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STRUCTURE OF CINERARIN, A TETRA-ACYLATED ANTHOCYANIN ISOLATED FROM THE BLUE GARDEN CINERARIA, SENECIO CRUENTUS

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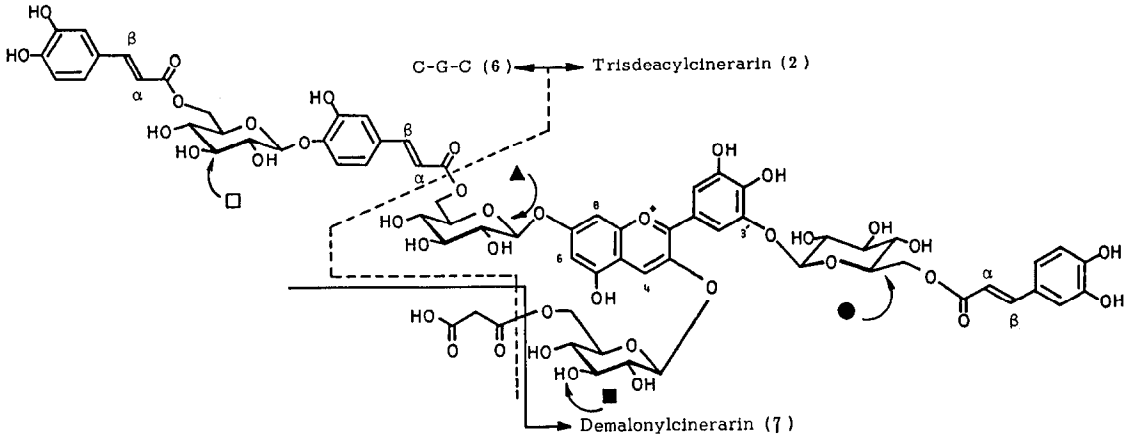
Structure of cinerarin is determined to be 3-O-(6-O-malonyl-β-D-glucopyranosyl)-7-O-(6-O-(4-O-(6-O-caffeyl-β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)-3'-O-(6-O-caffeyl-β-D-glucopyranosyl)delphinidin.

Cinerarin, which was isolated in 1974 by Yoshitama et al.[1] from blue flowers of garden cineraria, Senecio cruentus DC., is one of the heavily acylated anthocyanins that are unusually stable in neutral aqueous solutions.[2] They suggested its structure to be dicaffeyldelphinidin 3,7,3'-triglucoside,[3] but our study has disclosed that it is actually malonyltricaffeyldelphinidin tetraglucoside. We have established its complete structure and stereochemistry as described below.

Blue flower of cineraria was frozen in liquid nitrogen and powdered. It was extracted with cold methanol containing 3% trifluoroacetic acid (TFA) (when usual HCl-MeOH was used for extraction, the pigment was easily esterified and then lost the malonic acid component). After concentration, the extract was diluted with ether and the aqueous layer separated was poured on an Amberlite XAD-7 column, which was eluted with a gradient mixture of water and methanol (H_2O to MeOH). The pigment was eluted with 50% methanol. It was dissolved in a small amount of 3% TFA-MeOH and purified by means of ODS-HPLC (10-20 μ; solvent, TFA:AcOH:CH_3CN:H_2O = 0.5:10:12.5:77) to give TFA salt of cinerarin (1).[8]

FAB-MS spectrum gave a molecular peak at m/z 1523. PMR spectrum of (1)[8] showed the presence of a delphinidin nucleus, four glucose moieties, and three caffeic acid residues. The molecular weight of cinerarin (1523) indicated besides the above constituents an additional malonic acid in the molecule, which was confirmed by a quantitative GC analysis of dimethyl malonate (yield 81%) produced by treatment of (1) with 4% HCl-MeOH at 70°C for 3.5 hr. PRFT-PMR spectrum[4,8] of (1) clearly showed that four -CH_2O- groups of the four glucose moieties are all acylated.

Hydrolysis of cinerarin (1) was carried out with 50% methanol containing 2% NaOH at 0°C under nitrogen atmosphere for 25 min. After acidification with 3% TFA-MeOH, the mixture was chromatographed on an Amberlite XAD-7 column (gradient: water to methanol) and then purified by ODS-HPLC, giving the products A to E. Product B:[9] FAB-MS [m/z 951 (M^+)] and PMR spectra showed the presence of delphinidin, caffeic acid and three molecules of glucose as its compo-



Cinerarin (1)

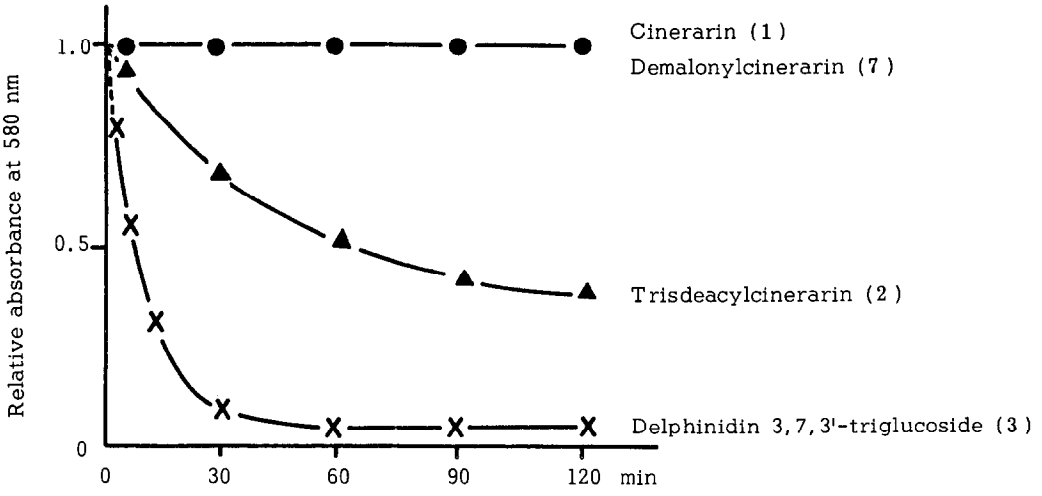


Fig. 1. Stability of Cinerarin and Deacylcinerarins

pH 6.46, conc. ca 5 x 10^{-5}M

nents. That a pair of signals assignable to $-CH_2O-$ group of a glucose unit appeared at lower fields (ca 0.8 ppm) than the other $-CH_2O-$ signals indicated that the $-CH_2O-$ group is esterified with caffeic acid. NOE between each of the anomeric protons and the protons on the delphinidin nucleus could be observed at 0°C, which indicated that product B is caffeyldelphinidin 3,7,3'-triglucoside (trisdeacylcinerarin). That two protons on the B-ring are non-equivalent (δ 7.88 & 7.77) also indicated the presence of glucose on 3'-position. NOE and proton spin-spin decoupling experiments showed that the $-CH_2O-$ group appeared at the lower fields belongs to ●-glucose moiety. All of the vicinal coupling constants of three glucose moieties are 8 - 10 Hz, indicating that all of the glucose moieties have β-glucopyranoside structure. Thus, product B is 3'-(6-O-caffeyl-β-D-glucopyranosyl)-3,7-di(β-D-glucopyranosyl)delphinidin (**2**) (trisdeacylcinerarin). Product A: PMR spectrum showed its structure to be 3,7,3'-tri(β-D-glucopyranosyl)delphinidin (**3**).[3] Product C: This was determined to be methyl 4-(β-D-glucopyranosyl)caffeate (**4**) by comparison with an authentic sample.[5] Product D: This was methyl caffeate (**5**) by comparison with an authentic sample. Product E:[10] FAB-MS [m/z 519 (M+1)] and PMR spectra showed the presence of two (E)-caffeic acid moieties ($J_{a,b}$ = 16 Hz), a β-D-glucopyranosyl unit, and a methoxy group. Further alkaline hydrolysis of product E gave (**4**) and (**5**). Structure of product E was thus established to be methyl 4-O-(6-O-caffeyl-β-D-glucopyranosyl)caffeate (**6** methyl ester).

The above results indicated that cinerarin (**1**) is trisdeacylcinerarin (**2**) esterified at the two free $-CH_2OH$ groups with the caffeylglucosylcaffeic acid (**6**) (C-G-C) and malonic acid. To determine the positions of esterification, demalonylcinerarin (**7**)[11] was prepared by acid hydrolysis of cinerarin (**1**) with 2% aq HCl at 80°C for 4.5 hr. NOE and proton spin-spin decoupling experiments[11] of (**7**) clearly showed that the $-CH_2O-$ group of ▲-glucose moiety is esterified with (**6**). Thus, structure of cinerarin (**1**) was established to be 3-O-(6-O-malonyl-β-D-glucopyranosyl)-7-O-(6-O-(4-O-(6-O-caffeyl-β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)-3'-O-(6-O-caffeyl-β-D-glucopyranosyl)delphinidin.

Cinerarin (**1**) and demalonylcinerarin (**7**) are quite stable in aqueous solution at pH 6.46 at a concentration of ca 5 x 10^{-5}M, whereas the decaffeylated products, (**2**) and (**3**), are fairly unstable (Fig. 1). The caffeyl groups in (**1**) have a strong effect on the stabilization of the anthocyanidin nucleus, mechanism of which must be similar to the case of gentiodelphin[6] and platyconin.[7]

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8. Cinerarin (1): UV (0.01% HCl-CH_3OH) nm (ε) 549 (26,100), 328 (35,700), 290 (40,800); PMR (500 MHz, 3% CF_3COOD-CD_3OD at 42.5°C) δ_{ppm} (J in Hz) (delphinidin) 8.41 (1H, s, H-4), 8.05 (1H, br.s, H-2'), 7.32 (1H, br.s, H-6'), 6.76 (2H, s, H-6 & 8); (3 caffeic acids) 7.30, 7.24 & 6.98 (each IH, d, J = 16, 3 x B-H), 6.17, 6.04 & 5.78 (each 1H, d, J = 16, 3 x α-H); (4 Glc) 5.43, 5.05, 4.89 & 4.85 (each 1H, d, J = 7-8, H-l x 4), 5.11, 5.01, 4.68 & 4.66 (each 1H, dd, J = 2 & 12, H-6a x 4), 4.57, 4.17, 4.15 & 4.02 (each 1H, dd, J ▫ 8.5-10 & 12, H-6b x 4); the proton signals of glucose moieties shown were confiemed by PRFT-PMR.

9. Trisdeacylcinerarin (2): UV (0.01% HCl-CH_3OH) nm (ε) 538 (25,800), 334 (14,000). 286 (20,300); PMR (500 MHz, 3% CF_3COOD-CD_3OD at 0°C) δ_{ppm} (J in Hz) (delphinidin) 8.59 (1H, s, H-4). 7.88 (1H, br.s, H-6'), 7.77 (1H, br.s, H-2'), 6.92 (1H, br.s, H-81, 6.74 (1H, br.s, H-6): (caffeic acid) 7.02 (1H, d, J ▫ 16). 6.49 (1H, d, J = 8), 6.35 (1H, br.s), 6.28 (1H, br.d, J = 8), 5.93 (1H, d, J = 16); (glucose) 5.26 (1H, d, J = 7.5, ●-1), 3.64 (1H, dd, J = 7 & 9, ●-2), 3.70 (1H, t, J ▫ 9, ●-3), 3.35 (1H, t, J ▫ 9, ●-4), 3.97 (1H, br.t, J = 9, ●-5), 4.92 (1H, br.d, J ▫ 11, ●-6a), 4.14 (1H, br.dd, J = 9 & 11, ●-6b), 5.10 (1H, d, J = 7.5, A-l), 4.08 (1H, br.d, J ▫ 11, ▲-6a), 3.76 (▲-6b); all proton signals of ●-glucose were correlated by spin-spin decoupling experiments; 5.10 (1H, d, J = 7.5, ■-1), 4.04 (1H, br.d, J = 11, ■-6a), 3.83 (1H, dd, J ▫ 6 & 12, ■-6b); NOE ●-1 ⌒ H-2' & H-6' (-23% & -6%, respectively), ▲-1 ⌒ H-6 & 8 (-12% & -18%, respectively), ■-1 ⌒ H-4 (-22%).

10. C-G-C methyl ester (6 methyl ester): UV (CH_3OH) nm (ε) 321 (25,200), 293 (26.500). 240 (19,900); PMR (500 MHz, CD_3OD at 27°C) δ_{ppm} (J in Hz) 7.56 & 7.42 (each 1H, d, J = 16), 7.11 (1H, d, J = 8.5), 7.05 (1H, d, J ▫ 2), 7.03 (1H, d, J ▫ 2), 6.94 (1H, dd, J ▫ 2 & 8.5), 6.86 (1H, dd, J = 2 & 8.5), 6.80 (1H, d, J = 8.5), 6.28 & 6.13 (each 1H, d, J = 16), 4.85 (1H, d, J = 7.5), 4.54 (1H, dd, J = 2.5 & 12), 4.39 (1H, dd, J ▫ 8 & 12), 3.76 (3H. s).

11. Demalonylcinerarin (7): UV (0.01% HCl-CH_3OH) nm (E) 549 (24,100), 328 (31,000). 290 (36,600); PMR (500 MHz, 3% CF_3COOD-CD_3OD at 55°C) δ_{ppm} (J in Hz) 5.41 (1H, m, ▲-1), 3.72 (2H, m, ▲-2 8 3), 3.47 (1H, m, ▲-4), 3.99 (1H, td, J = 2 8 9, ▲-5), 4.98 (1H, dd, J ▫ 2 & 11, ▲-6a), 4.17 (1H, dd, J = 9 & 11, ▲-6b); all proton signals of&glucose moiety were correlated by spin-spin decoupling experiments: NOE (at -20°C) ●-1 ⌒ H-2' (-7.8%). a-l ⌒ H-4 (-26.7%), ▲-1 ⌒ H-6 8 8 (-2.5% 8 -27.211, respectively).

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