STRUCTURE OF TERNATIN A2, ONE OF CLITORIA TERNATEA FLOWER ANTHOCYANINS HAVING THE UNSYMMETRICAL SIDE CHAINS

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Abstract ---- The structure of ternatin A2 was identified as 3-O-(6-O-malonyl-β-D-glucopyranosyl)-3'-O-(6-O-(E-4-O-(6-O-(E-4-O-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl)-5'-O-(6-O-(E-4-O-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl)delphinidin.

From blue butterfly pea (Clitoria ternatea L. Leguminosae) flowers, the six anthocyanins (ternatins A1, A2, B1, B2, D1 and D2) were isolated and estimated as polyacylated derivatives of delphinidin 3,3',5'-triglucoside (Da-T).[1,2] Of the anthocyanins, ternatins A1 and D1 were completely determined to be the structures having symmetrical p-coumaric acid (C)-glucose (G) side chains, such as CG and CGCG, at 3' and 5'-glucoses on B-ring of the delphinidin (Dp) nucleus.[3,4] This paper reports the structure determination of ternatin A2, one of the ternatins bearing the unsymmetrical side chains.

Ternatin A2 (**1**)[mp > 300°C (blackened over 180°C); λ_{max} (0.1% HCl-MeOH) nm (log ε) 548 (4.40, no shift with $AlCl_3$), 460 (sh, 3.92), 288 (4.79), $E_{310}/E_{vis.max}$ = 2.02] gave fab-ms data (molecular ion peak at m/z 1799 as a flavylium cation corresponding to $C_{81}H_{91}O_{46}^+$) and nmr data shown in Figure 1.[5,6] Alkaline hydrolysis of 1 with 2N NaOH afforded one molecule of Da-T, three molecules of E-4-O-β-D-glucopyranosyl-p-coumaric acid (CG)[1] and one molecule of

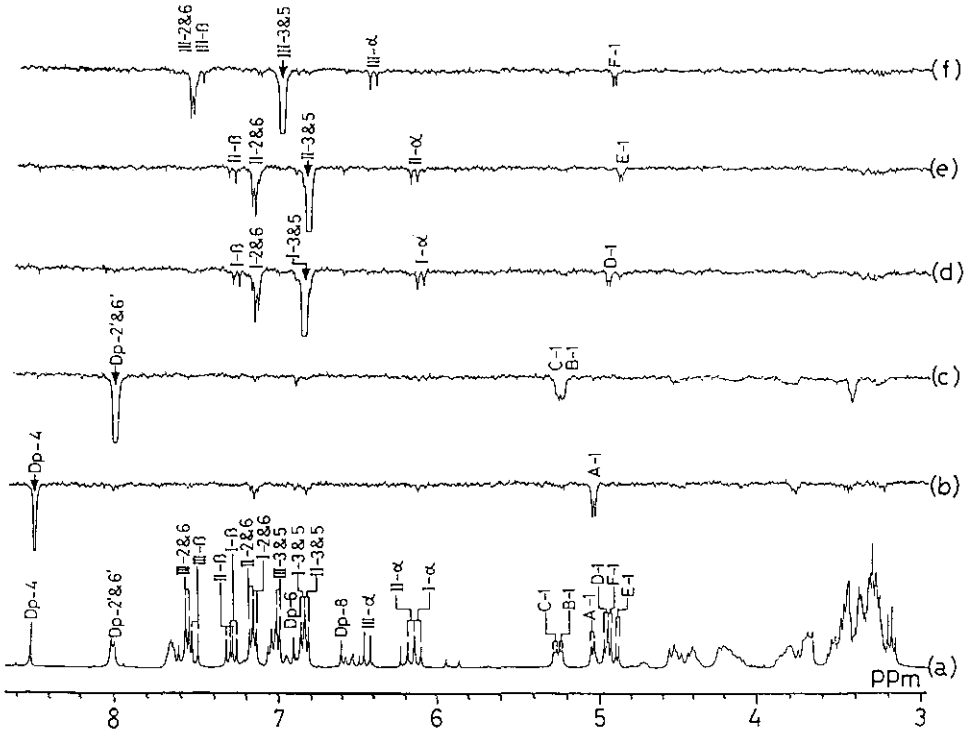

Figure 1. ^{1}H-Nmr (400 MHz) DIFNOE spectra of ternatin A2 (1) in DMSO-d_6-CF_3COOD = 9 : 1 at room temperature (a) Normal spectrum; (b) (f) DIFNOE spectra by irradiation at H-4 of Dp, H-2' and 6' of Dp, H-3 and 5 of I, H-3 and 5 of II and H-3 and 5 of III, respectively (Irradiation positions are indicated by the arrows).

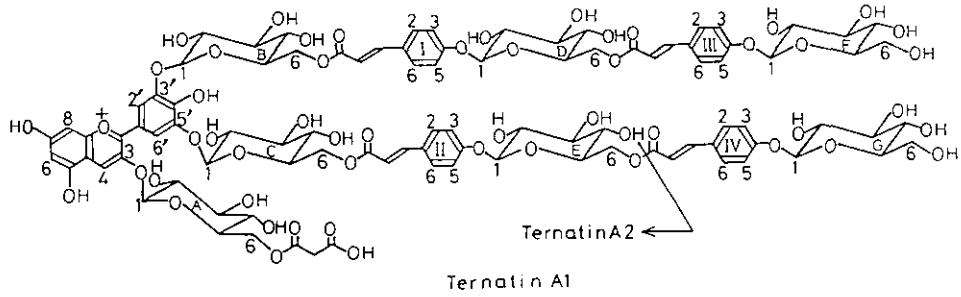

Figure 2. Structure of ternatin A2 (1).

malonic acid. Moreover by H_2O_2 oxidation **1** gave 6-O-malonyl-D-glucopyranose (MG), indicating that malonic acid attached to 3-glucose of Dp nucleus of **1**.[7] Based on the molecular weight and degradation examinations, 1 was presented to consist of delphinidin, six molecules of D-glucose, three molecules of p-coumaric acid and malonic acid. In the proton nmr spectrum[5] (Figure 1a), since all the anomeric protons are observed around 5 ppm with the coupling constants (J) of 7∿8 Hz, and since the sugar configurations of CG and MG parts were determined as D-glucopyranoside forms,[1] all six glucose moieties of 1 must be β-D-glucopyranoside. The methylene protons at 6-position of A∿D-glucoses are shifted to the low magnetic field (4∿5 ppm), indicating these four 6-CH_2-OHs are acylated but not those of the remaining two (E and F) glucosyl residues. The other sugar protons and malonyl-CH_2- protons have observed as heavily overlapped signals with the integrate intensity corresponding to 30 protons. In the p-coumaryl moieties as all α- and β-protons have large coupling constants (J = 16 Hz), the olefinic parts of all p-coumaryl moieties have trans (E) configuration. Moreover, the characteristic $(AX)_2$ couplings (J = ca 9 Hz) indicate the aromatic protons of p-coumaryl moieties, and the remaining five aromatic protons signals can be assigned to delphinidin skeleton.

DIFNOE spectra of **1** made clear the linking correlations of three sugars (A, B, and C-glucose) and other parts. As shown in Figures 1a∿c, NOEs between H-4 (Dp) and H-1 (A-glucose), H-2' (Dp) and H-1 (B-glucose), and H-6' (Dp) and H-1 (C-glucose) show that A-, B- and C-glucoses are attached to 3, 3', and 5'-OH of Dp through glycosidic bonds, respectively. Irradiations of ring protons (H-2 and 6, and H-3 and 5) of I, II and III-p-coumaryl moieties lead NOEs of anomeric protons of D, E and F-glucosyl moieties (Figures 1d∿f), respectively, indicating the presence of glucosyl-p-coumaric (CG) units such as I-D, II-E and III-F in 3',5'-side chains. In order to decide the positions of the three units in the side chains, the chemical shifts of anomeric protons of D-, E- and F-glucoses, and I, II and III-p-coumaryl protons were compared with those of ternatin A1.[4] The each proton peak of I-D and III-F of ternatin A2 was almost same as that of I and II-D and E, and III and IV-F and G of ternatin A1 whereas corresponding units to IV-G of A1 are absent, therefore I-D and III-F units must be located at inner and outer positions, respectively, in same side chain while II-E unit in other side chain as shown in Figure 2. Thus, the structure of ternatin A2 was proposed to be 3-O-(6-O-malonyl-β-D-glucopyranosyl)-3'-O-(6-O-(E-4-O-(6-O-(E-4-O-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl)-p-coumaryl)-β-D-glucopyranosyl)-5'-O-(6-O-(E-4-O-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl) delphinidin.

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5 Proton nmr of ternatin A2 (400 MHz, DMSO-d_6:CF_3COOD=9:1, δ ppm) 8.54 (1H, s, H-4 of Dp), 8.04 (1H, s, H-2' of Dp), 8.02 (1H, s, H-6'of Dp), 7.59 (2H, d, J=9Hz, H-2 and 6 of III), 7.54 (1H, d, J=16Hz, H-β of III), 7.32 (1H, d, J=16Hz, H-β of II), 7.30 (1H, d, J=16Hz, H-β of I), 7.19 (2H, d, J=9Hz, H-2 and 6 of II), 7.17 (2H, d, J=9Hz, H-2 and 6 of I), 7.02 (2H, d, J=9Hz, H-3 and 5 of III), 6.92 (1H, s, H-6 of Dp), 6.87 (2H, d, J=9Hz, H-3 and 5 of I), 6.84 (2H, d, J=9Hz H-3 and 5 of II), 6.64 (1H, s, H-8 of Dp), 6.46 (1H, d, J=16Hz, H-α of III), 6.18 (1H, d, J=16Hz, H-α of II), 6.14 (1H, d, J=16Hz, H-α of I), 5.28 (1H, d, J=7Hz, H-1 of C), 5.25 (1H, d, J=7Hz, H-1 of B), 5.07 (1H, d, J=8Hz, H-1 of A), 4.98 (1H, d, J=8Hz, H-1 of D), 4.95 (1H, d, J=7Hz, H-1 of F), 4.90 (1H, d, J=8Hz, H-1 of E), 4.4∿4.6 (4H, m, H-6a of A∿D), 4.1∿4.3 (4H, m, H-6b of A∿D), 3.1∿3.9 (30H, m, H-2∿5 of A∿F + 6-CH_2- of E, F + malonyl-CH_2-).

6 Carbon-13 nmr of ternatin A2 (100 MHz, DMSO-d_6:CF_3COOD=9:1, δ ppm) 167.90 (s), 167.00 (s), 166.49 (s), 166.37 (s), 166.28 (s), 166.12 (s), 166.09 (s), 159.28 (s), 159.22 (s), 158.91 (s), 158.88 (s), 158.70 (s), 158.53 (s), 158.15 (s), 157.76 (d), 157.15 (s), 155.42 (s), 145.88 (d), 145.74 (d), 145.24 (s), 144.51 (d), 144.36 (d), 144.25 (d), 144.08 (d), 143.58 (d), 130.07 (d), 130.01 (d), 129.88 (d), 129.48 (d), 129.36 (d), 127.81 (s), 127.69 (s), 127.36 (s), 119.39 (s), 118.14 (s), 116.58 (d), 116.52 (s), 116.31 (s), 116.25 (s), 115.85 (d), 115.78 (d), 113.65 (s), 112.18 (s), 110.78 (s), 101.92 (d), 100.40 (d), 100.14 (d), 100.03 (d), 99.84 (d), 99.73 (d); 77.08 (d), 76.96 (d), 76.57 (d), 76.48 (d), 76.29 (d), 75.79 (d), 75.75 (d), 74.29 (d), 74.18 (d), 73.85 (d), 73.21 (d), 70.16 (d), 69.77 (d), 69.71 (d); 69.59 (d), 64.45 (t), 63.73 (t), 63.52 (t), 63.29 (t), 62.84 (t), 60.69 (t); 41.11 (malonyl-CH_2-).

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