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(DELPHINIDIN 3-GENTIOBIOSYL) (LUTEORIN 7-GLUCOSYL) MALONATE FROM THE FLOWERS OF *EICHHORNIA CRASSIPES*

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Abstract – An additional new pigment exhibiting anthocyanin and flavone disubstituted malonate structure, was isolated from the blue-purple flowers of *Eichhornia crassipes* as a minor anthocyanin. This pigment was identified as (6’’’-*O*-(delphinidin 3-*O*-(6’’-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl))) (6’’-*O*-(luteolin 7-*O*-(β-D-glucopyranosyl))) malonate by chemical and spectroscopic methods. The λmax of this pigment is 547 nm in 0.1% HCl-MeOH, where the negative cotton effect is also observed as similar to that of Eichhornia anthocyanin A, (delphinidin 3-gentiobiosyl) (apigenin 7-glucosyl) malonate previously isolated from the same flowers. This result supports that the new pigment also takes a folding conformation between delphinidin (chromophore) and luteorin (co-pigmnet).

In the course of the investigation of the flower color variation in *Eichhornia crassipes*, we firstly found a new acylated anthocyanin, a disubstituted malonate with delphinidin 3-gentiobioside and apigenin 7-glucoside, as the major pigment in the purple-blue flowers of this plant.[1]

As a part of our ongoing program, we have isolated another new anthocyanin pigment together with a major anthocyanin, Eichhornia anthocyanin A,[1] from the same flowers of this plant. In this paper, we wish to report the structure determination of a new disubstituted malonate with delphinidin

3-gentiobioside and luteolin 7-glucoside. For the sake of convenience, the new anthocyanin was named as Eichhornia anthocyanin B. Dried central petals (*ca*. 60 g) of *Eichhornia crassipes* were immersed in 5% AcOH at room temperature over night and the pigments were extracted. The concentrated extract was purified by Diaion HP-20 column chromatography (C.C.) with 1% AcOH and AcOH-MeOH-H_2O (1:6:12) as described previously.[1] Purified elute was fractionated over Sephadex LH-20 CC using AcOH-MeOH-H_2O (1:6:12). The fractions of anthocyanin pigments were further purified by PC (n-BuOH-AcOH-H_2O, 4:1:2 and 15% AcOH) and by preparative ODS-HPLC. Preparative HPLC was performed on Hitachi 6200 system using Inertsil ODS-2 (20ϕ x 250 mm) column and AcOH solvent system. Eichhornia anthocyanin B (*ca*. 10mg) was obtained as a minor pigment as well as Eichhornia anthocyanin A (*ca*. 15mg).[1] Eichhornia anthocyanin A was identified by the analysis of TLC and HPLC[2] with the authentic Eichhornia anthocyanin A obtained previously.[3]

The chromatographic data and spectral properties of Eichhornia anthocyanin B (EAB) are shown in reference.[4] On acid hydrolysis, EAB gave delphinidin, luteolin, glucose and malonic acid. Alkaline hydrolysis of EAB under nitrogen yielded a deacylanthocyanin, luteolin 7-glucoside and malonic acid. The deacylanthocyanin was identified to be delphinidin 3-gentiobioside[5] by HPLC in comparison with the data of the authentic delphinidin 3-gentiobioside which was obtained from EAA by the similar process of an alkaline hydrolysis. Luteolin 7-glucoside was also identified by chromatographic and spectral analyses[6] with the authentic sample obtained from *Pharbitis nil*.[7]

The FAB mass spectral measurement of EAB showed a $[M]^+$ at 1143 *m/z* and $[M –H + Na]^+$ 1165 *m/z*.

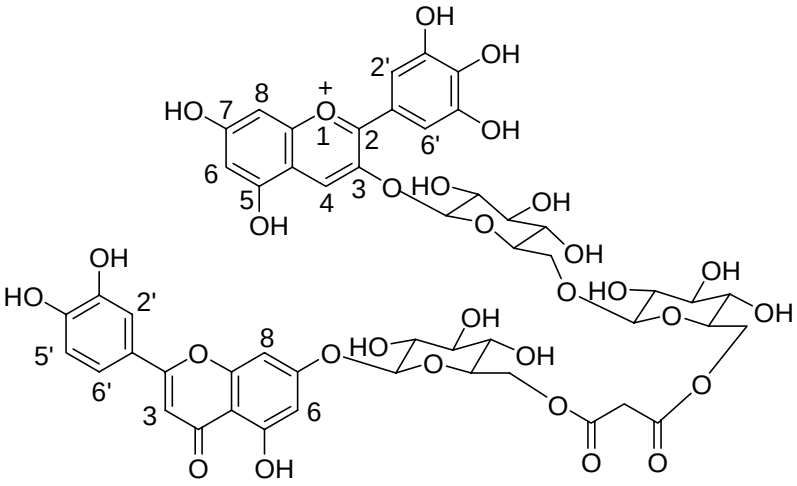

Figure 1. Eichhornia anthocyanin B.

The $[M]^+$ mass is good agreement with the mass calculated for $C_{51}H_{51}O_{30}$ (1143 *m/z*), suggested that EAB was consisted of one molecule each of delphinidin 3-gentiobioside, luteolin 7-glucoside and malonic acid,

respectively. Its exact elemental composition was further confirmed by measurement of a high-resolution mass spectrum. The 500MHz proton FT-NMR and ^{1}H-^{1}H COSY spectra of EAB were measured in TFA-d_1-DMSO-d_6 with TMS as the internal standard, and its structure was determined by the analysis of these spectra as follows. The ^{1}H-NMR spectrum of EAB is superimposable on that of EAA except the signals of luteolin moiety.[8] The signals of sugar parts were observed in the region of δ 5.42 - 3.20, and three anomeric protons appeared at δ 5.42 (d, *J* = 7.9 Hz, Glu A), δ 5.23 (d, *J* = 7.0 Hz, Glu B) and δ 5.12 (d, *J* = 7.3 Hz, Glu C) were assigned with the vicinal coupling constants of 7.0 – 7.9 Hz. Therefore, all the glucose units must be β-glucopyranose. By analysis of the ^{1}H-^{1}H COSY spectrum at the sugar moieties, four characteristic protons appeared in the lower magnetic fields at δ 4.47 and 4.42 (Glu B) and δ 4.47 and 4.42 (Glu C) with the geminal coupling constants (*J* = 10.1 Hz) were assigned to the C-6 methylenes of glucoses B and C indicating the malonyl moiety to be attached to both the OH-6 groups of glucoses units B and C. Since two anomeric protons of Glu B (δ 5.23) and Glu C (δ 5.12) were correlated to both methylene protons (δ 4.47 and 4.42 of Glu B, and δ 4.47 and 4.42 of Glu C) of these glucoses, the OH-6 of Glu B in delphinidin 3-gentiobioside and the OH-6 of Glu C in luteolin 7-glucoside must be acylated with malonic acid as in the case of Eichhornia anthocyanin A.[1] Consequently, this pigment is determined to be (6’’’-*O*-(delphinidin 3-*O*-(6’’-*O*-(β-D-glucopyranosyl)- β-D-glucopyranosyl)) (6’’-*O*-(luteolin 7-*O*-(β-D-glucopyranosyl))) malonate, which is a new anthocyanin in plants.[9]

There are three reports of disubstituted dicarbonates with anthocyanins and flavonoids such as a disubstituted malonate with delphinidin 3-gentiobioside and apigenin 7-glucoside in *Eichhornia crassipes*,[1] four disubstituted malonates with cyanidin 3-glucoside and kaempferol 3-glycosides in *Allium schoenoprasum*,[10] and a disubstituted succinate with delphinidin 3-*p*-coumarylglucoside-7-glucoside and kaempferol 3,4’-diglucoside-7-xyloside in *Agapanthus praecox* sp. *orientalis*.[11] Thus, this will be the third report of the occurrence of a disubstituted malonate with an anthocyanin and a flavonoid glycoside.

Eichhornia anthocyanin B exhibited a strong shift of the visible λmax at 549 nm in contrast to the λmax (538 nm) of its deacylanthocyanin in the 0.1 % HCl-MeOH. Furthermore, a negative Cotton effect was observed at 537 nm by the CD spectrum measurement of this pigment in the same solution. Therefore, the structure of this pigment is presumed to keep the folding conformation between the anthocyanin moiety and the flavone in the solution.[1, 12]

REFERENCES AND NOTES

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2. Analytical HPLC was performed on a Inertsil ODS-2 column (4.6ϕ× 250 mm) at 35ºC with a flow rate of 0.8 mL/min monitoring at 530 nm. Solvent systems used were as follows: a linear gradient elution for 40 min from 25 to 85% solvent B (15% H_3PO_4, 20% HOAc and 25% MeCN in H_2O) in solvent A (15% H_3PO_4).
3. Eichhormia anthocynin A: UV-VIS λmax (0.1% HCl-MeOH); 548, 342, 272 nm. R*f* values (×100; TLC); BAW (n-BuOH-AcOH-H_2O, 4:1:5): 16; BuH (n-BuOH-2M HCl, 1:1): 4; 1% HCl: 2; HOAc-HCl (AcOH-HCl-H_2O, 13:3:82): 14. HPLC; R*t* (min) 27.3. FAB-MS; $[M]^+$ 1127 *m/z*.
4. Eichhormia anthocynin B: UV-VIS λmax (0.1% HCl-MeOH); 547, 351, 272 nm. R*f* values (×100; TLC); BAW: 10; BuH: 3; 1% HCl: 1; HOAc-HCl: 6. HPLC; R*t* (min) 26.0. FAB-MS; $[M]^+$ 1143 *m/z*; High resolution FAB-MS; Calcd for $C_{51}H_{51}O_{30}$ $[M]^+$ 1143.2466, found 1143.2443. Circular dichroism measurement; CD spectrum was determined at 25ºC in the range of 210-800 nm with 10 nm/min, and used a cell having path length of 1 cm. EAB was dissolved at 0.265 mg/200 μL (Mr = 1143) in 0.5% TFA-MeOH.
5. Delphinidin 3-gentiobioside: UV-VIS λmax (0.1% HCl-MeOH); 538, 281 nm. R*f* values (×100; TLC); BAW: 11; BuH: 1; 1% HCl: 8; HOAc-HCl: 28. HPLC; R*t* (min) 4.6. FAB-MS; $[M]^+$ 627 *m/z*.
6. Luteolin 7-glucoside: UV-VIS λmax (0.1% HCl-MeOH); 350, 254 nm. R*f* values (×100; TLC); BAW: 53; 15% AcOH: 6; H_2O: 0; PhOH: 63. HPLC; R*t* (min) 24.4.
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8. ^{1}H-NMR Spectral data for EAB (500 MHz, DMSO-d_6-CF_3COOD, 9:1): δ 8.76 (1H, s, delphinidin H-4), 7.87 (2H, br s, delphinidin H-2', 6'), 7.49 (1H, br s, flavone H-2'), 7.48 (1H, d, *J*=8.6 Hz, flavone H-6'), 7.29 (1H, br s, delphinidin H-8), 6.99 (1H, d, *J*=8.6 Hz, flavone H-5'), 6.82 (1H, br s, flavone H-8), 6.80 (1H, br s, delphinidin H-6), 6.73 (1H, s, flavone H-3), 6.51 (1H, br s, flavone H-6), 5.42 (1H, d, *J*=7.9 Hz, Glu A-1), 5.23 (1H, d, *J*=7.0 Hz, Glu B-1), 5.12 (1H, d, *J*=7.3 Hz, Glu C-1), 4.47 (2H, d, *J*=10.1 Hz, Glu B-6a and Glu C-6a), 4.42 (2H, m, Glu B-6b and Glu C-6b), 3.63-3.69 (1H, m, Glu A-2), 3.40-3.44 (1H, m, Glu B-2), 3.34-3.38 (1H, m, Glu C-2), 3.30-3.50 (2H, m, malonic CH_2), 3.20-3.90 (11H, m, sugar protons).
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