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STRUCTURE OF ZEBRININ, A NOVEL ACYLATED ANTHOCYANIN ISOLATED FROM ZEBRINA PENDULA

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Abstract: Structure of zebrinin isolated from Zebrina pendula was determined to be 3-O-(6-O-(2,5-di-O-caffeyl-α-L-arabinofuranosyl)-β-D-glucopyranosyl)-7,3'-di-O-(6-O-caffeyl-β-D-glucopyranosyl)cyanidin.

Anthocyanins[1] are usually very unstable in weakly acidic and neutral media, so that the colors quickly disappear, but some acylated anthocyanins are known to be modelately stable[2]. For the stabilization it has been suggested that plural aromatic acids in the anthocyanins play important roles. Recently we have revealed that the stabilization is caused by a hydrophobic interaction among anthocyanidin and the aromatic nuclei of the acyl groups, forming an intramolecular sandwich type stacking[3].

From the flowers of Tradescantia reflexa Yoshitama[4]isolated purple and blue anthocyanins, of which the structures were suggested to be ferulylcaffeyl(2:1 or 1:2)cyanidin- and tricaffeyl-delphinidin-3,7,3'-triglucoside. Stirton and Harborne[5] also isolated similar pigments and tricaffeyl-cyanidin-3,7,3'-triglucoside from Zebrina pendula.

We wish to report a novel structure for an anthocyanin isolated from Zebrina pendula, which contains L-arabinose and four molecules of caffeic acid. We name the pigment zebrinin.

Fresh leaves (6.39 Kg) of Z. pendula were deep-frozen with liquid nitrogen, powdered by a blender and extracted twice with 1% HCl-MeOH at room temperature. The combined extracts were chromatographed on an Amberlite XAD-7 column using stepwise elution from H_2O to MeOH containing 0.5% trifluoroacetic acid (TFA). The 65% MeOH fraction containing an anthocyanin as a major component was dried up and treated with MeOH-Et_2O as usual to give a crude pigment, which was purified by preparative ODS-HPLC (Nomura Develosil ODS, 5 μ) using a mixture of AcOH:CH_3CN:TFA:H_2O (5.4:6.8:0.5:87.5) and precipitated with MeOH (containing 1% HCl)-Et_2O to afford a pure pigment as its chloride (zebrinin,**1**) [dark red powder, mp 117-123 °C, λ_{max} (ε) (0.01% HCl-MeOH) 532 (25000), 329 (51800), and 292 nm (47300)]. FABMS of **1** showed a molecular ion peak at m/z 1553 (corresponding to $C_{74}H_{73}O_{37}$). ^1H NMR spectrum (Table 1, in 3% CF_3COOD-CD_3OD) suggested that **1** is composed of cyanidin, four molecules of caffeic acid, three molecules of hexoses and one molecule of a pentose.

Table 1. ^{1}H NMR spectrum of zebrinin (**1**) in CD_3OD containing 3% TFA-d at 25 °C

cyanidin	H-4	8.39(s)[a]	H-8	6.72(d, 2)	H-5'	7.00(d, 9.0)				
	H-6	6.75(d, 2)	H-2'	7.50(d, 1.5)	H-6'	8.43(dd, 2 & 9)				
caffeic acids	H-2"	6.93	6.86	6.29	6.16	H-α[b]	7.45	7.33	7.06	6.96
	H-5"	6.65	6.61	6.28	6.60	H-β	6.14	5.95	6.06	5.75
	H-6"	6.74	6.63	5.96	6.12					
		●	△	▲		■				
sugars	H-1	5.36	5.10	5.04	H-1	5.19(s)				
	H-2	3.66	3.65	3.72	H-2	5.17(d, 2.5)				
	H-3	3.70	3.69	3.65	H-3	4.06(dd, 2.5 & 6.0)				
	H-4	3.42	3.37	3.49	H-4	4.26(ddd, 2.5, 5.0 & 6.0)				
	H-5	3.94	3.80	3.84	H-5a	4.45(dd, 2.5 & 12)				
	H-6a	4.94	5.13	4.20	H-5b	4.30(dd, 5.0 & 12)				
	H-6b	3.98	3.95	3.83						

a: multiplicity and J-values (Hz). b: all $J_{\alpha,\beta}$ values are 16 Hz.

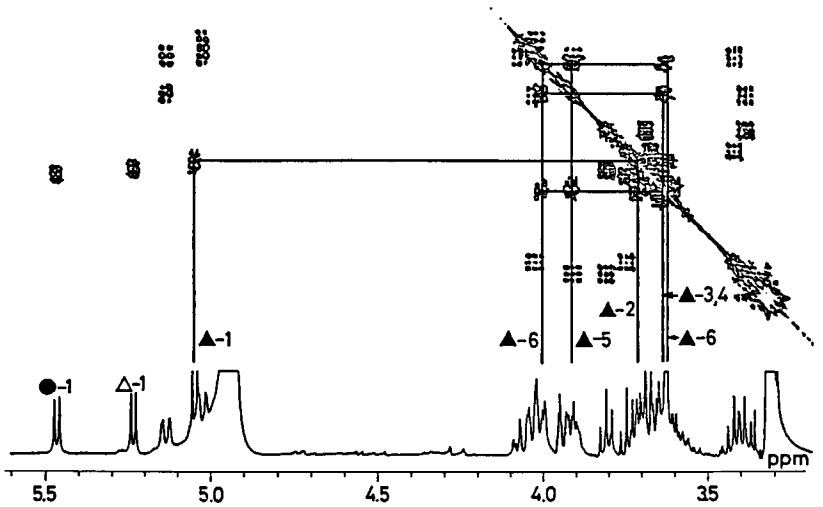
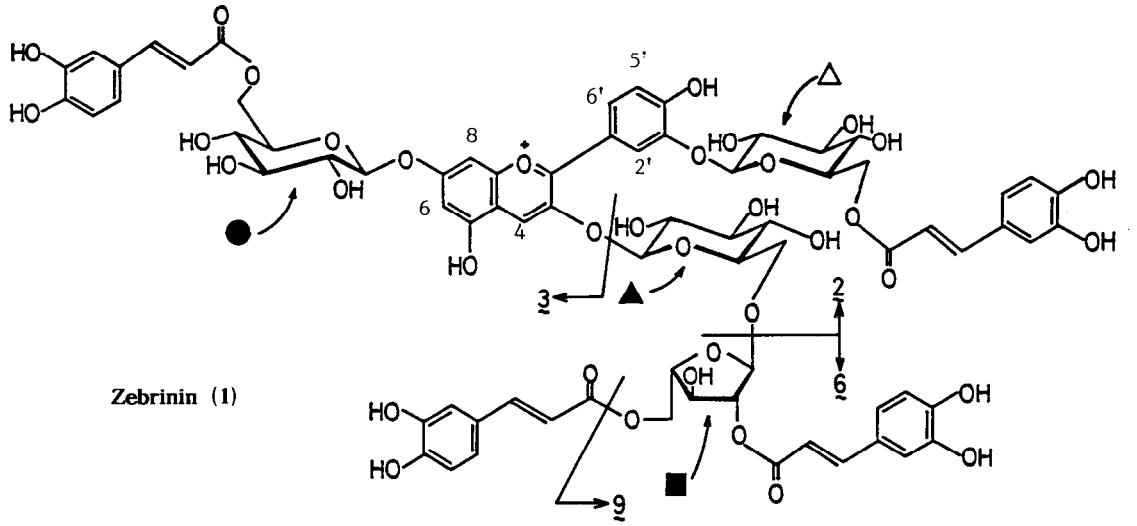


Figure 1. ^{1}H COSY spectrum in the sugar moiety of **2**

Hydrolysis of **1** (77.3 mg) with 0.7 N HCl-H_2O (6 ml) at 60 °C for 36 h followed by separation by ODS-HPLC using stepwise elution with AcOH:CH_3CN:TFA:H_2O (A; 4:5:0.5:90.5, then B; 6:7.5:0.5:86) to give four fractions of pigments, [bisdeacylzebrinin A (**2**), bisdeacylzebrinin B (**3**), trisdeacylzebrinin A (**4**) and the unchanged **1**], which were dried up and treated with MeOH (containing 1% HCl)-Et_2O. Each fraction gave a pigment as a dark-red amorphous precipitate: **2** (17.1 mg), **3**[6] (3.1 mg), and **4**[7] (3.3 mg).

^{1}H NMR and FABMS of **2** (m/z 1097) showed the presence of a cyanidin, two caffeic acids and three hexoses, of which all vicinal coupling constants were 7.5-9.0 Hz. Therefore, all of the hexoses must be β-glucopyranosides. By analysis of the ^{1}H COSY spectrum (Figure 1) at the sugar moieties, ●(5.47 ppm) and △(5.24 ppm) anomeric protons were finally related to the two acylated -CH_2O-, ●(5.04 and 4.08 ppm) and △(5.14 and 4.04 ppm), respectively. In order to determine the position of the glycosidic linkages, a low temperature difference NOE (at 0 °C) was measured. Irradiation at ●-1, △-1 and ▲-1 reduced intensities of H-8 (-1%) and H-6 (-9%), H-2' (-10%), and H-4 (-7%), respectively. Thus, the structure of **2** must be 7,3'-di-O-(6-O-caffeyl-β-D-glucopyranosyl)-3-O-(β-D-glucopyranosyl)cyanidin.

1 (101 mg) was hydrolyzed with 2% NaOH in MeOH:H_2O (1:1) at -20 °C for 17 h, followed by treatment with 6% TFA-MeOH. Separation of the hydrolyzate by ODS-HPLC eluted with AcOH:CH_3CN:TFA:H_2O (1:1.3:0.5:97.2) gave the tetradecaffeylzebrinin (**5**)[8] (FABMS m/z 905, yield 26.1 mg as its chloride) and methyl caffeate. No other esters were found. The fragment ion of **5** at m/z 773 (M^+-132) strongly suggested the presence of a pentose.



Zebrinin (1)

1 (50 mg) was treated with 0.05 N HCl-MeOH (50 ml) at 65 °C for 6 h, then neutralized with 0.1 N NaOH, desalted by column chromatography of Amberlite XAD-7, and separated by ODS-HPLC using a solvent system of CH_3CN:H_2O (1:5) to afford methyl glycoside of **6** (**7** , yield 1.5 mg) besides four anthocyanins (**1** - **4**). **7** was hydrolyzed with 0.5 N NaOMe in MeOH followed by TLC to give caffeic acid and methyl α-L-arabinofuranoside (**8**), which was converted by treatment with benzoyl chloride-pyridine to the corresponding tribenzoate. It was identified by CD ($[\theta]_{235nm}$+31500°), and ^{1}H NMR spectrum of authentic methyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranoside (CD $[\theta]_{236nm}$+37700°)[9]. FABMS (m/z 489, M+1) and ^{1}H NMR of **7**, in which the signals of the ■-2 methine and ■-5 methylene were shifted more than 0.5 ppm toward lower-field than those of **8**, indicated that **7** is methyl 2,5-di-O-caffeyl-α-L-arabinofuranoside.

Since ^{1}H NMR spectrum[8] of the pentoside part of **5** ($J_{1,2}$=1.0, $J_{2,3}$=4.0, $J_{3,4}$=6.0, $J_{4,5a}$= 3.0 and $J_{4,5b}$=6.0 Hz) is closely resemble to that of methyl α-arabinofuranoside ($J_{1,2}$=1.0, $J_{2,3}$= 3.5, $J_{3,4}$=6.0, $J_{4,5a}$=3.0 and $J_{4,5b}$=5.5 Hz), and not to that of the β-isomer ($J_{1,2}$=4.5, $J_{2,3}$=7.0, $J_{3,4}$=7.5, $J_{4,5a}$=4.0 and $J_{4,5b}$=7.5 Hz), the anomeric linkage was determined to have an α configuration[10].

By spin decoupling, 2D COSY, and especially a difference NOE all of the protons in the ^{1}H NMR spectrum of **1** could be assigned (Table 1). Irradiating at ■-1 of **1** produced a negative NOE (at +10 °C) on ▲-6a overlapped with ▲-5 and ▲-6b [-20% and -7% at 3.86 ppm (2H) and 4.18 ppm (1H), respectively], indicating that the arabinose part is glycosidically linked to 6-OH of ▲-glucose. Thus, the structure of **1** was determined to be 3-O-(6-O-(2,5-di-O-trans-caffeyl-α-L-arabinofuranosyl)-β-D-glucopyranosyl)-7,3'-di-O-(6-O-trans-caffeyl-β-D-glucopyranosyl)cyanidin.

In the flowers and stems of Z. pendula, there exists, besides of **1**, a minor pigment, of which the structure was determined to be monodecaffeylzebrinin (**9**), lost the caffeic acid molecule at ■-5 of **1**.

Zebrinin (**1**) and bisdeacylzebrinin A (**2**) are quite stable in a neutral aq. solution, whereas tetradecaffeylzebrinin (**5**) is quickly decolorized (Figure 2). The color stability of **1** evidently depends upon the acyl groups.

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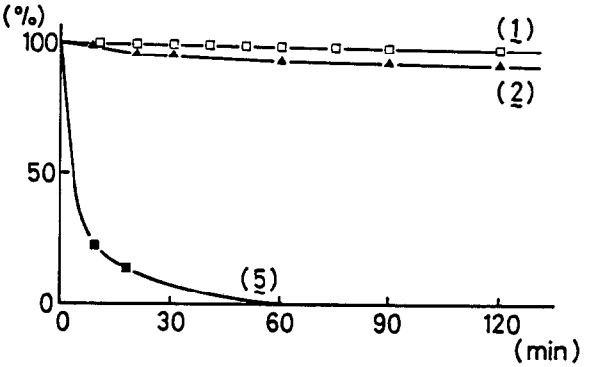

Figure 2. Stability of anthocyanins (**1**, **2**, and **5**) in 1/30 M phosphate buffer solution at pH 6.5 (conc $2X10^{-5}$ M ; observed at λ_{max} of the visible absorption).

References and footnotes

1. R. Brouillard, In "Anthocyanins as Food Colors", ed. P. Markakis, 1982, Academic Press, Inc., Chapter 1, pp 1-40.
2. N. Saito, Y. Osawa and K. Hayashi, Phytochem., **10**, 445 (1971); Bot. Mag. Tokyo, **85**, 105 (1972).
3. T. Goto, H. Tamura, T. Kawai, T Hoshino, N. Harada and T. Kondo, Annals New York Acad. Sci., **471**, 155 (1986); see also R. Brouillard, Phytochem., **20**, 143 (1981).
4. K. Yoshitama, Bot. Mag. Tokyo, **91**, 207 (1978).
5. J. Z. Stirton and J. B. Harborne, Biochem. System. Ecology., **8**, 285 (1980).
6. FABMS (m/z 935) of **3** and the disappearance of the signals attributable to ▲-glucose in the ^1H NMR spectrum of **3** in comparison with that of **2** indicated that the structure of **3** is 3-O-monodeglucoside of **2**.
7. The structure of **4** is suggested by ^1H NMR to be a mono caffeate of 3,7,3'-tri-O-glucosyl-cyanidin.
8. **5** was slowly decomposed under the acid condition of NMR measurement. In 2D COSY all the proton signals of the arabinose part could be asssigned. The positions of the glucoside-linkages were able to be determined by NOE experiments [irradiation of ▲-1, ●-1 and △-1 at -35°C caused negative NOEs at H-4(-35%), H-8(-46%), and H-2'(-35%), respectively].
9. CD of methyl 2,3,5-tri-O-benzoyl-α-D-arabinofuranoside shows $[\theta]_{237}$ -41900°.
10. By the similarity of ^1H NMR of **5** [δ 4.85(H-1), 3.99(H-2), 3.80(H-3), 3.90(H-4), 3.70(H-5a) and 3.60(H-5b)] with that of authentic methyl 6-O-α-L-arabinofuranosyl-4-O-α-D-glucopyranosyl-β-D-glucopyranoside [$J_{1,2}$=1.0, $J_{2,3}$=4.0, $J_{3,4}$=6.0, $J_{4,5a}$=3.0 and $J_{4,5b}$=6.0Hz ; δ 4.93(H-1), 3.91(H-2), 3.72(H-3), 3.87(H-4), 3.65(H-5a) and 3.54(H-5b)] the anomeric configuration of the arabinoside moiety of **5** was further confirmed to be α [T. Fujiwara, T. Takeda and Y. Ogiwara, Carbohyd. Res., **141**, 168 (1985)].

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