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STRUCTURE OF TERNATIN A1, THE LARGEST TERNATIN IN THE MAJOR BLUE ANTHOCYANINS FROM CLITORIA TERNATEA FLOWERS

Norihiko Terahara*, Norio Saito*+, Toshio Honda#, Kenjiro Toki$ and Yutaka Osajima++

Department of Food Science and Technology, College of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan; +Chemical Laboratory, Meiji-gakuin University, Totsuka-ku, Yokohama 224, Japan; #Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142, Japan; $Department of Horticulture, College of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan; ++Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

The structure of ternatin A1 has been identified as 3-O-(6-O-malonyl-β-D-glucopyranosyl)-3',5'-di-O-(6-O-((E)-4-O-(6-O-((E)-4-O-β-D-glucopyranosyl-p-coumaryl)-β-D-glucopyranosyl)-p-coumaryl)-β-D-glucopyranosyl)-delphinidin by application of the negative NOE difference (DIFNOE) spectroscopy.

In the course of structure determination of the blue flower anthocyanins of Clitoria ternatea L., the six polyacylated derivatives of delphinidin 3,3',5'-triglucoside (Da-T), named ternatin A1, A2, B1, B2, D1 and D2, were isolated by preparative ODS-HPLC[1]. Among them, the structure of ternatin D1 was determined first as 2 (Figure 2)[2]. This paper reports the structure elucidation of ternatin A1 (1) by ^{1}H-NMR, ^{13}C-NMR and DIFNOE spectroscopy[3].

Ternatin A1 (1) [mp > 300 °C (blackened over 200 °C); λ_{max} (0.1% HCl-MeOH) nm (log ε) 550 (4.50, no shift with $AlCl_3$), 460 (sh, 3.98), 287 (5.00), $E_{310}/E_{vis.max}$=2.32] exhibited a molecular ion peak at m/z 2107 as a flavylium ion corresponding to $C_{96}H_{107}O_{53}^{+}$ in its FABMS spectrum. Alkaline hydrolysis of 1 afforded Da-T, (E)-4-O-β-D-glucopyranosyl-p-coumaric acid (CG)[1] and malonic acid (M). Moreover H_2O_2 oxidation of 1 gave 6-O-malonyl-D-glucopyranose (MG), indicating that M is attached to the glucose at the 3-position (3-G) on delphinidin (Dp) nucleus of 1[4]. Based on the molecular weight and the degradation experiments, 1 was presented to consist of a molecule of Dp, seven molecules of D-glucose (G), four molecules of p-coumaric acid (C) and a molecule of malonic acid.

Proton NMR[5] and ^{13}C-NMR[6] spectra suggested that 3'- and 5'-side chains on 1 possessed the equal GCGCG units because of their highly symmetrical signal patterns as observed in ternatin D1 (2)[2]. Especially, in the ^{1}H-NMR

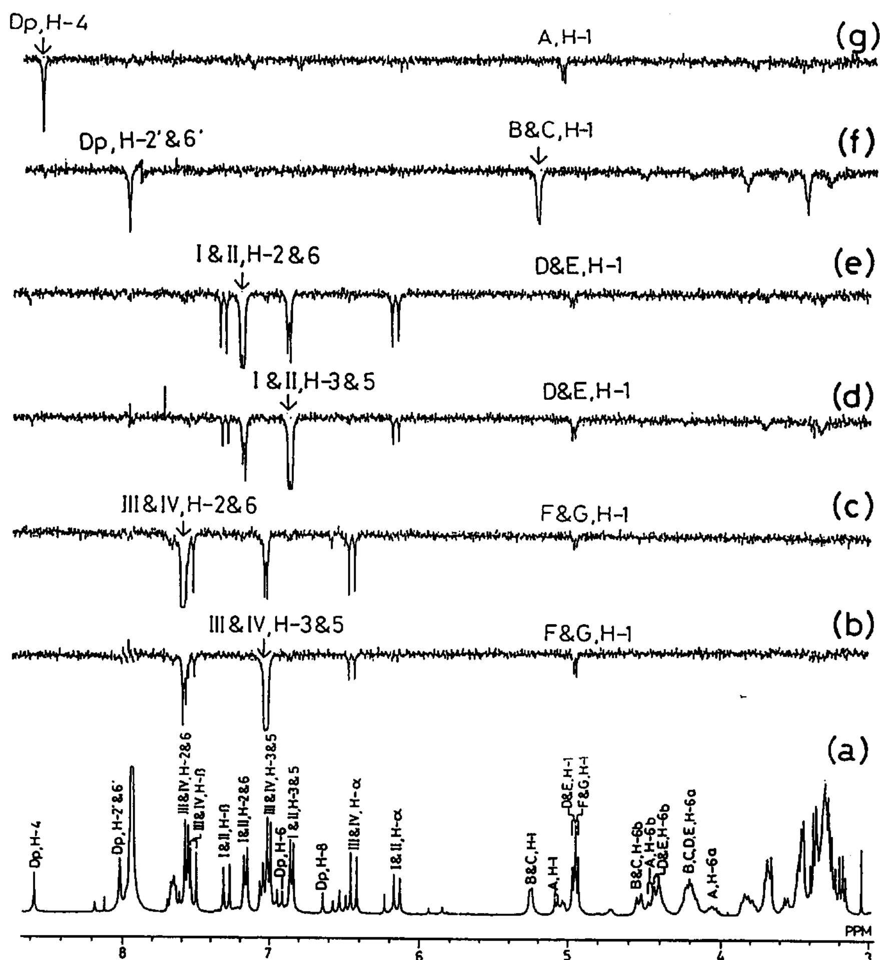

Figure 1. ^{1}H-NMR (400 MHz) NOE difference spectra of ternatin A1 (1) in DMSO-d_6 : CF3COOD = 9 : 1 at r.t. (a) Normal spectrum; (b)-(g) DIFNOE spectra by irradiation at H-3&5 of III&IV, H-2&6 of III&IV, H-3&5 of I&II, H-2&6 of I&II, H-1 of B&C and H-4 of Dp, respectively (Irradiation positions are indicated by the arrows).

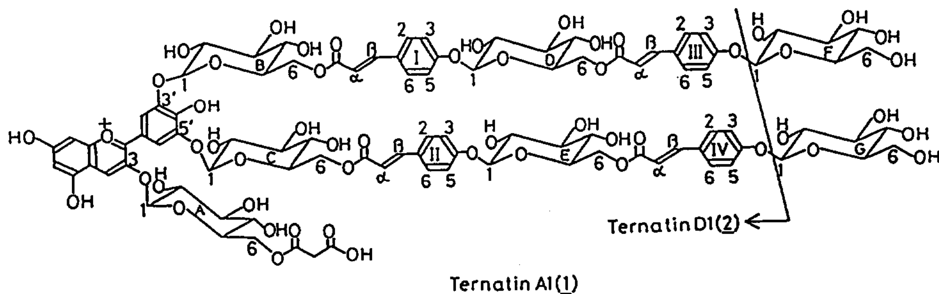

Figure 2. Structure of ternatin A1 (1).

spectrum of 1 the proton signals of three couples of glucoses (B&C, D&E and F&G) showed them to be completely duplicated respectively as well as those of two p-coumaric acid pairs (I&II and III&IV) as shown in Figure 1a. Conerning the sugar protons, the anomeric protons of all glucoses (A∼G) and 6-CH_2O- protons of the five glucoses (A∼E) appeared as the separated NMR signals in the low magnetic field while the other sugar protons and malonyl -CH_2- protons gave heavily overlapped signals with the integrated intensity corresponding to 34 protons. Since the anomeric protons were observed around 5 ppm with the coupling constants (J) about 8 Hz, and the sugar configurations of CG and MG moieties took D-glucopyranoside forms[1], all glucose moieties in 1 must be β-D-glucopyranoside forms. The signals of methylene protons at the 6-positions of A∼E-glucose moieties were shifted to the low magnetic field (4-5 ppm), indicating these five 6-CH_2OHs were acylated but not those of the remaining F and G-glucose moieties. This finding suggested that F and G-glucoses were located at terminal positions in 1. The characteristic A_2X_2 system (J = 9 Hz) corresponding to H-2&6 and H-3&5 supported the presence of p-coumaryl moieties of which double bond geometries are all (E) configuration ($J_{\alpha,\beta}$= 16 Hz). The remaining five aromatic proton signals could be assigned as H-2', 6', 4, 6 and 8 of delphinidin skeleton.

The measurement of DIFNOE spectra of 1 made clear the more detailed structure of 1. Irradiation of the signals of the H-2 and H-6 of I and II-p-coumaryl moieties (H-2&6 of I&II) or H-3&5 of I&II gave the DIFNOEs on the H-1 of D&E-glucose moieties (H-1 of D&E) in addition to the aromatic and the olefinic protons of I&II (Figure 1d, e). Thus I and D (or II and E) were linked with the glycosidic bond between 4-OH of I and 1-OH of D (or 4-OH of II and 1-OH of E). Similarly the presence of glucosyl-p-coumaric acid (CG) bonds such as III-F and IV-G units was also confirmed by NOE spectra (Figure 1b, c). NMR shift data of protons on I&II , III&IV and H-1 of D&E in 1[5] were respectively similar to those of the corresponding protons in 2[2]. These findings indicated the two CG units (I-D and II-E) to be located at the inner positions in 3',5'-side chains on 1 while the other two units (III-F and IV-G) at the outer ones as shown in Figure 2. Furthermore three glucoses (A, B and C) on Dp-ring were determined to be located at 3, 3' and 5'-OHs of Dp, respectively, due to the observations of NOEs as shown in Figure 1g and 1f. All of the seven glucose moieties on 1 were consequently correlated with delphinidin and four p-coumaryl moieties.

Ternatin Al is not only one of the most stable anthocyanins such as Heavenly Blue anthocyanin etc. in neutral aqueous solution[7] but also the largest molecule in the known monomeric anthocyanins[3,8].

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4. H_2O_2 oxidation of ternatin A1 was performed according to the modified Chandler and Harper method (B. V. Chandler and K. A. Harper, Aust. J. Chem., 1961, **14**, 586.) and Rf values [Rfx100 : 43 in EtOAc-AcOH-H_2O (3:1:1), 39 in n-BuOH-AcOH-H_2O (4:1:2), 31 in EtOH-NH_4OH-H_2O (16:1:3), 44 in Phenol-HCOOH-H_2O (75:1:25), 31 in Et_2O-HCOOH-H_2O (5:2:1)] on cellulose-TLC of the oxidation product were consistent with those of the authentic 6-malonylglucose[1].
5. Proton NMR of ternatin A1 (400 MHz, DMSO-d_6:CF_3COOD=9:1, δ ppm) 8.58 (1H, s, H-4 of Dp), 8.02 (2H, s, H-2'&6'of Dp), 7.57 (4H, d, J=9Hz, H-2&6 of III&IV), 7.52 (2H, d, J=16Hz, H-β of III&IV), 7.30 (2H, d, H-β of I&II), 7.17 (4H, d, J=9Hz, H-2&6 of I&II), 7.00 (4H, d, J=9Hz, H-3&5 of III&IV), 6.92 (1H, s, H-6 of Dp), 6.85 (4H, d, J=9Hz H-3&5 of I&II), 6.64 (1H, s, H-8 of Dp), 6.44 (2H, d, J=16Hz, H-α of III&IV), 6.15 (2H, d, J=16Hz, H-α of I&II), 5.24 (2H, br d, J=8Hz, H-1 of B&C), 5.07 (1H, d, J=7Hz, H-1 of A), 4.96 (2H, d, J=8Hz, H-1 of D&E), 4.94 (2H, d, J=8Hz, H-1 of F&G), 4.53 (2H, d, J=10Hz, H-6b of B&C), 4.45 (1H, d, J=12Hz, H-6b of A), 4.42 (2H, d, J=12Hz, H-6b of D&E), 4.1-4.3 (4H, m, H-6a of B, C, D & E), 4.0-4.1 (1H, m, H-6a of A), 3.1-3.9 (34H, m, H-2∿H-5 of A∿G + H-6 of F&G + malonyl-CH_2-).
6. Carbon-13 NMR of ternatin A1 (100 MHz, DMSO-d_6:CF_3COOD=9:1, δ ppm) 167.85 (s), 166.94 (s), 166.47 (s), 166.35 (s), 166.28 (s), 166.15 (s), 159.75 (s), 159.23 (s), 158.99 (s), 158.90 (s), 158.70 (s), 158.50 (s), 158.12 (s), 157.75 (s), 155.47 (d), 145.84 (s), 144.65 (s), 144.36 (d), 144.25 (d), 144.11 (s), 143.57 (d), 130.00 (d), 129.48 (d), 129.48 (d), 128.02 (s), 127.83 (s), 127.69 (s), 127.46 (s), 119.37 (s), 118.14 (s), 116.59 (d), 116.50 (s), 116.31 (s), 115.79 (d), 113.63 (s), 112.71 (s), 112.19 (s), 110.77 (s), 102.80 (d), 101.92 (d), 100.57 (d), 100.14 (d), 100.05 (d), 99.76 (d); 77.08 (d), 76.57 (d), 75.73 (d), 74.34 (d), 74.20 (d), 73.84 (d), 73.21 (d), 70.21 (d), 69.71 (d) ; 64.44, 64.10, 63.70, 63.27 (t), 62.80, 60.69 (t); 41.06 (malonyl-CH_2-).

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