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STRUCTURE OF GENTIODELPHIN, AN ACYLATED ANTHOCYANIN ISOLATED FROM GENTIANA MAKINOI, THAT IS STABLE IN DILUTE AQUEOUS SOLUTION

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Structure of gentiodelphin is determined to be 5,3'-di-O-(6-O-trans- caffeoyl-β-D-glucosyl)-3-O-(β-D-glucosyl)delphinidin The anthocyanin is stable in dilute neutral aqueous solution This stabilization may be caused from intramolecular hydrophobic interactions among the aromatic nuclei, the anthocyanidin being sandwiched in between two caffeic acids

Anthocyanins are usually unstable in neutral or weakly acidic aqueous solutions, but recently a few acylated anthocyanins have been found to be stable in such aqueous solutions, they are platyconin,[1] cineraria,[2] heavenly blue anthocyanin,[3] etc [4,5] For clarification of the cause of such stabilization, complete structure and stereochemistry of the pigments must be determined, but structure of none of these pigments has been clarified completely The anthocyanin of Gentiana makinoi (Japanese name oyama-rindo) is one of such pigments We report herewith the complete structure of this anthocyanin which we named gentiodelphin

Gentiodelphin (1) was isolated as its chloride[6] from the flower petals of Gentiana makinoi by extraction of the fresh flower petals with 0 1% HCl-MeOH The extracts were concentrated and washed with ether to remove ether-soluble materials, and the residue was absorbed on a column packed with Amberlite XAD-7, which was then eluted with aqueous methanol to give crude pigment It was purified by ODS (Nomura Develosil) HPLC[7] to give pure gentiodelphin (1) chloride [mp 189-190 °C, FD mass m/z 1114 (M+1), 951, 790, UV-VIS (0 01% HCl-MeOH) nm (log ε) 538 (4 49), 328 (4 45), 297 (4 42), 280 (4 40), E_{328}/E_{538} = 0 933, E_{440}/E_{538} = 0 153, PMR[8] (Fig 1), CMR[8]] Complete acid hydrolysis of 1 afforded delphinidin, glucose and caffeic acid This result coupled with its physical data[8] indicates that 1 consists of delphinidin, three molecules of glucose and two molecules of caffeic acid

Gentiodelphin (1) chloride was hydrolyzed by treatment with 4% NaOH in 50% MeOH at 0 °C under Ar atmosphere for 30 min The mixture was acidified with 5% HCl-MeOH and evaporated to dryness The residue was treated with 0 1% HCl-MeOH and centrifuged to remove NaCl The resulted supernatant was diluted with ether, when red pigment precipitated The supernatant was evaporated and fractionated by preparative HPLC (ODS) to afford methyl caffeate[9] identical with the authentic sample [10] The precipitated pigment was desalted by passing it through a column of Amberlite XAD-7 using 1% HCl giving crude bis-deacylgentiodelphin (2) chloride, which was purified by HPLC (ODS) [mp 165-166 °C, FD mass m/z 789 (M^+), 626 (M -

$C_6H_{11}O_5$), UV-VIS (0 01% HCl-MeOH) nm (log ε) 528 (4 64), 340 (3 49), 295 (sh 3 98), 274 (4 27), E_{440}/E_{528} = 0 144, PMR (100 MHz, 1% DCl-CD_3OD) ppm 9 16 (1H, s, H-4), 8 12 (1H, d, J = 2 Hz, H-2' or 6'), 7 98 (1H, d, J = 2 Hz, H-2' or 6'), 7 17 (1H, br s, H-6 or 8), 7 08 (1H, br s, H-6 or 8), 5 30, 5 17, and 5 02 (each 1H, d, J = 8 Hz, 3 x anomeric H)]

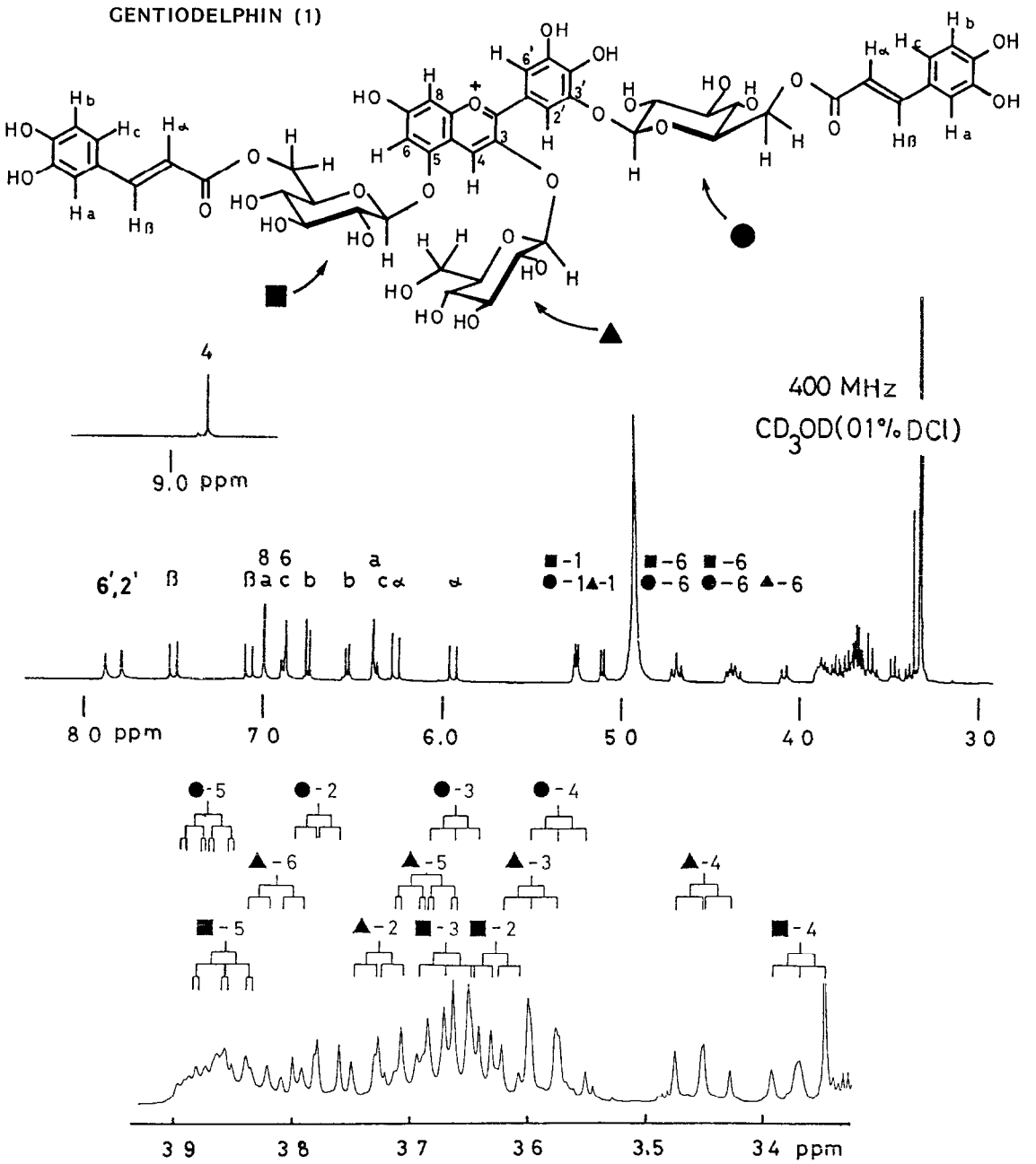

Fig 1 PMR spectra of gentiodelphin (1)

Acid hydrolysis of bis-deacylgentiodelphin (2) chloride with 1 5N HCl in 50% MeOH at 60 °C for 7 h afforded 3,5-di(β-D-glucopyranosyl)delphinidin (3) chloride [mp 188-191 °C (dec) , UV-VIS (0 01% HCl-MeOH) nm (log ε) 538 (4 29), 345 (3 30), 300 (sh, 3 68), 275 (3 96), E_{440}/E_{538} = 0 111, PMR (100 MHz, 1% DCl-CD_3OD) ppm 9 04 (1H, s, H-4), 7 79 (2H, s, H-2' and 6'), 7 04 (2H, s, H-6 and 8), 5 34 and 5 14 (each 1H, d, J = 8 Hz, 2 x anomeric H)], whose structure was confirmed by comparison of these spectra as well as mp with those of authentic sample prepared from awobanin (4)[11] chloride by deacylation with 4% NaOH-50% MeOH Non-equivalence in the chemical shifts of 2'-H and 6'-H of B-ring of 2 deduced the structure of 2 to be 3,5,3'-tri-O-glucosyldelphinidin

The structure of gentiodelphin (1) was determined as follows 1) the pigment consists of bis-deacylgentiodelphin (2) and two molecules of caffeic acid as evident from FD-mass and PMR spectrum, 2) position of attachment of each glucose moiety was determined by measurement of NOE between one of the anthocyanidin protons and the anomeric proton of each glucose moiety,[12] 3) complete analysis of the PMR signals (Fig 1) corresponding to protons of the sugar moieties using spin-spin decoupling technique including INDOR method indicated that the all three glucoside moieties excist in β-pyranosyl form, and 4) the methylene groups at 6-position of two glucose moieties show PMR signals lower than 4 3 ppm, indicating that two caffeic acids are attached at the 6-position of two glucose moieties, which are assigned to be ● and ■, ▲ glucose being not acylated Thus, the structure of gentiodelphin (1) is determined to be 5,3'-di-O-(6-O-trans-caffeoyl-β-D-glucosyl)-3-O-(β-D-glucosyl)delphinidin

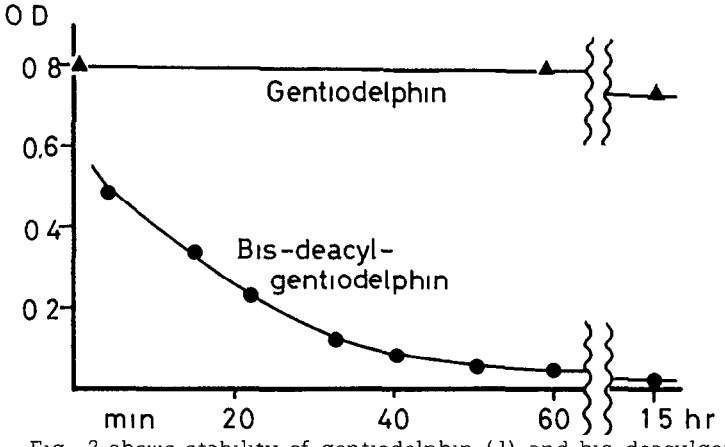

Fig 2 Stability of gentiodelphin (1) and bis-deacylgentiodelphin (2) in aq solution at pH 6 50 (conc 4 27 x 10^{-5}M in 1/15 M phosphate buffer, O D at λ_{max}, 1 at 618 nm and 2 at 591 nm)

Fig 2 shows stability of gentiodelphin (1) and bis-deacylgentiodelphin (2) in aqueous solution It indicates that the acyl groups in gentiodelphin (1) quinonoid base strongly stabilizes the anthocyanidin chromophore as has been pointed out in the other acylated anthocyanidins, intramolecular interaction between anthocyanidin and the organic acid moieties has been suggested [13] We propose that intramolecular hydrophobic interaction among the aromatic nuclei is an important factor for the stabilization, the anthocyanidin nucleus being sandwiched in between two benzene rings of caffeic acids Similar hydrophobic interactions have been proposed for stabilization of co-pigmented complexes and self-associations of anthocyanins [14]

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6 Genuine gentiodelphin as well as its chloride were obtained as crystals Genuine anthocyanin was extracted from dried petals with 65% EtOH, purified by Sephadex LH 20 column and obtained as purplish blue needles By treatment with 0 5% HCl, the chloride was obtained as red needles

7 A modified method of N Akavia and D Strack [Z Naturforsch , 35c, 16 (1980)]

8 PMR (400 MHz, 0 1% DCl in CD_3OD) ppm 8 80 (1H, s), 7 89 (1H, d, J = 2 Hz), 7 80 (1H, d, J = 2 Hz), 7 51 (1H, d, J = 16 Hz), 7 09 (1H, d, J = 16 Hz), 7 01 (2H, d, J = 2 Hz), 6 90 (1H, dd, J =2 and 8 Hz), 6 87 (1H, d, J = 2Hz), 6 77 (1H, d, J = 8 Hz), 6 54 (1H, d, J = 8 Hz), 6 40 (1H, d, J = 2 Hz), 6 39 (1H, dd, J = 2 and 8 Hz), 6 28 (1H, d, J = 16 Hz), 5 96 (1H, d, J = 16 Hz), 5 23 (1H, d, J = 7 5 Hz), 5 24 (1H, d, J = 7 5 Hz), 5 08 (1H, d, J = 7 5 Hz), 4 68 (1H, dd, J = 2 and 12 Hz), 4 65 (1H, dd, J = 2 and 12 Hz), 4 36 (1H, dd, J = (7 and 12 Hz), 4 33 (1H, dd, J = 9 and 12 Hz), 4 06 (1H, dd, J = 2 and 12 Hz), for other signals, see Fig 1, NOE (400 MHz) H-4 ▲ -1 = -6%, H-6 ■ -1 = -5%, H-2' ● -1 = -14%, (100 MHz) H-4 ▲ -1 = +5%, CMR (25 MHz, 0 1% DCl-CD_3OD) ppm 169 6 (s), 169 0 (s), 168 4 (s), 162 3 (s), 156 5 (s), 156 2 (s), 149 5 (s), 149 0 (s), 147 7 (s), 147 3 (d x 2), 146 8 (s), 146 5 (s x 2), 146 1 (s), 145 9 (d), 145 7 (s), 135 1 (d), 127 6 (s), 127 0 (s), 123 1 (d), 122 8 (d), 119 7 (s), 116 5 (d x 2), 115 4 (d), 115 3 (d), 114 7 (d), 113 7 (s), 111 8 (d), 106 5(d), 103 8 (d), 102 3(d), 101 0 (d), 97 5 (d), 78 9 (d), 78 3 (d), 77 7 (d), 77 3 (d), 76 2 (d), 74 6 (d), 72 6 (d), 71 5 (d), 64 6 (t), 64 4 (t), 62 8 (t)

9 PMR (100 MHz, CD_3OD) ppm 7 54 (1H, d, J = 16 Hz, H), 7 02 (1H, d, J = 2 Hz, H-2), 6 94 (1H, dd, J = 2 and 8 Hz, H-6), 6 74 (1H, d, J = 8 Hz, H-5), 6 24 (1H, d, J = 16 Hz, H-), 3 75 (3H, s, Me)

10 An authentic sample was prepared from caffeic acid by refluxing in methanol containing c-HCl, mp 152-155 °C, PMR spectrum is identical with that of ref 9

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12 See NOE data in ref 8

13 See references 1 to 4

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