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STRUCTURE OF MALONYLAWOBANIN, THE REAL ANTHOCYANIN PRESENT IN BLUE-COLORED FLOWER PETALS OF COMMELINA COMMUNIS¶

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The real anthocyanin in Commelina communis is not awobanin, but malonylawobanin, whose structure was determined to be 3-O-(6-O-(trans-p-coumaroyl)-β-D-glucosyl)-5-O-(6-O-malonyl-β-D-glucosyl)delphinidin (1).

In 1931, Kuroda[1] isolated from flower petals of Commelina communis an anthocyanin, awobanin, whose structure was proposed by Kuroda[2] and by Takeda and Hayashi[3] to be delphinidin 3-(p-coumaroyl-D-glucosido)-5-D-glucoside. Its complete structure and stereochemistry were determined by us[4] to be 3-O-(6-O-(trans-p-coumaroyl)-β-D-glucosyl)-5-O-(β-D-glucosyl)delphinidin (3). Hayashi et al.[5] isolated from the flower a blue-colored complex pigment, commelinin, and analyzed it to be composed of two molecules each of awobanin (3) and a flavone, flavocommelin, and one atom each of Mg and K.[6] We have now found that the true anthocyanin present in the flower is not awobanin (3), but a new anthocyanin that we named malonylawobanin (1). Thus, awobanin (3) is an artifact produced from malonylawobanin (1) during extraction from the flower petals or from commelinin with methanolic hydrochloric acid.

Awobanin (3) was usually obtained as its flavylium chloride from commelinin by treatment with methanolic hydrochloric acid followed by precipitation with ether. However, careful treatment of commelinin with the acid afforded three pigments, A, B, and C, whose Rf values on Avicel tlc ($AcOH:HCl:H_2O$ = 5:1:40 or $n\text{-}BuOH:HCl:H_2O$ = 7:2:5) were quite close each other. Transformation of A to C via B was observed with time. We have succeeded in isolating of these three pigments as their trifluoroacetate by decomposition of commelinin with an appropriate acid (for A, 1.5% H_3PO_4 at room temp. for a few hours; for B and C, 1% HCl in MeOH at room temp. for 7 h and 3 days, respectively) and then by HPLC separation on an

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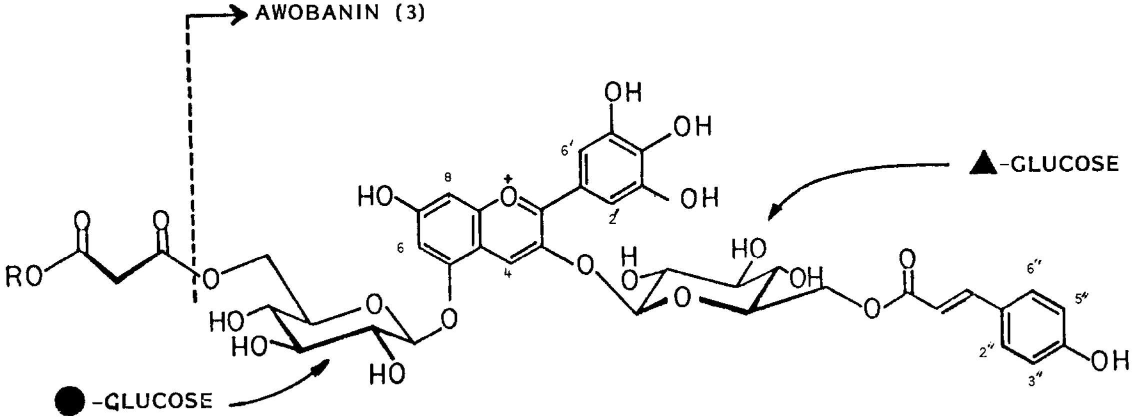
¶ Reported to the 25th Symposium on the Chemistry of Natural Products, Tokyo, Oct. 1982. Symposium papers pp 375-382 (1982). In this abstract we named this pigment "protoawobanin", but we propose to change this name to "malonylawobanin", because "proto-" was already used in "protocyanin" in a different sense [E. Bayer, Angew. Chem. Internat. Ed., 5, 791 (1966)].

ODS (Nomura, 10-20 μ) column using a solvent composed of $AcOH:CH_3CN:H_2O:CF_3CO_2H$ (10: 12.5:77:0.5). PMR spectra of the sugar region of these pigments are shown in Fig. 1 (other signals, see Footnotes 7, 8, and 9).

Pigment C [mp 176-177 °C (dec); FAB-MS m/z 773 (M^+)]^7 was identified with awobanin (3) by comparision of their physical data. Pigment A [mp 161-165 °C; FAB-MS m/z 859 (M^+), 611, 551, 303]^8 and B [mp 148 °C; FAB-MS 873 (M^+), 611, 565, 303]^9 have larger molecular weights than that of pigment C (awobanin) by 86 and 100, respectively. The PMR spectrum (Fig. 1) of awobanin (3) shows signals for only one $-CH_2O-$ group of a glucose unit at lower fields than 4.1 ppm, indicating that the group is acylated, whereas signals for two $-CH_2O-$ groups are found in the lower fields in the case of pigments A and B, indicating that each of the later two pigments has two acyl groups attached to 6 positions of both glucose units in its molecule. In addition, pigment B has a singlet signal at 3.72 ppm corresponding to a methoxy group. These data indicate that pigment A and B have malonic acid and malonic acid monomethyl ester, respectively, as an additional component besides awobanin (3).



MALONYLAWOBANIN (R = H) (1)

MALONYLAWOBANIN METHYL ESTER (R = CH_3) (2)

Malonic acid in pigment A was detected by gas chromatography [Carbowax 20M (5%) on Chromosorb W and Silicon XE 60 (5%) on Chromosorb W] as its dimethyl ester (yield ca 26%) which was obtained from the pigment by heating with 2N HCl at 100 °C, extracting with ether, and evaporating the ether solution followed by treatment with methanolic hydrochloric acid. That p-coumaric acid in these pigments exists in the trans form was clearly shown by the coupling constant between H-α and H-β (J = 16 Hz).

Pigment A, which we named malonylawobanin (1), must, therefore, be 3-O-(6-O-(trans-p-coumaroyl)-β-D-glucosyl)-5-O-(6-O-malonyl-β-D-glucosyl)delphinidin, and pigment B its methyl ester (2).

Monardaein[10] (= salvianin ?)[11] was the only instance of anthocyanin so far reported that it contained malonic acid in its molecule, but later Harborne[12] reported that it was erroneous and that no anthocyanins having malonic acid moiety had been found in nature. We believe that monardaein has malonic acid, but Harborne overlooked the acid due to improper isolation procedure of the pigment.

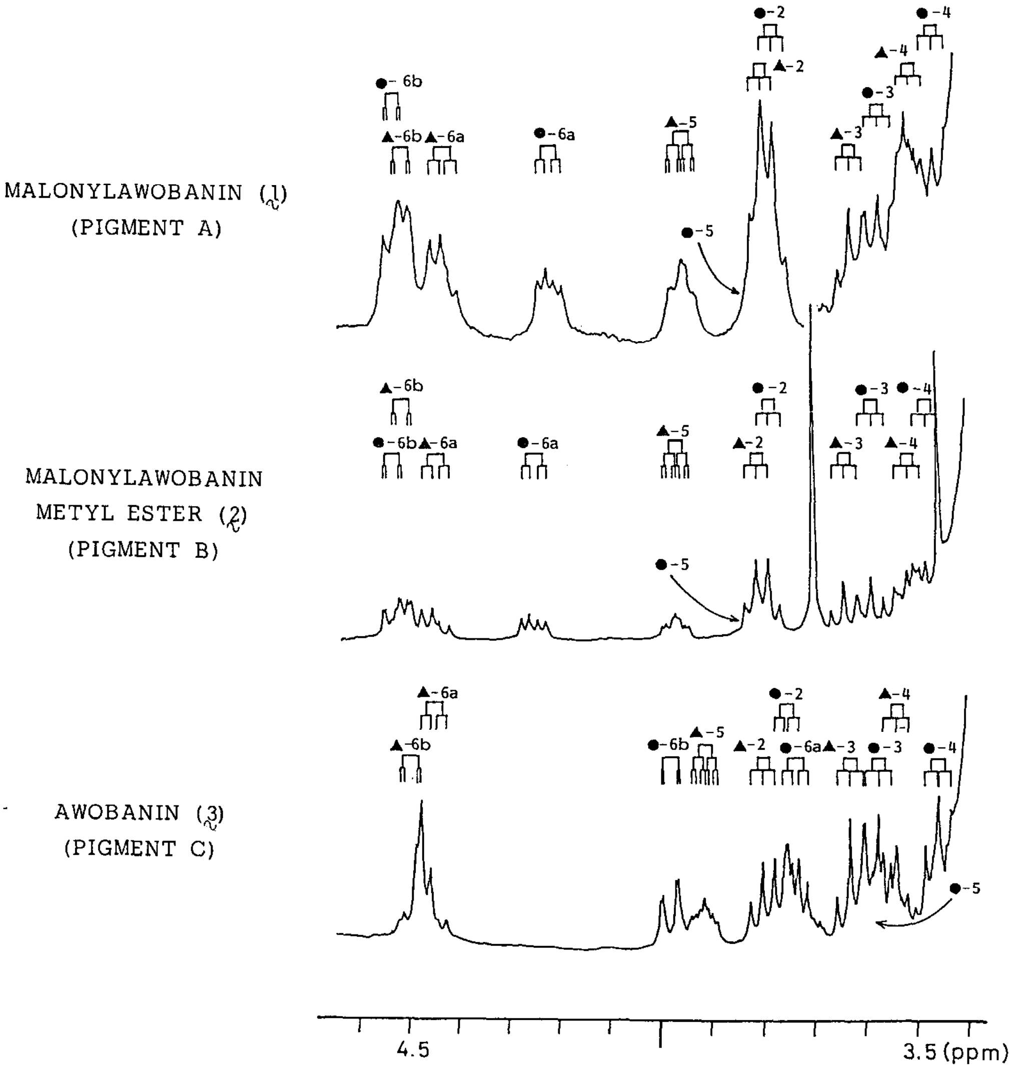


Fig. 1. PMR spectra of the pigments in CD_3OD containing 0.5% CF_3COOD at 360 MHz at room temp. Assigments of the signals corresponding to the sugar moieties in the PMR spectra of (2) and (3) were done by spin-spin decoupling experiments. Assignments of the signals for (1) were done by comparing with the signals of (2).

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5. K. Hayashi, Y. Abe and S. Mitsui, Proc. Japan Acad., 34, 373 (1958).
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7. Awobanin (3) (pigment C): $\lambda_{max}^{0.01\%\ HCl}$ nm (log ε) 524 (4.42), 312 (4.23), 295 (4.26), 277 (4.08), $E_{312}/E_{vis\text{-}max} = 0.616$, $A_{440}/A_{vis\text{-}max} = 0.201$; PMR (360 MHz, CD_3OD cont'ng 0.5% CF_3COOD) ppm 8.89 (1H, s, H-4), 7.75 (2H, s, H-2' & 6'), 7.38 (1H, d, J = 16 Hz, H-β) 7.28 (2H, d, J = 8.5 Hz, H-2" & 6"), 7.00 (1H, br.s, H-6 or 8), 6.89 (1H, br.s, H-8 or 6), 6.76 (2H, d, J = 8.5 Hz, H-3" & 5"), 6.23 (1H, d, J = 16 Hz, H-α), 5.43 (1H, d, J = 7.5 Hz, anomeric ▲-H), 5.15 (1H, d, J = 7.5 Hz, anomeric ●-H).
8. Malonylawobanin (1) (pigment A): $\lambda_{max}^{0.01\%\ HCl}$ nm (log ε) 525.5 (4.44), 312 (4.19), 295 (4.23), 277 (4.31), $E_{312}/E_{vis\text{-}max} = 0.545$, $A_{440}/A_{vis\text{-}max} = 0.166$; PMR (360 MHz, CD_3OD cont'ng 0.5% CF_3CO_2D) ppm 8.87 (1H, s, H-4), 7.77 (2H, s, H-2' & 6'), 7.37 (1H, d, J = 16 Hz, H-β), 7.23 (2H, d, J = 8.5 Hz, H-2" & 6"), 7.00 (1H, br.s, H-6 or 8), 6.93 (1H, br.s, H-8 or 6), 6.73 (2H, d, J = 8.5 Hz, H-3" & 5"), 6.27 (1H, d, J = 16 Hz, H-α), 5.44 (1H, d, J = 7.5 Hz, anomeric ▲-H), 5.18 (1H, d, J = 7.5 Hz, anomeric ●-H).
9. Malonylawobanin methyl ester (2) (pigment B): $\lambda_{max}^{0.01\%\ HCl}$ nm (log ε) 527 (4.40), 312 (4.17), 295 (4.21), 278 (4.29), $E_{312}/E_{vis\text{-}max} = 0.585$, $E_{440}/E_{vis\text{-}max} = 0.190$; PMR (360 MHz, CD_3OD cont'ng 0.5% CF_3COOD) ppm 8.87 (1H, s, H-4), 7.77 (2H, s, H-2' & 6'), 7.37 (1H, d, J = 16 Hz, H-β), 7.23 (2H, d, J = 8.5 Hz, H-2" & 6"), 6.98 (1H, br.s, H-6 or 8), 6.92 (1H. br. s, H-8 or 6), 6.72 (2H, d, J =8.5 Hz, H-3" & 5"), 6.27 (1H, d, J = 16 Hz, H-α), 5.44 (1H, d, J = 7.5 Hz, anomeric ▲-H), 5.19 (1H, d, J = 7.5 Hz, anomeric ●-H), 3.72 (3H, s, OCH_3).
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11. R. Willstätter and E. K. Bolton, Ann., 412, 113 (1917); Karrer and Widmer[10] identified salvianin to be monardein, whereas Harborne[12] reported that salvianin was different from monardein.
12. J. B. Harborne, Phytochem., 3, 151 (1964).

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