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STRUCTURES OF A SUCCINYL ANTHOCYANIN AND A MALONYL FLAVONE, TWO CONSTITUENTS OF THE COMPLEX BLUE PIGMENT OF CORNFLOWER CENTAUREA CYANUS

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Structures of a new anthocyanin and a new flavone isolated from the complex pigment of blue flower of cornflower Centaurea cyanus were determined to be 3-O-(6-O-succinyl-β-D-glucosyl)-5-O-(β-D-glucosyl)cyanidin (1) (succinylcyanin) and apigenin 4'-O-(6-O-malonyl-β-D-glucoside) 7-O-β-D-glucuronide (3), respectively.

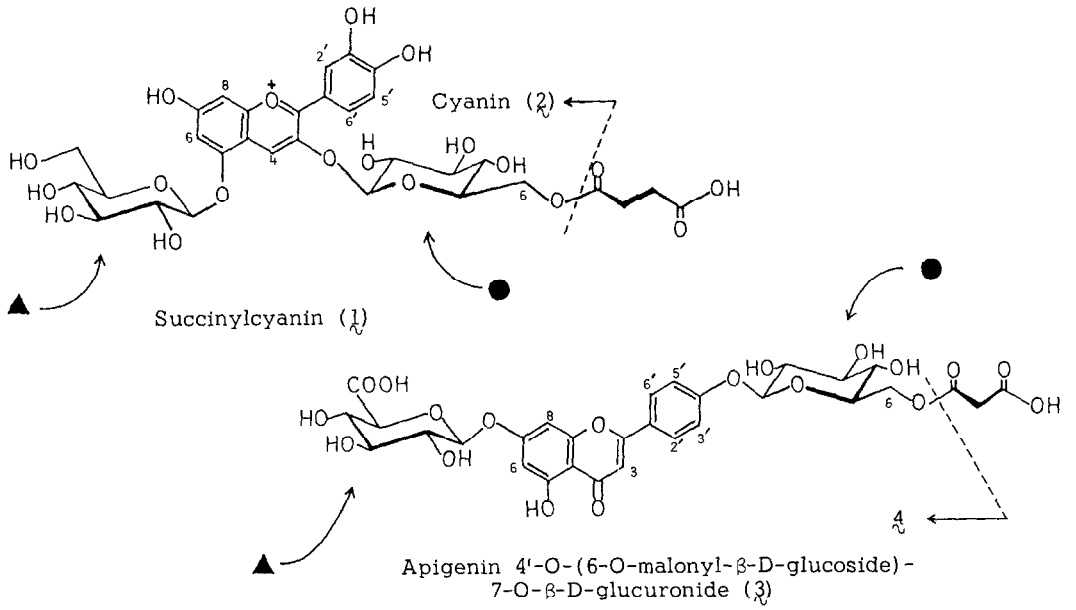
In 1913 Willstätter and Everest[1] isolated cyanin from cornflowers and determined its structure to be 2. The blue color of cornflower is actually produced from a complex pigment that consists of not only such an anthocyanin but also other substances. In 1958, Bayer[2] isolated an amorphous blue pigment named protocyanin, which contained cyanin (2), a polysaccharide, and Fe and Al metal ions. Hayashi et al.[3] reported that protocyanin, that they isolated in a crystalline form, on hydrolysis yielded cyanin (2), a flavone, a carbohydrate, a peptide as well as Fe, Mg, and K ions. Asen and Jurd[4] isolated from cornflowers a crystalline blue pigment and analyzed it to be an iron complex of four molecules of cyanin and three molecules of a bisflavone glucoside. They thought that this pigment differed markedly from protocyanin, and named it cyanocentaurin. Later, Asen and Horowitz[5] reported that the "bisflavone glucoside" was actually monomeric apigenin 4'-O-β-D-glucoside 7-O-β-D-glucuronide (4).

We have obtained from the blue complex pigment of cornflowers a new anthocyanin and a new flavone, structures of which were determined to be 3-O-(6-O-succinyl-β-D-glucosyl)-5-O-(β-D-glucosyl)cyanidin (1), which we named succinylcyanin, and apigenin 4'-O-(6-O-malonyl-β-D-glucoside) 7-O-β-D-glucuronide (3), respectively. The reason why the other investigators have isolated only cyanin (2) and the flavone 4 may be that the succinic and malonic acid half esters were easily hydrolyzed in methanolic hydrochloric acid that was used for extraction of the pigments. We have used trifluoroacetic acid instead of hydrochloric acid for extraction. Also, we have not used methanol, because esterfication of the free carboxyl group in these acids would make hydrolysis of the other end more easily, as has been found

in the case of malonylawobanin.[6]

The blue pigment isolated from cornflowers was decomposed by dissolving it in a mixed solvent composed of trifluoroacetic acid : acetic acid : acetonitrile : water (0.5 : 6.3 : 7.9 : 85.3) at room temp for 0.5 h and then separated by HPLC on an ODS column using the mixed solvent to give an anthocyanin and a flavone; almost no other pigment was found on the HPLC.

The anthocyanin (trifluoroacetate, amorphous, mp 86-90 °C)[7] gave its molecular ion at m/z 711 on the SI mass spectrum, which indicated that the anthocyanin may consist of cyanin (2) and succinic acid. This assumption was supported from its PMR spectrum, which showed a 4H-signal at ca 2.5 ppm corresponding to the two methylene groups of succinic acid moiety. Presence of succinic acid in the anthocyanin was proved as follows. The anthocyanin was refluxed in 1% HCl/MeOH for 2 h, and the reaction mixture was extracted with CH_2Cl_2. After evaporation of the solvent, remaining dimethyl succinate was analyzed by GLC (carbowax 20M 5% on Chromosorb W); the yield being 29%.



The complete structure of the anthocyanin, succinylcyanin, was determined by analysis of its PMR spectrum. In the spectrum taken in CF_3COOD/DMSO-d_6 (2:3), most of the signals could be assigned (Fig. 1).[7] Glucose ●-1 signal showed an NOE(~20% in 200 MHz) with H-4, indicating that ●-glucose is attached to 3-position of the anthocyanidin nucleus. All of the signals corresponding to the protons on ●-glucose could be assigned by homo-decoupling experiments and two-dimensional analysis (Fig. 2). The CH_2-O- protons of ●-glucose appeared at a lower field than other signals of ●-glucose, indicating that the CH_2-O- group is acylated with succinic acid. Thus, succinylcyanin must have the structure of 1.

The flavone (darkened gradually over 240 °C without melting)[8] showed the $[M + 1]^+$ peak at 695 in its SI mass spectrum, suggesting the presence of a malonic acid moiety on 4.

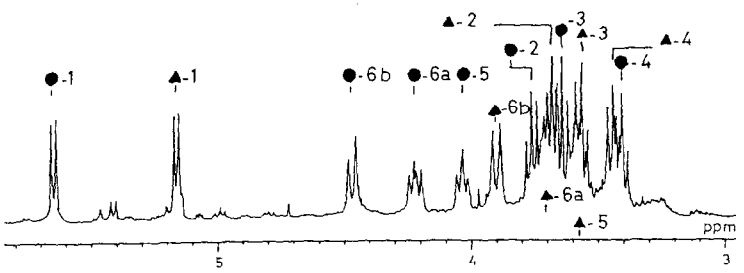


Fig. 1. PMR spectrum of succinylcyanin (1) at 400 MHz in $CF_3COOD/DMSO-d_6$ (2:3).

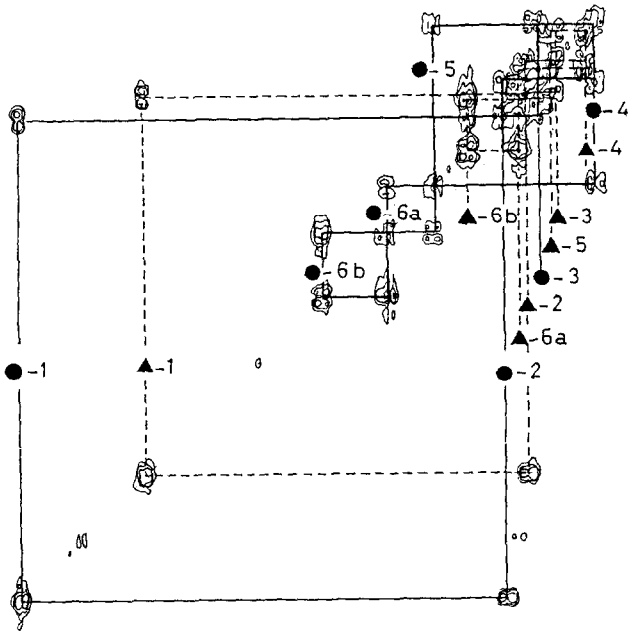


Fig. 2. $^1H-^1H$ shift correlated spectroscopy (COSY) of 1. The complicated contour plots were analyzed using the exact chemical shift values obtained from 2D-J (^1H J-resolved two dimensional NMR spectroscopy) except ▲-5 and ▲-6a.

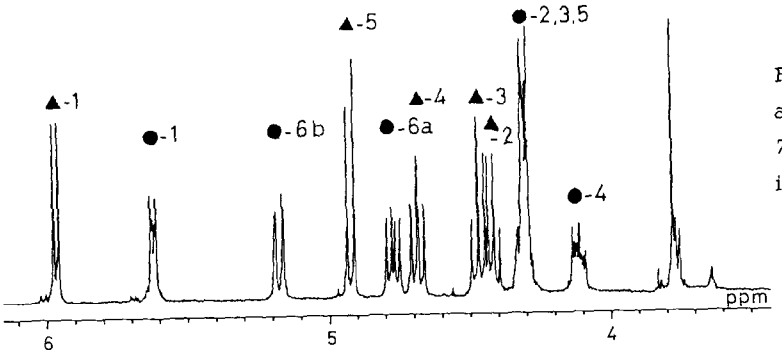


Fig. 3. PMR spectrum of apigenin 4'-O-malonylglucoside 7-O-glucuronide (3) at 400 MHz in pyridine-d_5.

Malonic acid was detected in the flavone as follows. The flavone was dissolved in DMSO and the solution was diluted with 5% HCl/MeOH. The mixture was refluxed for 2 h, extracted with CH_2Cl_2, and evaporated to yield dimethyl malonate, which was analyzed by GLC (same as above); the yield being 28%. The complete structure of the flavone was determined by analysis of its PMR spectrum (Fig. 3); the signals of ●-6a and ●-6b protons of glucose moiety appeared at a lower field than other signals of ●-glucose, indicating that the malonyl moiety is attached at 6 position of the glucose unit. Thus, the flavone must have the structure of 3.

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7. Succinylcyanin (1): UV (0.1% HCl) nm (ε) 510 (20,200), 320 (2,400), 290sh (8,400), 277 (13,300); $\lambda_{440}/\lambda_{vis}$ = 0.205, $\lambda_{320}/\lambda_{vis}$ = 0.119; PMR (400 MHz, CF_3COOD/DMSO-d_6 =2:3)ppm 8.86 (1H, s, H-4), 8.19 (1H, br.d, J=9 Hz,H-6'), 8.14 (1H, br.s, H-2'), 7.11 (1H, d, J=9 Hz, H-5'), 7.08 (1H, s, H-8), 7.00 (1H, s, H-6), 5.65 (1H, d, J=8 Hz, ●-1), 5.16 (1H, d, J=8 Hz, ▲-1), 4.47 (1H, br.d, J=12 Hz, ●-6b), 4.21 (1H, dd, J=9 & 12, ●-6a), 4.03 (1H, m, J=9, 9 & <1, ●-5), 3.90 (1H, br.d, J=12 Hz, ▲-6b), 3.76 (1H, t, J=9 Hz, ●-2), 3.70 (1H, dd, J=4 & 12 Hz, ▲-6a), 3.67(1H,t, J=9 Hz,▲-2),3.64 (1H, t, J=9 Hz, ●-3), 3.58 (1H, m, J=9, 4 & <1 Hz, ▲-5), 3.57 (1H, t, J=9 Hz, ▲-3), 3.43 (1H, t, J=9 Hz, ▲-4), 3.40 (1H, t, J=9 Hz, ●-4), 2.5 (4H, m, $-CH_2CH_2-$); NOE (200 MHz) ●-1 → H-4 ~20%, ▲-1 → H-6 ~11%.
8. The malonyl flavone 3: UV (0.5% DMSO/MeOH) nm (ε) 317 (15,100), 269 (17,000); PMR (400 MHz, pyridine-d_5)ppm 7.88 (2H, d, J=10 Hz, H-2' & 6'), 7.39 (2H, d, J=10 Hz, H-3' & 5'), 7.06 (1H, d, J=3 Hz, H-6 or 8), 6.88 (1H, d, J=3 Hz, H-8 or 6), 6.79 (1H, s, H-3), 5.97 (1H, d, J=8.5, ▲-1), 5.63 (1H, d with virtual couplings, J=8 Hz, ●-1), 5.18 (1H, dd, J=13 & 2Hz, ●-6b), 4.93 (1H, d, J=11 Hz, ▲-5), 4.78 (1H, dd, J=13 & 8 Hz, ●-6a), 4.69 (1H, t, J=10 Hz, ▲-4), 4.48 (1H, t, J=10 Hz, ▲-3), 4.43 (1H, dd, J=10 & 8.5 Hz, ▲-2), 4.3 (3H, m, ●-2, 3 & 5), 4.12 (1H, m, ●-4), 3.78 (2H, s, malonate CH_2); the following NOE's (400 MHz) were observed: ●-1 → H-3' & 5'; ▲-1 → H-6 & 8.

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