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STRUCTURE OF TERNATIN D1, AN ACYLATED ANTHOCYANIN FROM CLITORIA TERNATEA FLOWERS

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The structure of ternatin D1 was identified as 3-O-(6-O-malonyl-β-D-glucopyranosyl)-3',5'-di-O-(6-O-(E-4-O-(6-O-E-p-coumaryl-β-D-glucopyranosyl)-p-coumaryl)-β-D-glucopyranosyl)delphinidin by ^{1}H-NMR, ^{13}C-NMR and negative nuclear Overhauser effect difference spectroscopy.

Ternatins extracted from blue petals of the butterfly pea, Clitoria ternatea, were composed of the six major anthocyanins(T-A1~D2) and showed high stability in neutral aqueous solution[1]. In the course of their structure determination, ternatin D1(1) was isolated by preparative ODS-HPLC as a trifluoroacetate form. This paper reports the structure elucidation of 1 by ^{1}H-NMR, ^{13}C-NMR and negative nuclear Overhauser effect difference spectroscopy[2].

Ternatin D1; mp>300℃ (blackened over 220℃); λmax (0.1% HCl-MeOH) nm (log ε) 548(4.37, no shift with $AlCl_3$), 460(sh, 3.86), 292(4.83), E_{440}/Evis=0.26, E_{310}/Evis=2.50; had molecular weight $[M]^+$=1783 (as a flavylium cation corresponding to $C_{84}H_{87}O_{43}^+$) as determined by FABMS and was estimated to consist of a molecule of delphinidin(Dp), five molecules of D-glucose(G), four molecules of p-coumaric acid(C) and a molecule of malonic acid[3]. Alkaline hydrolysis of 1 afforded delphinidin 3,3',5'-triglucoside(Da-T)[1], E-4-O-p-coumaryl-β-D-glucopyranoside(CG)[4] and malonic acid; the structures of Da-T and CG being determined to be 2 and 3, respectively. Moreover, by H_2O_2 oxidation 1 gave 6-O-malonyl-D-glucopyranose(MG), indicating that malonic acid attached to 3-glucose of 1[4]. Thus, the structure of 1 was proposed as Da-T acylated with malonic acid at 3-glucose and with CGC-side chains at 3'- and 5'-glucoses[3].

Proton NMR(400 MHz)[5] and ^{13}C-NMR(100 MHz)[6] spectra suggest that 3'- and 5'-side chains on 1 have the equal GCGC unit due to their high symmetrical signal patterns. Especially, in the ^{1}H-NMR spectrum of 1 the proton signals of inner side glucoses(B and C) or the outer couple(D and E) are completely duplicate respectively as well as those of inner p-coumaric acid pair(I and II) or the terminal pair(III and IV)(Figure 2). In sugar region, the anomeric and 6-CH_2O- protons of five sugars appear as the separated signals in the downfield while the other sugar protons and malonyl -CH_2- protons give overlapped signals with the integrate intensity corresponding to 22 protons. Since the anomeric protons are observed near δ 5ppm with the coupling constants(J) about 8 Hz, and since the sugar configurations of CG and MG parts were both D-glucopyranose forms[3,4], five glucoses all are determined to be β-D-glucopyranose form. All methylene protons at 6-position are shifted to low magnetic field(4-5 ppm), indicating these five -CH_2OHs are acylated[5].

In the p-coumaryl moieties in the side chains, as all α- and β-protons have large coupling constants(J=ca 16 Hz), the olefinic parts of all p-coumaric acids have trans(E) configuration. The type of A_2X_2 couplings(J=ca 9Hz) between H-2, H-6 and H-3, H-5 confirm that aromatic moieties of all p-coumaric acids are p-substituted benzene rings. The remaining aromatic proton signals(5 protons) show a flavylium skeleton with the B-ring trihydroxylated symmetrically, delphinidin.

To know the more detailed stereostructure, the negative nuclear Overhauser effect difference(DIFNOE) spectrum(400 MHz) of 1 was measured. Irradiation of the proton signal of the H-3 and H-5 on I - and II -p-coumaryl moieties gives the DIFNOE spectrum as Figure 1c, in which the anomeric protons of D- and E-glucose moieties as well as 2, 6, α- and β-proton signals of the I and II indicate the DIFNOE. Therefore, it shows that I - and II -p-coumaric acids are linked with D- and E-glucoses through glycosidic bond, respectively, and that I and II are located in the inner positions of the GCGC-side chains. Similarly, irradiation of H-3 and H-5 of III - and IV -p-coumaryl moieties(Figure 1b) indicates that the phenolic OHs of III - and IV -p-coumaric acids are free, and III and IV are therefore located at terminals of the GCGC-side chains because NOE with anomeric protons of any glucoses are not observed. The three glucose units, A-, B- and C-glucose, are attached to the 3, 3', 5'-OH positions, respectively, on the delphinidin nucleus, as deduced from the observation of DIFNOE between H-4 of delphinidin and H-1 of A-glucose, and between H-2' and H-6' of delphinidin and anomeric protons of B- and C-glucose(Figure 1e and 1d). Thus, all of the five anomeric protons of the glucose moieties were correlated with delphinidin and four p-coumaric acid moieties.

The exceptional color stability of ternatin D1(1) in a neutral aqueous solution is attributed to the intramolecular stacking between delphinidin nucleus and the GCGC-side chains, whereby the chromophore moiety of 1(in its quinonoidal base form) is protected from attacks of nucleophiles such as water molecule etc[7]. The structure elucidations of other ternatins are in progress.

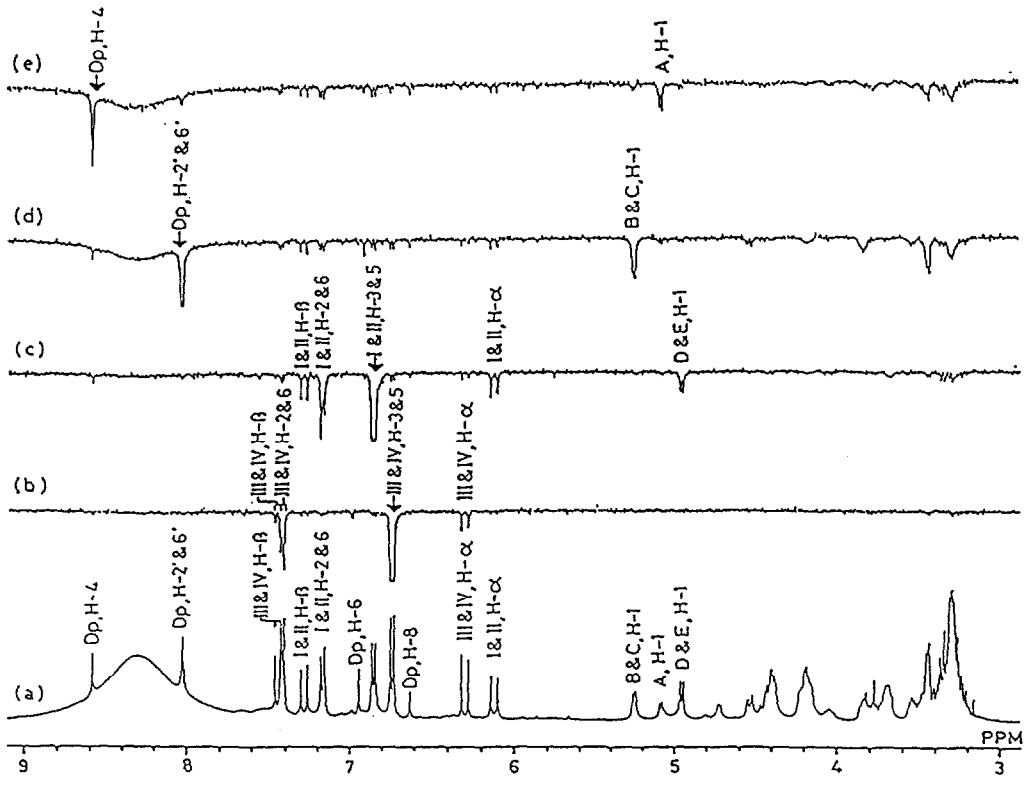

Figure 1. ^{1}H-NMR(400MHz) NOE difference spectra of ternatin D1(1) in DMSO-d_6 : CF_3COOD = 9 : 1 at r.t.. (a) Normal spectrum; (b)-(e) DIFNOE spectra by irradiation at H-3 & 5 of III & IV, H-3 & 5 of I & II, H-2'& 6' of Dp and H-4 of Dp, respectively (Irradiated positions are indicated by the arrows).

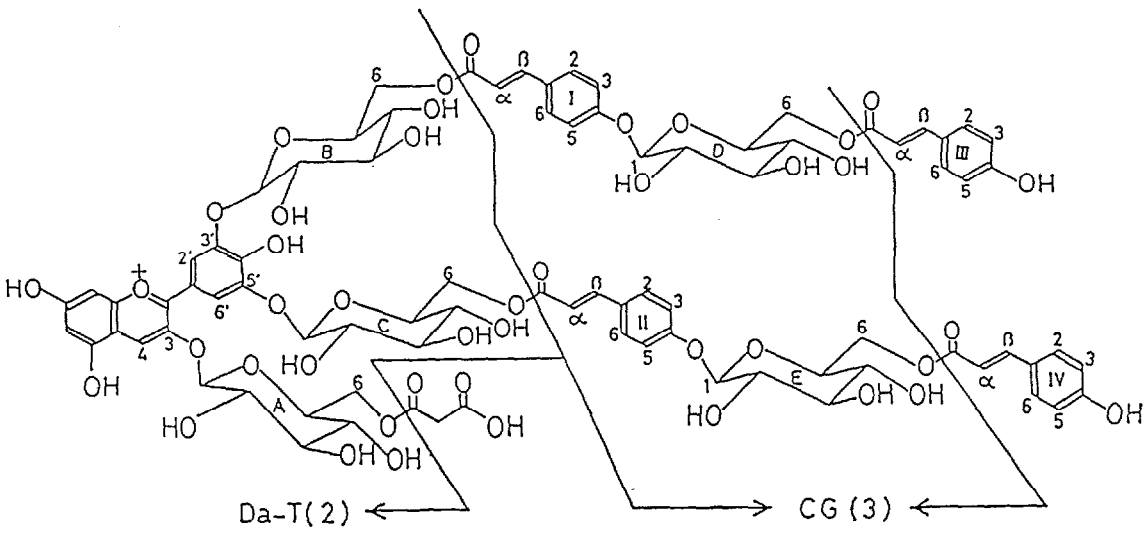

Figure 2. Ternatin D1(1)

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5. Proton NMR (400 MHz, DMSO-d_6 : CF_3COOD = 9 : 1 , δ ppm)
   8.59(1H, s, H-4 of Dp), 8.04(2H, s, H-2'&6' of Dp), 7.46(2H, d, J=16Hz, H-β of Ⅲ&Ⅳ), 7.43(4H, d, J=9Hz, H-2&6 of Ⅲ&Ⅳ), 7.29(2H, d, J=16Hz, H-β of Ⅰ&Ⅱ), 7.17(4H, d, J=9Hz, H-2&6 of Ⅰ&Ⅱ), 6.93(1H, br s, H-6 of Dp), 6.86(4H, d, J=9Hz, H-3&5 of Ⅰ&Ⅱ), 6.75(4H, d, J=9Hz, H-3&5 of Ⅲ&Ⅳ), 6.65(1H, br s, H-8 of Dp), 6.31(2H, d, J=16Hz, H-α of Ⅲ&Ⅳ), 6.13(2H, d, J=16 Hz, H-α of Ⅰ&Ⅱ), 5.27(2H, d, J=7Hz, H-1 of B&C), 5.08(1H, d, J=8Hz, H-1 of A), 4.96(2H, d, J=8Hz, H-1 of D&E), 4.56(2H, d, J=11Hz, H-6b of B&C), 4.47(1H, d, J=12Hz, H-6b of A), 4.44(2H, d, J=11Hz, H-6b of D&E), 4.19(4H, dd, J=5, 11Hz, H-6a of B,C,D&E), 4.07(1H, dd, J=7, 12Hz, H-6a of A), 3.2~3.9(22H, m, H-2~5 of A,B,C,D,E & malonyl-CH_2-).
6. Carbon-13 NMR (100 MHz, DMSO-d_6 : CF_3COOD = 9 : 1 , δ ppm)
   41.02(malonyl-CH_2-); 69.00, 69.57, 69.72, 69.87, 69.98(sugars 6-CH_2O- × 5); 73.09, 73.76, 73.84, 74.11, 74.23, 75.67, 75.75, 76.25(sugars C-2~5); 99.64, 99.90, 100.58, 101.80(sugars anomeric C); 110.78, 112.13, 112.75, 113.65, 113.94, 114.04, 115.70, 115.81, 116.02, 116.23, 116.46, 116.52, 118.08, 119.39, 124.912, 125.08, 127.39, 127.92, 129.45, 129.99, 130.12, 130.29, 143.49, 144.05, 144.77, 144.87, 145.18, 145.80, 157.24, 157.71, 158.09, 158.47, 158.63, 158.85, 158.91, 159.81; 166.09, 166.35, 166.44, 166.90, 167.78, 167.84(C=O × 6).
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