin chromophores must stack in a right-handed or left-handed screw axis to cause this type of splitting bands (Fig. 3).[10] Magnitudes of [θ] in each of the anthocyanins are strongly intensified by increasing concentration,[6] thus indicating the formation of stacked molecules more in the more concentrated solutions.

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The factor determining the stacking to the right-handed or left-handed screw axis is not yet clarified, but it may say that 4'-monosubstituted anthocyanin stacks in a right-handed screw manner, while a left-handed screw axis is found in the cases of 3',4',5'-trisubstituted anthocyanins. The chirality of 3',4'-disubstituted anthocyanins is affected by minor changes in the structure (compare cyanin with peonin). Shifts of the visible spectra towards longer or shorter wavelengths by formation of the chiral aggregates are also noted (Table 1). Thus, the stacking affects variation of the flower colors that consist of such anthocyanins.

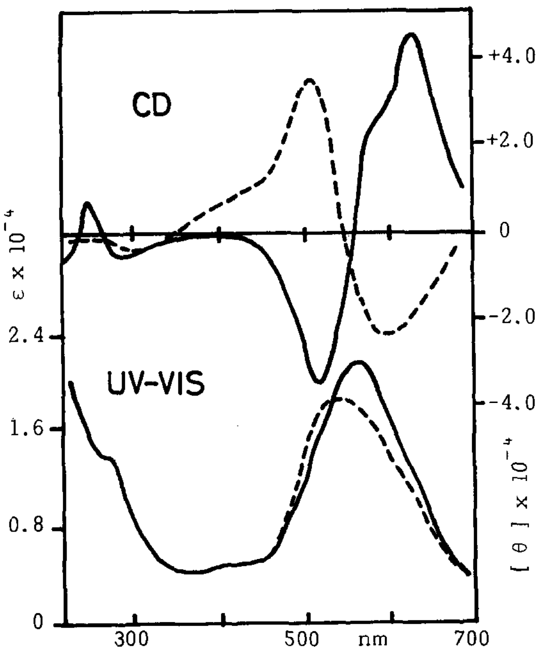


Fig. 1. Electronic and CD spectra of cyanin and delphin in aqueous solution at pH 7.0
—— Cyanin; ----- Delphin (5×10^{-4} M)

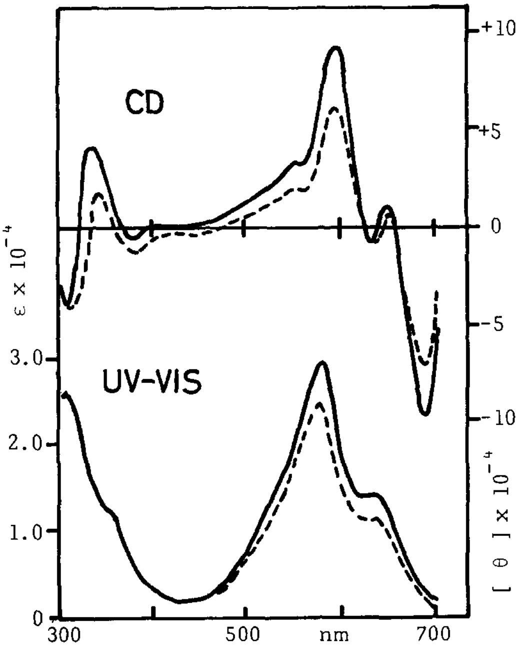
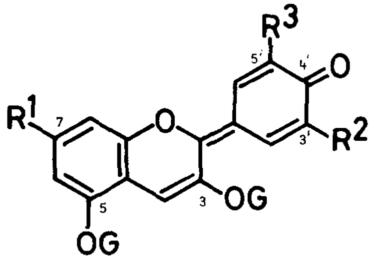


Fig. 2. Electronic and CD spectra of commelinin in vitro and in vivo
—— Commelinin in aq soln. (5×10^{-5} M)
----- A petal of Commelina (arbitrary units on both spectra)



G = β-D-glucosyl

* 4'-Hydroxy-7-keto form may be present in the case of R^1 = OH

ANTHOCYANIN	SUBSTITUENTS $R^1(7)^*$	$R^2(3')$	$R^3(5')$
PELARGONIN	OH	H	H
CYANIN[5]	OH	OH	H
PEONIN	OH	OCH_3	H
DELPHIN	OH	OH	OH
HIRSUTIN[6]	OCH_3	OCH_3	OCH_3
MALVIN[6]	OH	OCH_3	OCH_3

Table 1. Visible spectra and circular dichroism of anthocyanidin 3,5-diglucosides in 0.1 M phosphate buffer at pH 7.0

ANTHOCYANIN	5 x 10^{-5} M		5 x 10^{-4} M		5 x 10^{-4} M¶			
					First Cotton		Second Cotton	
	λ_{max}	$\varepsilon^{\dagger}$	λ_{max}	$\varepsilon^{\dagger}$	λ_{ext}	[θ]	λ_{ext}	[θ]
PELARGONIN	544	14,800	560*	16,400	630*	+200,000*	550*	-66,000*
CYANIN	572	16,800			634*	+46,000*	520*	-36,000*
(CYANIN**			586	12,000	630	+260,000	515	-252,000)
PEONIN	572	18,100	550	17,000	630	-12,000	525	+10,000
DELPHIN	572	20,800	544	19,000	600	-22,000	510	+37,000
HIRSUTIN	562	8,800	528	8,900	575	-6,000	490	+6,000
MALVIN	596	21,200	564	19,200	650	-12,000	516	+18,000

¶ CD spectra were determined with a Jasco J-500C spectropolarimeter equipped with a model DP-500 data processor for rapid measurements in the range of 200-700 nm within 60 sec.

† ε **values** were determined within 5 min after dissolving.

* The values were obtained when [θ] came into the maximum value (pelargonin: ca 10 min; and cyanin: ca 60 min after dissolving).

** Aggregated precipitates obtained from aq. solution at pH 7.0 (see Ref. 5).

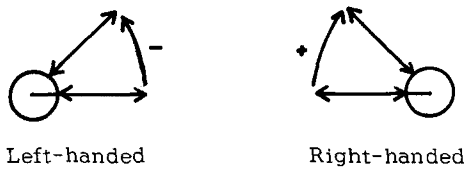


Fig. 3. Screw axis

Previously we proposed that commelinin, the sky-blue color of Commelina petals, consists in a stacked pair of awobanin (an anthocyanin) and flavocommelin (a flavone) molecules; the pairs may aggregate further to the dimer, trimer, and so on.[11] The CD spectrum of commelinin (Fig. 2) shows clearly an exciton splitting curve (though it is a little complex), indicating that the pairs form further aggregates in a helical sense.[12] A live petal of Commelina showed identical CD spectrum with that of commelinin in aqueous solution as shown in Fig. 2.

REFERENCES AND NOTES

1. R. Brouillard and J.-E. Dubois, J. Am. Chem. Soc., 99, 1359 (1977); R. Brouillard and B. Delaporte, J. Am. Chem. Soc., 99, 8461 (1977).
2. G. M. Robinson and R. Robinson, Biochem. J., 25, 1687 (1931); S. Asen, R. N. Stewart, and K. H. Norris, Phytochem., 10, 171 (1971); 11, 1139 (1972); S. Asen, K. H. Norris, and R. N Stewart, J. Am. Soc. Hort. Sci., 96, 770 (1971); S. Asen. R. N. Stewart, K. H. Norris, and D. R. Hassie, Phytochem., 9, 619 (1970); P. Scheffeldt and G. Hrazdina, J. Food Sci., 43, 517 (1978); M. Williams and G. Hrazdina, J. Food Sci., 44, 66 (1979); L.-J. Chen and G. Hrazdina, Phytochem., 20, 297 (1981).
3. T. Hoshino, U. Matsumoto, and T. Goto, Phytochem., 19, 663 (1980).
4. K. Hayashi, "The Anthocyanins" in "The Chemistry of Flavonoid Compounds", Ed. T. A. Geissman, p. 248, Pergamon Press, New York, 1962; K. Hayashi and K. Takeda, Proc. Japan Acad., 46, 535 (1970); E. Bayer, H. Eggeter, A. Fink, K. Nether, and K. Wegmann, Angew. Chem., 78, 834 (1966).
5. T. Hoshino, U. Matsumoto, and T. Goto, Tetrahedron Lett., 21, 1751 (1980).
6. T. Hoshino, U. Matsumoto, and T. Goto, Phytochem., in press.
7. Asen et al. reported some indication of self-association of anthocyanin flavilium ions in acidic concentrated solutions (pH 3.16) by observing chenges in the absorption spectra with dilution or moderate heat. [S. Asen, R. N. Stewart, and K. H. Norris, Phytochem., 11, 1139 (1972); 14, 2677 (1975)].
8. Flavilium ions of these anthocyanins also show exciton type CD spectra, suggesting that flavilium ions also form stacking structures. These results will be reported elsewhere.
9. N. Harada and K. Nakanishi, Acc. Chem. Res., 5, 257 (1972); N. Harada, Y. Takuma, and H. Uda, J. Am. Chem. Soc., 100, 4029 (1978); N. Harada, Y. Tamai, and H. Uda, J. Am. Chem. Soc., 102, 506 (1980).
10. For examples, (dinucleotides) M. Ikehara, S. Uesugi, and M. Yamamoto, J. Am. Chem. Soc., 92, 4735 (1970); (biopolymer-dye systems) M. Hatano, M. Yoneyama, and Y. Sato, Biopolymers, 12, 2423 (1973).
11. T. Goto, T. Hoshino, and S. Takase, Tetrahedron Lett., 2905 (1979).
12. In the previous paper[11] we suggested as one of the interpretations on the origin of the large CD of commelinin the twisting of the B-ring of both of the flavonoid nuclei in a same direction, but it is found that the split-type CD band cannot be interpretable with it. Preliminary calculation of this type of structures gave only very small magnitudes on CD.

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