Five New Anthocyanins, Ternatins A3, B4, B3, B2, and D2, from *Clitoria ternatea* Flowers

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Five new ternatins **1**−**5** have been isolated from *Clitoria ternatea* flowers, and the structures have been determined by chemical and spectroscopic methods as delphinidin 3-malonylG having 3′-GCG-5′-GCG, 3′-GCG-5′-GC, 3′-GCGCG-5′-GC, 3′-GCGC-5′-GCG, and 3′-GCGC-5′-GC side chains, respectively, in which G is D-glucose and C is *p*-coumaric acid. Pigment **1** had symmetric 3′,5′-side chains. Compounds **3** and **4** are structural isomers. These ternatins were shown to form an intramolecular stacking between the aglycon ring and the 3′,5′-side chains in solution.

The anthocyanins extracted from the bluish purple flowers of *Clitoria ternatea* L. (Leguminosae), "Butterfly pea" in English and "Cho-mame" in Japanese, are known to be exceptionally stable in weakly acidic or neutral aqueous solution[1−3] and are used as food colorants in Southeast Asia.[4] The six major anthocyanins ternatins A1, A2, B1, B2, D1, and D2, were isolated, and these structures have been characterized as malonylated delphinidin 3,3′,5′-triglucosides having 3′,5′-side chains with alternating D-glucose and *p*-coumaric acid units.[5,6] These structures, except ternatin B2, have been determined completely by Kondo *et al.* (ternatins A1, B1, and D1)[7] and Terahara *et al.* (ternatins D1, A1, and A2).[8−10] Yoda *et al.* reported the complete structure of ternatin D2.[11] Recently we isolated five new ternatins A3 (**1**), B4 (**2**), B3 (**3**), B2 (**4**), and D2 (**5**) from the same plant. In this paper, we describe the structure elucidation of these anthocyanins using a combination of chemical analyses, FABMS, and ^{1}H- and ^{13}C-NMR spectroscopies.

Results and Discussion

Dried petals of *C. ternatea* were extracted with 80% MeOH to give a pigment extract that contained nine or more anthocyanins by HPLC analysis. Compounds **1**−**5** were isolated as reddish purple powders of trifluoroacetic acid (TFA) salts. On acid hydrolysis, **1**−**5** gave delphinidin (Dp) as the aglycon and D-glucose (G) as the sugar. When $AlCl_3$ was added to each HCl−MeOH solution of **1**−**5**, no bathochromic shift of the visible absorption maxima ($\lambda_{vis_{max}}$) around 545 nm was observed, in spite of their being anthocyanins based on Dp with three vicinal OHs on the B-ring. This suggested that the 3′- and 5′-OH in the B-ring of the aglycon moieties of **1**−**5** were substituted. In the UV region, **1**−**5** had a large absorption due to acylation with an aromatic acid. The ratios (E_{310}/E_{vis}) of absorbance at 310 nm to absorbance at $\lambda_{vis_{max}}$ suggested that **3**, **4**, and **5** had three molecules of *p*-coumaric acid (C) and **1** and **2** had two molecules of C, respectively.[12] On alkaline hydrolysis, all pigments gave delphinidin 3,3′,5′-triglucoside as deacylternatin (Da-T) and malonic acid and 4-glucosyl-*p*-coumaric acid (CG) as the acyl components. As an additional acyl component, **2**−**5** gave C, indicating that C was linked through an ester bond to at least one terminal of 3′- and 5′-side chains of each ternatin, while **1** belonged to the ternatin A group with sugars in both terminals.[6] On H_2O_2 oxidation, **1**−**5** gave 6-malonylglucose, showing the connection of malonic acid on 3-G of the Dp nucleus. Moreover, the fragmentation ions [M − G − malonate]$^+$ such as *m/z* 1243, 1081, 1389, 1389, and 1227 in respective FABMS spectra of **1**−**5** suggested the presence of malonylglucose residue in these ternatin molecules. The pigments **1**−**5** gave the molecular ion peaks *m/z* 1491, 1329, 1637, 1637, and 1475 as a flavylium cation corresponding to $C_{66}H_{75}O_{39}^+$, $C_{60}H_{65}O_{34}^+$, $C_{75}H_{81}O_{41}^+$, $C_{75}H_{81}O_{41}^+$, and $C_{69}H_{71}O_{36}^+$, respectively, in the FABMS spectra. The result showed that **3** and **4** were isomers of each other, and **1**−**5** were composed of malonylated Da-T with the side chains having two Gs and two Cs, one G and two Cs, two Gs and three Cs, two Gs and three Cs, and one G and three Cs, respectively. Therefore, **2**−**4** and **5** belonged to the

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ternatin B- and D-groups, respectively.[6] The ODS-HPLC elution order of **1**−**5** supported this result as shown in the Experimental Section. Thus, hydrophobic ternatins **2**−**5** were retained more strongly than hydrophilic **1**, and in the same ternatin B-series, **2** with the smallest molecular weight, eluted faster than **3** and **4**.[6] In the case of ternatins with the same hydrophilicity and molecular weight, **3** eluted faster than **4**, suggesting that **3** had a more asymmetrical side-chain structure than **4**. The detailed structures of **1**−**5** were established through ^{1}H- and ^{13}C-NMR containing DQF-COSY, NOE difference spectroscopy (NOEDS), HOHAHA, HSQC,[13] and HMBC techniques. To compare the chemical shifts, Da-T was remeasured in the solvent system DMSO-d_6−CF_3COOD (9:1), and the shifts were compared to those measured in CD_3OD−DCl.[5] 1D ^{1}H-NMR spectra showed rather separated proton signals of Dp and C in the low magnetic region (> δ 6 ppm), while overlapped signals of G and M were observed in the high magnetic region (<δ 6 ppm). Characteristic singlets of Dp ring protons appeared in the range of 6.63−8.52 ppm, but the 2′ and 6′ proton peaks in **2**, **3**, and **5** were split. This fact suggested that the asymmetrical side-chain structures in **2**, **3**, and **5** had, to a large extent, a nonequivalent environment around these protons.[7] Two (**1** and **2**) and three (**3**−**5**) pairs of olefinic doublet signals with large coupling constants (16 Hz) indicated all Cs in **1**−**5** to have a *trans E* geometrical configuration. Assignment of aglycon and C protons was carried out with the aid of DQF-COSY. Sugar protons were assigned by DQF-COSY and HOHAHA methods. In the high magnetic region, four (**2** and **5**) and five (**1**, **3**, and **4**) anomeric protons and the ring proton signals with large coupling constants (7−8 Hz) demonstrated all sugar residues to be present as β-D-glucopyranosyl types in each pigment molecule. As three (**1** and **2**) and four (**3**−**5**) 6-methylene proton signals (6a and 6b) showed the downfield shifts arising from the proton deshielding, these sugars were proved to be acylated on the 6-methylene OHs. In the heavily overlapped region, the malonyl methylene protons of **1**−**5** were observed as intense singlets at 3.30−3.34 ppm. Characteristic malonyl methylene carbons in **1**−**5** were also confirmed by ^{13}C-NMR signals at 41.33−41.43 ppm.

Attachment positions of G and C in **1**−**5** were ascertained by NOEDS. By irradiation of Dp-4 and Dp-2′,6′ protons, NOEs on G_a-1 and G_b, G_c-1 protons, respectively, were observed. This showed that G_a, G_b, and G_c were linked, respectively, through a glycosyl bond to Dp 3-OH, 3′-OH, and 5′-OH having Da-T moiety. Similarly, NOEs between C_I-3,5 and G_d-1 indicated that C_I 4-OH was glycosidating with G_d; that is, they showed the presence of a C_I−G_d unit in the side chains. Irradiation of C_{II}-3,5 furnished NOEs of G_e-1 in **1** and **4** but no NOE in **2**, **3**, and **5**, respectively. This confirmed that G_e attached to C_{II} 4-OH in **1** and **4** (having C_{II}-G_e unit) but C_{II} was at a terminal spot in **2**, **3**, and **5**. Irradiation at C_{III}-3,5 of **3**−**5** resulted in NOE difference of only **3** G_f-1, indicating glycosylation of G_f on C_{III} 4-OH (having C_{III}-G_f unit) and that **4** and **5** C_{III} were terminal. Therefore, **2**−**4** were confirmed to be B-series ternatins having C and G on each terminal of 3′,5′-side chains, which were asymmetrical structures, and also **3** and **4** were confirmed as structural isomers with the side chains having the terminal G at the different positions. In addition, **5** was a D-type ternatin, while **1** was an A-type ternatin with symmetrical side chains.

These findings were also verified by analogy between the chemical shift values of C protons (α, β, 2,6, and 3,5 protons) in **1**−**5** and corresponded to those of ternatins A1 and D1 (T-A1 and T-D1) bearing the symmetric side chains, 3′,5′-GCGCG and 3′,5′-GCGC, respectively.[7,8,10] As listed in Table 1, chemical shifts of C_I protons in **1**−**5** are consistent with one another within δ 0.06 ppm and also with those of T-A1 and T-D1, showing that C_I is located on the inner site in the 3′-side chain in each ternatin, that is **1**−**5** have a partial connectivity −GC_IG−. The chemical environment of C_{III} protons in **3** is similar to that in T-A1 within 0.02 ppm, but it is considerably different from that of **4** and T-D1, with a minimum of 0.07 ppm. C_{III} proton shifts of **4** and **5** are similar to those of T-D1 but fairly different from those of **3** and T-A1. All of the above observations demonstrated that the structures of the 3′-side chain in **3** and **4** are the same as in T-A1 (−$GC_IGC_{III}G$) and T-D1 (−GC_IGC_{III}), respectively. The chemical shifts of C_{II} protons in **2**, **3**, and **5** are analogous with one another, but these are appreciably different from those of **1** and **4** and also from those of T-A1 and T-D1. The results revealed that C_{II} attaches to the terminal position, and, therefore, the structures of the 5′-side chains in **2**, **3**, and **5** are deduced to be −GC_{II} and those of **1** and **4** as 5′-GC_{II} G.

Consequently, ternatins A3, B4, B3, B2, and D2 were unambiguously determined as 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl)-3′,5′-bis-*O*-[6-*O*-((*E*)-4-*O*-β-D-glucopyranosyl-*p*-coumaryl)-β-D-glucopyranosyl]delphinidin, 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl)-3′-*O*-[6-*O*-((*E*)-4-*O*-β-D-glucopyranosyl-*p*-coumaryl)-β-D-glucopyranosyl]-5′-*O*-(6-*O*-(*E*)-*p*-coumaryl-β-D-glucopyranosyl)delphinidin, 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*)-*p*-coumaryl-β-D-glucopyranosyl)delphinidin, 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl)-3′-*O*-[6-*O*-[(*E*)-4-*O*-(6-*O*-(*E*)-*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, and 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl)-3′-*O*-[6-*O*-[(*E*)-4-*O*-(6-*O*-(*E*)-4-*O*-*p*-coumaryl-β-D-glucopyranosyl)-*p*-coumaryl]-β-D-glucopyranosyl]-5′-*O*-(6-*O*-(*E*)-*p*-coumaryl-β-D-glucopyranosyl)delphinidin, respectively (Figure 1).

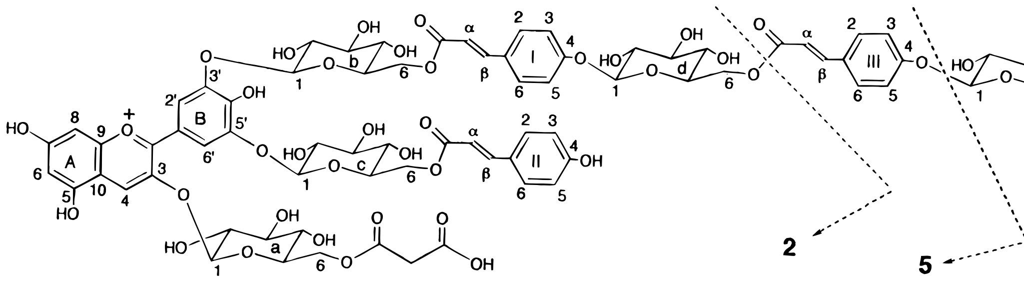
The three-dimensional information of **1**−**5** in solution was also deduced from NOE and chemical shift data. In **1** and **4**, weak NOEs were also observed between the Dp-4 proton and C_I/C_{II} ring protons, which were present on the inner side of the 3′,5′-side chains. Moreover, the C_I/C_{II} ring protons shifted farther upfield than those of the outer C_{III} in **3**, **4**, T-A1, and T-D1 (Table 1). This tendency is clarified by comparing the chemical shift for C moieties of ternatins with that of 4-glucosyl-*p*-coumaric acid (CG) as the reference, which is a simple molecule[6] as shown in Table 2. However, the proton chemical shift difference ($\Delta\delta$) values of the sugar region are plus (downfield shift) or 0 (data not shown). Delphinidin ring protons are also shifted farther upfield compared with those of Da-T. These phenomena can be attributable to the diamagnetic anisotropy shielding effect of aromatic ring currents based on orienting the

Table 1. ^{1}H NMR Spectral Data of the Pigments **1−5** and the Related Ternatins A1 and D1 (400 MHz, in DMSO-d_6−CF_3COOD, 9:1; δ ppm from TMS; and *J* Hz in parentheses)[a]

H	1	2	3	4	5	T-Al[b]	T-D1[b]
Dp-4	8.44 (s)	8.44 (s)	8.47 (s)	8.52 (s)	8.48 (s)	8.58 (s)	8.59 (s)
Dp-2′	8.05 (s)	8.02 (s)	8.03 (s)	8.05 (brs)	8.04 (s)	8.02 (s)	8.04 (s)
Dp-6′	8.05 (s)	7.99 (s)	7.98 (s)	8.05 (brs)	8.00 (s)	8.02 (s)	8.04 (s)
Dp-6	6.93 (s)	6.89 (s)	6.88 (s)	6.93 (s)	6.87 (s)	6.92 (s)	6.93 (s)
Dp-8	6.63 (s)	6.64 (s)	6.68 (s)	6.64 (s)	6.67 (s)	6.64 (s)	6.65 (s)
I-α	6.12 (d, 16)	6.12 (d, 16)	6.09 (d, 16)	6.08 (d, 16)	6.06 (d, 16)	6.15 (d, 16)	6.13 (d, 16)
II-α	6.12 (d, 16)	6.00 (d, 16)	6.02 (d, 16)	6.17 (d, 16)	6.02 (d, 16)	6.15 (d, 16)	6.13 (d, 16)
III-α			6.44 (d, 16)	6.31 (d, 16)	6.30 (d, 16)	6.44 (d, 16)	6.31 (d, 16)
I-*β*	7.28 (d, 16)	7.29 (d, 16)	7.27 (d, 16)	7.26 (d, 16)	7.25 (d, 16)	7.30 (d, 16)	7.29 (d, 16)
II-*β*	7.28 (d, 16)	7.17 (d, 16)	7.18 (d, 16)	7.32 (d, 16)	7.18 (d, 16)	7.30 (d, 16)	7.29 (d, 16)
III-*β*			7.53 (d, 16)	7.46 (d, 16)	7.45 (d, 16)	7.52 (d, 16)	7.46 (d, 16)
I-2&6	7.12 (d,9)	7.12 (d,9)	7.10 (d,8)	7.16 (d,9)	7.10 (d,8)	7.17 (d,9)	7.17 (d,9)
II-2&6	7.12 (d,9)	6.98 (d, 8)	6.98 (d,8)	7.13 (d,9)	6.99 (d,8)	7.17 (d,9)	7.17 (d,9)
III-2&6			7.56 (d,9)	7.41 (d,9)	7.40 (d,9)	7.57 (d,9)	7.43 (d,9)
I-3&5	6.85 (d,9)	6.85 (d,8)	6.87 (d,9)	6.85 (d,8)	6.86 (d,9)	6.85 (d,9)	6.86 (d,9)
II-3&5	6.85 (d,9)	6.57 (d,8)	6.56 (d,8)	6.86 (d,8)	6.56 (d,8)	6.85 (d,9)	6.86 (d,9)
III-3&5			7.02 (d,8)	6.76 (d,8)	6.75 (d,8)	7.00 (d,9)	6.75 (d,9)
a-1	4.97 (d,8)	4.94 (d,8)	4.96 (d,8)	5.03 (d,7)	4.96 (d,7)		
b-1	5.33 (d,7)	5.30 (d,8)	5.30 (d,8)	5.30 (d,8)	5.30 (d,8)		
c-1	5.33 (d,7)	5.32 (d,7)	5.32 (d,8)	5.31 (d,7)	5.31 (d,8)		
d-1	4.95 (d,8)	4.95 (d,8)	5.00 (d,8)	4.98 (d,8)	4.99 (d,7)		
e-1	4.95 (d,8)			4.93 (d,8)			
f-1			4.98 (d,7)				
a-2	3.60 (d,7)	3.58 (d,7)	3.61 (d,8)	3.58 (d,7)	3.61 (d,8)		
b-2	3.52 (t,7)	3.48 (d,8)	3.51 (d,8)	3.50 (d,8)	3.54 (d,8)		
c-2	3.52 (t,7)	3.56 (d,7)	3.51 (d,8)	3.50 (d,7)	3.54 (d,8)		
d-2	3.33 (t,8)	3.32 (d,7)	3.44 (d,8)	3.43 (d,8)	3.51 (d,8)		
e-2				3.35 (d,7)			
f-2			3.37 (d,7)				
a-3	3.43 (d,7)	3.50 (t,7)	3.53 (t,8)	3.55 (t,8)	3.46 (t,7)		
b-3	3.39 (t,7)	3.49 (m)	3.51 (m)	3.56 (m)	3.43 (m)		
c-3	3.39 (t,7)	3.49 (m)	3.51 (m)	3.56 (m)	3.43 (m)		
d-3	3.37 (t,8)	3.30 (d,9)	3.39 (t,8)	3.37 (t,8)	3.42 (m)		
e-3	3.37 (t,8)			3.31 (d,8)			
f-3			3.34 (t,8)				
a-4	3.20−3.70 (m)	3.35 (t,8)	3.32 (m)	3.30 (m)	3.38 (t,7)		
b-4	3.25 (t,8)	3.27 (m)	3.40 (m)	3.33 (m)	3.31 (m)		
c-4	3.25 (t,8)	3.27 (m)	3.40 (m)	3.33 (m)	3.28 (t,8)		
d-4	3.20−3.70 (m)	3.23 (t,9)	3.34 (t,8)	3.35 (t,8)	3.36 (m)		
e-4	3.20−3.70 (m)			3.24 (t,8)			
f-4			3.24 (t,8)				
a-5	3.70−3.90 (m)	3.80−3.90 (m)	3.77−3.80 (m)	3.84 (m)	3.71−3.90 (m)		
b-5	3.70−3.90 (m)	3.80−3.90 (m)	3.77−3.80 (m)	3.92 (m)	3.71−3.90 (m)		
c-5	3.70−3.90 (m)	3.80−3.90 (m)	3.77−3.80 (m)	3.92 (m)	3.71−3.90 (m)		
d-5	3.20−3.70 (m)	3.53 (m)	3.73 (m)	3.73 (m)	3.71−3.90 (m)		
e-5	3.20−3.70 (m)			3.45 (m)			
f-5			3.44 (m)				
a-6a	4.10−4.30 (m)	4.15−4.22 (m)	4.10−4.30 (m)	4.16 (m)	4.10−4.44 (m)		
b-6a	4.10−4.30 (m)	4.15−4.22 (m)	4.10−4.30 (m)	4.19 (m)	4.10−4.44 (m)		
c-6a	4.50−4.70 (m)	4.15−4.22 (m)	4.10−4.30 (m)	4.19 (m)	4.10−4.44 (m)		
d-6a	3.80 (m)	3.70−3.80 (m)	4.10−4.30 (m)	4.23 (m)	4.10−4.44 (m)		
e-6a	3.80 (m)			3.57 (m)			
f-6a			3.53 (brd,13)				
a-6b	4.50−4.70 (m)	4.58−4.64 (m)	4.55 (brd, 11)	4.54 (brd,12)	4.48 (brd,11)		
b-6b	4.50−4.70 (m)	4.58−4.64 (m)	4.63 (brd,12)	4.61 (brd,12)	4.64 (brd,12)		
c-6b	4.50−4.70 (m)	4.58−4.64 (m)	4.60 (brd,13)	4.61 (brd,12)	4.61 (brd,12)		
d-6b	3.58 (m)	3.70−3.80 (m)	4.49 (brd,12)	4.47 (brd,12)	4.55 (brd,11)		
e-6b	3.58 (m)			3.79 (m)			
f-6b			3.72 (brd,12)				
−CH$_2$−	3.33 (s)	3.30 (s)	3.34 (s)	3.31 (s)	3.32 (s)		

[a] Abbreviations: T-A1, T-D1, Dp, and −CH$_2$− = ternatins A1, D1, delphinidin, malonyl methylene, respectively; s, d, t, m, brs, brd = singlet, doublet, triplet, multiplet, broad singlet and broad doublet, respectively. [b] Only required values were cited from references T-A1[10] and T-D1.[8]

C$_{\mathrm{I}}$ and C$_{\mathrm{II}}$ rings and the olefinic moieties above and below the Dp pyrylium ring as reported in.[3,11] The fact proves the presence of an intramolecular sandwich-type stacking between the Dp pyrylium ring and the inner C rings on the 3′,5′-side chains in solutions of **1−5** and it efficiently blocks nucleophilic hydration on Dp C-2,[14,15] leading to the colorless secondary species such as the hemiacetal water 2-adduct and the retrochalcones.[16] Indeed, these ternatins demonstrated high stability in neutral aqueous solution (data not shown). In particular, C$_{\mathrm{II}}$ ring protons of **2**, **3**, and **5** have stronger ring-current effect than the corresponding protons of the same inner C$_{\mathrm{I}}$, and simultaneously Dp-6 protons as well as Dp-4 of the same ternatins are shifted strongly (Table 2). However, the effect is unclear in C$_{\mathrm{II}}$ ring protons in **1** and **4** and also in T-A1 and D1. In contrast to the



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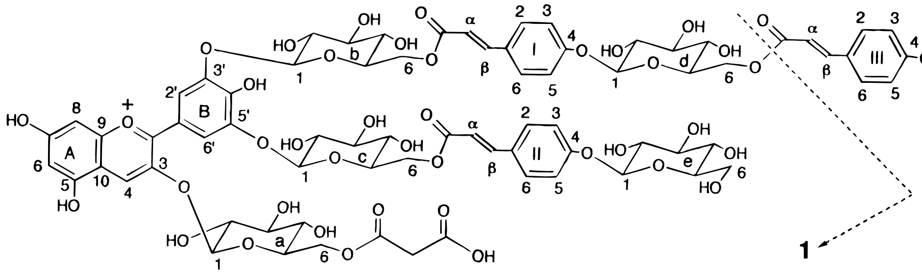

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Figure 1. Structures of ternatins A3 (**1**), B4 (**2**), B3 (**3**), B2 (**4**), and D2 (**5**).

Table 2. Proton Chemical Shift Differences Between Ternatins and the Related Compounds Da-T and CG

	Δδ (proton chemical shift differences, ppm)								δ (ppm)	
H	1	2	3	4	5	T-A1[a]	T-D1[a]	T-A2[a]	Da-T	CG[a]
				delphinidin moiety						
Dp-4	−0.60	−0.60	−0.57	−0.52	−0.56	−0.46	−0.45	−0.50	9.04	
Dp-2′	−0.12	−0.15	−0.14	−0.12	−0.13	−0.15	−0.13	−0.13	8.17	
Dp-6′	−0.12	−0.18	−0.19	−0.12	−0.17	−0.15	−0.13	−0.15	8.17	
Dp-6	−0.18	−0.22	−0.23	−0.18	−0.24	−0.19	−0.18	−0.19	7.11	
Dp-8	−0.14	−0.13	−0.09	−0.13	−0.10	−0.13	−0.12	−0.13	6.77	
				p-coumaryl moiety						
I-α	−0.26	−0.26	−0.29	−0.30	−0.32	−0.23	−0.25	−0.24		
II-α	−0.26	−0.38	−0.36	−0.21	−0.36	−0.23	−0.25	−0.20		6.38
III-α			0.06	−0.07	−0.08	0.06	−0.07	0.08		
I-*β*	−0.23	−0.22	−0.24	−0.25	−0.26	−0.21	−0.22	−0.21		
II-*β*	−0.23	−0.34	−0.33	−0.19	−0.33	−0.21	−0.22	−0.19		7.51
III-*β*			0.02	−0.05	−0.06	0.01	−0.05	0.03		
I-2&6	−0.48	−0.48	−0.50	−0.44	−0.50	−0.43	−0.43	−0.43		
II-2&6	−0.48	−0.62	−0.62	−0.47	−0.61	−0.43	−0.43	−0.41		7.60
III-2&6			−0.04	−0.19	−0.20	−0.03	−0.17	−0.01		
I-3&5	−0.19	−0.19	−0.17	−0.19	−0.18	−0.19	−0.18	−0.17		
II-3&5	−0.19	−0.47	−0.48	−0.18	−0.48	−0.19	−0.18	−0.20		7.04
III-3&5			−0.02	−0.28	−0.29	−0.04	−0.29	−0.02		

[a] Abbreviations: T-A1, T-D1, T-A2, Da-T, CG, and Dp = ternatins A1, D1, A2, deacylternatin, 4-glucosyl-*p*-coumaric acid, and delphinidin, respectively. Chemical shift values were cited from references T-A1,[10] T-D1,[8] T-A2,[9] and CG.[6]

result of the report,[11] the inner terminal C stacks more tightly over the whole surface of Dp pyrylium and A rings than does the inner glycosylated C or the outer terminal and/or glycosylated C in ternatins in solution. Similarly, **4**, **5**, and T−D1 C_{III} ring protons have stronger ring-current effects, suggesting that the terminal C_{III} is stacking with the inner C_I ring more tightly than do those of other ternatins. These results suggest that at least one side chain has two C folds at the flexible sugar moieties and stacks the inner C and the outer C as well as the inner C and Dp nucleus.

Experimental Section

General Experimental Procedures. TLC was carried out as noted in a previous publication,[6] and open column chromatographies were applied on HP-20 (Diaion) and PVP (polyvinylpyrrolidone, Polyclar AT, GAF Chemicals Co.). HPLC was performed on an L-6200 intelligent pump system (Hitachi). Analytical HPLC was run on an Inertsil ODS-2 (4.6 i.d. × 50 mm + 4.6 i.d. × 250 mm, GL Sciences Inc.) column at 35 °C with a flow rate of 1 mL/min, monitoring at 312 nm for UV-absorbing compounds and at 530 nm for anthocyanins. Solvent systems employed were as follows: a linear gradient elution for 45 min from 25% to 70% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). Preparative HPLC was carried out on an Inertsil ODS (20 i.d. × 250 mm, GL Sciences Inc.) column with a flow rate 7−10 mL/min by an isocratic elution using mixture of solvent A (15% AcOH in H_2O) and solvent B (15% AcOH, 30% MeCN in H_2O), A:B = 64:35−90:10 at 530 nm. Preparative MPLC was performed by a YFLC-540 pump system (Yamazen) on YFLC gel column (ODS, 40 μm, 20 i.d. × 300 mm, MM column) with 15−20 mL/min in A:B = 50:50. UV−vis spectra were recorded on an MPS-2000 (Shimadzu) spectrophotometer in 0.01% HCl−MeOH. The bathochromic shift test was carried out by the addition of 5% $AlCl_3$−MeOH. FABMS spectra were recorded on JMS SX-102 (JEOL) in MeOH with the Magic Bullet (a dithioerythritol−dithiothreitol mixture, $C_4H_{10}O_2S_2$ = 154) as a matrix and measured on a positive mode. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were run on α-400 (JEOL) and JMN GX-400 (JEOL) in DMSO-d_6: CF_3COOD (9:1) with TMS as the internal standard.

Plant Materials. *Clitoria ternatea* L. was grown on a farm at Minami-Kyushu University and the flower petals were collected during July and Oct 1994, dried at 45 °C overnight, and stored in a Si gel desiccator until used for extraction.

Isolation of Pigments. The dried petals (200 g) were macerated overnight in 300 mL of 80% MeOH and filtered. This operation was repeated four times. The combined crude extract contained nine or more anthocyanins with the following retention times (min) (contents %) by HPLC analysis: ternatins A3 (**1**), 13.9 (3); B4 (**2**), 18.9 (4); A2, 23.3 (7); B3 (**3**), 25.9 (4); A1, 26.3 (2); B2 (**4**), 32.1 (16); B1, 33.4 (16); D2 (**5**), 35.1 (11); and D1 40.0 (12). The extract was evaporated to dryness *in vacuo* and redissolved in 300 mL of 50% MeOH. The solution was washed and subsequently fractionated by $CHCl_3$ and then *n*-BuOH. The aqueous solution was evaporated to dryness *in vacuo*, redissolved in 1% HOAc, adsorbed on a HP-20 resin column (60 i.d. × 450 mm), washed with 1% HOAc, and eluted with 1% HOAc in 70% EtOH. After evaporation, the residue was dissolved in 0.1 N HCl:MeOH (3:7) and chromatographed on a PVP (45 i.d. × 100 mm) column in the same solvent. The eluates were applied on an HP-20 column to remove HCl, were washed with 1% HOAc, and were eluted with 1% HOAc in 70% EtOH to give three fractions. Ternatins A1 (**1**), A2, and A3 were contained in fraction 1, ternatins B4 (**2**), B3 (**3**), B2 (**4**), and B1 in fraction 2, and ternatins D2 (**5**) and D1 in fraction 3. Pigments **1**−**5** were then purified by ODS-MPLC and isolated by preparative ODS-HPLC using an HOAc solvent system. The anthocyanin fractions were evaporated to dryness *in vacuo*, dissolved in a small amount of TFA, and precipitated with excess Et_2O to give the TFA salts of **1**−**5** as reddish purple powders.

Chemical Analyses. Acid and alkaline hydrolyses and H_2O_2 oxidation of isolated ternatins were performed according to previous methods.[6]

Ternatin A3 (1): UV−vis λ_{max} (0.01% HCl−MeOH) nm, 545 (no bathochromic shift with $AlCl_3$), 286, $E_{440}/E_{vis} = E_{440}/E_{545} = 33\%$, $E_{UV}/E_{vis} = E_{286}/E_{545} = 184\%$, $E_{310}/E_{545} = 142\%$; FABMS *m/z* 1491 [M = $C_{66}H_{75}O_{39}$]$^+$, 1405 [M − malonate]$^+$, 1243 [M − G − malonate]$^+$, 1513 [M + Na]$^+$ with Na^+ addition; ^{13}C NMR (DMSO-d_6−CF_3-COOD, 100 MHz) 169.04 (malonyl C═O), 168.27 (malonyl C═O), 166.30 (C_I, C_{II} C═O), 167.39 (Dp-7), 159.09 (C_I, C_{II}-4), 159.60 (Dp-2), 158.83 (Dp-9), 155.57 (Dp-5), 146.02 (Dp-3′,5′), 144.48 (Dp-4′), 144.34 (Dp-3), 143.72 (C_I, C_{II}-β), 136.35 (Dp-4), 129.55 (C_I, C_{II}-2,6), 127.55 (C_I, C_{II}-1), 121.73 (Dp-1′), 116.45 (C_I, C_{II}-3,5), 116.00 (C_I, C_{II}-α), 112.58 (Dp-2′,6′), 112.38 (Dp-10), 109.88 (Dp-6), 100.46 (G_b, G_c-1), 102.22 (G_a-1), 100.03 (G_d, G_e-1), 95.91 (Dp-8), 95.03, 93.84, 81.75, 77.35 (G_a-6), 77.19 (G_b, G_c-6), 76.68 (G_d, G_e-6), 76.00, 74.58, 73.57, 73.45, 70.57, 70.01, 69.74, 64.69, 63.88, 61.08, 41.43 (malonyl −CH_2−); ^{1}H NMR (DMSO-d_6−CF_3COOD, 400 MHz), see Table 1.

Ternatin B4 (2): UV-vis λ_{max} (0.01% HCl−MeOH) nm, 543 (no bathochromic shift with $AlCl_3$), 287, $E_{440}/E_{vis} = E_{440}/E_{543} = 34\%$, $E_{UV}/E_{vis} = E_{287}/E_{543} = 158\%$, $E_{310}/E_{543} = 132\%$; FABMS *m/z* 1329 [M = $C_{60}H_{65}O_{34}$]$^+$, 1483 [M + Magic Bullet]$^+$, 1081 [M − G − malonate]$^+$, 1021 [M − G − C]$^+$, 859 [M − 2G − C]$^+$; ^{13}C NMR (DMSO-d_6−CF_3COOD, 100 MHz), 169.23 (malonyl C═O), 168.33 (malonyl C═O), 167.43 (C_{II} C═O), 166.37 (C_I C═O), 166.34 (Dp-7), 159.85 (C_{II}-4), 159.35 (Dp-2), 159.10 (C_I-4), 157.41 (Dp-9), 155.60 (Dp-5), 146.07 (Dp-3′,5′), 145.91 (Dp-4′), 144.30 (dp-3), 144.22 (C_{II}-β), 143.75 (C_I-β), 134.19 (Dp-4), 129.83 (C_{II}-2,6), 129.59 (C_I-2,6), 127.51 (C_{II}-1), 124.81 (C_I-1), 118.42 (Dp-1′), 116.42 (C_I-3,5), 115.99 (C_I-α), 115.84 (C_{II}-3,5), 114.40 (C_{II}-α), 114.26 (Dp-2′), 113.37 (Dp-10), 112.46 (Dp-6′), 103.08 (Dp-6), 102.22 (G_a-1), 100.47 (G_c-1), 100.10 (G_b-1), 99.95 (G_d-1), 94.68 (Dp-8), 77.18 (G_c-6), 76.69 (G_b-6), 76.00 (G_a,G_d-6), 74.67 74.57, 73.45, 72.19, 71.84, 70.76, 70.58, 70.01, 69.76, 64.75, 63.95, 63.64, 61.09, 41.42 (malonyl −CH_2−); ^{1}H NMR (DMSO-d_6−CF_3COOD, 400 MHz), see Table 1.

Ternatin B3 (3): UV-vis λ_{max} (0.01% HCl−MeOH) nm, 548 (no bathochromic shift with $AlCl_3$), 290, $E_{440}/E_{vis} = E_{440}/E_{548} = 32\%$, $E_{UV}/E_{vis} = E_{290}/E_{548} = 322\%$, $E_{310}/E_{548} = 277\%$; FABMS *m/z* 1637 [M = $C_{75}H_{81}O_{41}$]$^+$, 1791 [M + Magic Bullet]$^+$, 1551 [M − malonate]$^+$, 1389 [M − G − malonate]$^+$, 1329 [M − G − C]$^+$; ^{13}C NMR (DMSO-d_6−CF_3COOD, 100 MHz) 169.41 (malonyl C═O), 168.40 (C_{III}C═O), 167.45 (C_{II}C═O), 166.61 (C_IC═O), 166.44 (Dp-7, C_{II}-4), 160.23 (C_{III}-4), 159.93 (Dp-2), 159.50 (C_I-4), 157.64 (Dp-9), 155.65 (Dp-5), 146.26 (Dp-3′,5′), 145.91 (Dp-4′), 144.60 (C_{III}-β), 144.36 (Dp-3, C_{II}-β), 144.27 (C_I-β), 143.77 (Dp-4), 130.34 (C_{III}-2,6), 130.16 (C_{II}-2,6), 129.86 (C_I-2,6), 129.74 (C_{II}-1), 127.95 (C_{III}-1), 127.62 (C_I-1), 124.87 (Dp-1′), 118.46 (C_{III}-3,5), 116.54 (C_I-3,5), 116.10 (C_I,C_{III}-α), 115.90 (C_{II}-3,5), 115.58 (C_{II}-α), 114.37 (Dp-2′,6′), 112.57 (Dp-10), 102.29 (Dp-6), 100.64 (G_a-1), 100.37 (G_c-1), 100.21 (G_b-1), 99.89 (G_d-1), 97.26 (G_f-1), 94.80 (Dp-8), 77.38 (G_c-6), 77.08 (G_b-6), 76.89 (G_a-6), 76.63 (G_d-6), 76.08 (G_f-6), 74.73, 74.14, 73.53, 70.85, 70.71, 70.23, 69.93, 64.86, 63.70, 60.93, 55.79, 41.43 (malonyl −CH_2−); ^{1}H NMR (DMSO-d_6−CF_3COOD, 400 MHz), see Table 1.

Ternatin B2 (4): UV−vis λ_{max} (0.01% HCl−MeOH) nm, 548 (no bathochromic shift with $AlCl_3$), 289, $E_{440}/E_{vis} = E_{440}/E_{548} = 28\%$, $E_{UV}/E_{vis} = E_{289}/E_{548} = 236\%$, $E_{310}/E_{548} = 211\%$; FABMS *m/z* 1637 [M = $C_{75}H_{81}O_{41}$]$^+$, 1791 [M + Magic Bullet]$^+$, 1551 [M − malonate]$^+$, 1389 [M − G − malonate]$^+$, 1329 [M − G − C]$^+$, 1167 [M − 2G − C]$^+$, 1021 [M − 2G − 2C]$^+$; ^{13}C NMR (DMSO-d_6−CF_3COOD, 100 MHz) 168.98 (malonyl C═O), 168.26 (C_{III} C═O), 167.32 (C_{II}C═O), 166.65 (C_IC═O), 166.34 (Dp-7,C_{II}-4), 160.09 (C_{III}-4), 159.76 (Dp-2), 159.07 (C_I-4), 157.38 (Dp-9), 155.66 (Dp-5), 146.10 (Dp-3′,5′), 145.95 (Dp-4′), 145.06 (C_{III}-β), 144.57 (Dp-3), 144.38 (C_{II}-β), 143.75 (C_I-β), 143.64 (Dp-4), 134.27 (C_{III}-2,6), 130.35 (C_{II}-2,6), 129.69 (C_I-2,6), 129.58 (C_{II}-1), 127.59 (C_{III}-1), 127.54 (C_I-1), 125.18 (Dp-1′), 118.48 (C_{III}-3,5), 116.45 (C_I-3,5), 116.39 (C_{III}-α), 116.09 (C_I-α), 116.00, (C_{II}-2,6), 114.19 (C_{II}-α,Dp-2′), 113.82 (Dp-6′), 112.37 (Dp-10), 103.10 (Dp-6), 102.17 (G_a-1), 100.69 (G_c-1), 99.96 (G_b-1), 99.82 (G_d-1), 94.69 (Dp-8), 77.16 (G_c-6), 76.63 (G_b-

6), 76.51 (G_e-6), 75.92 (G_a,G_d-6), 74.55, 74.44, 74.02, 73.39, 70.44, 70.21, 69.95, 69.75, 61.03, 41.33 (malonyl $-CH_2-$); ^{1}H NMR (DMSO-$d_6$$-$$CF_3COOD$, 400 MHz), see Table 1.

Ternatin D2 (5): UV$-$vis λ_{max} (0.01% HCl$-$MeOH) nm, 545 (no bathochromic shift with $AlCl_3$), 286, $E_{440}/E_{vis} = E_{440}/E_{545} = 33\%$, $E_{UV}/E_{vis} = E_{286}/E_{545} = 184\%$, $E_{310}/E_{545} = 142\%$; FABMS m/z 1475 [M = $C_{69}H_{71}O_{36}$]$^+$, 1629 [M + Magic Bullet]$^+$, 1389 [M $-$ malonate]$^+$, 1227 [M $-$ G $-$ malonate]$^+$, 1167 [M $-$ G $-$ C]$^+$; ^{13}C NMR (DMSO-$d_6$$-$$CF_3COOD$, 100 MHz) 169.25 (malonyl C=O), 168.23 (malonyl C=O), 167.32 (C_{III}C=O), 166.65 (C_{II} C=O), 166.36 (C_I C=O), 166.33 (Dp-7), 160.13 (C_{II}-4), 159.84 (C_{III}-4), 159.49 (Dp-2), 160.13 (C_I-4), 157.51 (Dp-9), 155.62 (Dp-5), 149.33 (Dp-3′), 146.18 (Dp-5′), 145.87 (Dp-4′), 145.05 (C_{III}-β), 144.60 (Dp-3), 144.29 (C_{II}-β), 144.22 (C_I-β), 143.62 (Dp-4), 130.32 (C_{III}-2,6), 129.78 (C_{II}-2,6), 129.65 (C_I-2,6), 127.60 (C_{II}-1), 125.42 (C_{III}-1), 125.19 (C_I-1), 124.82 (Dp-1′), 118.40 (C_{III}-3,5), 115.99 (C_I-3,5), 116.13 (C_{III}-α), 115.82 (C_I-α), 116.50 (C_{II}-3,5), 115.82 (C_{II}-α), 114.31 (Dp-2′), 112.23 (Dp-6′), 112.47 (Dp-10), 102.79 (Dp-6), 102.26 (G_c-1), 100.69 (G_b-1), 100.20 (G_a-1), 99.88 (G_d-1), 94.72 (Dp-8), 76.58 (G_a-6), 76.02 (G_b,G_c-6), 74.68 (G_d-6), 74.61, 74.51, 74.07, 73.38, 70.75, 70.56, 70.31, 69.81, 65.15, 64.74, 64.11, 63.52, 55.75, 41.34 (malonyl $-CH_2-$); ^{1}H NMR (DMSO-$d_6$$-$$CF_3$-COOD, 400 MHz), see Table 1.

Deacylternatin (Da-T): ^{1}H NMR (DMSO-$d_6$$-$$CF_3$-COOD, 400 MHz), 9.04 (1H, s, Dp-4), 8.17 (2H, s, Dp-2′,6′), 7.11 (1H, s, Dp-6), 6.77 (1H, s, Dp-8), 5.39 (1H, d, 7, a-1), 5.09 (2H, d,7, b,c-1), 3.54 (1H, d,8, a-2), 3.48 (2H, d,7, b,c-2), 3.36$-$3.44 (6H, m, a,b,c-3,5), 3.21 (1H, t, 8, a-4), 3.26 (2H, t, 9, b,c-4), 3.54 (1H, m, a-6a), 3.59 (1H, m, a-6b), 3.75 (1H, brd, 8, a-6b), 3.78 (2H, brd, 10, b,c-6b); ^{13}C NMR (DMSO-$d_6$$-$$CF_3COOD$, 100 MHz) 169.30 (Dp-7), 161.30 (Dp-2), 157.87 (Dp-9), 157.92 (Dp-5), 146.29 (Dp-3′,5′), 145.50 (Dp-4′), 145.36 (Dp-3), 144.40 (Dp-4), 120.03 (Dp-1′), 118.64 (Dp-2′,6′), 114.71 (Dp-10), 102.66 (Dp-6), 102.51 (G_a-1), 102.16 (G_b,G_c-1), 97.17 (Dp-8), 78.00 (G_a-5), 77.66 (G_b,G_c-5), 76.44 (G_a-2), 76.33 (G_b,G_c-2), 73.59 (G_b,G_c-3), 73.54 (G_a-3), 69.88 (G_b,G_c-4), 69.50 (G_a-4), 60.99 (G_b,G_c-6), 60.43 (G_a-6).

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References and Notes

(1) Saito, N.; Abe, K.; Honda, T.; Timberlake, C. F.; Bridle, P. *Phytochemistry* **1985**, *24*, 1583−1586.
(2) Goto, T. *Forschr. Chem. Org. Naturst.* **1987**, *52*, 114−158.
(3) Goto, T.; Kondo, T. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 17−21.
(4) Lowry, J. B.; Chew, L. *Econ. Bot.* **1974**, *28*, 61−62.
(5) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Phytochemistry* **1990**, *29*, 3686−3687.
(6) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Phytochemistry* **1990**, *29*, 949−953.
(7) Kondo, T.; Ueda, M.; Goto, T. *Tetrahedron* **1990**, *46*, 4749−4756.
(8) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Tetrahedron Lett.* **1989**, *30*, 5305−5308.
(9) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Heterocycles* **1990**, *31*, 1773−1776.
(10) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Tetrahedron Lett.* **1990**, *31*, 2921−2924.
(11) Yoda, K.; Haruyama, H.; Kuwano, H.; Saito, N. *Abstracts of Papers*, 28th NMR Symposium (Japan), 1989; pp 191−194.
(12) Harborne, J. B. *Biochem. J.* **1958**, *70*, 22−28.
(13) Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185−189.
(14) Goto, T.; Kondo, T.; Tamura, H.; Imagawa, H.; Iino, A.; Takeda, K. *Tetrahedron Lett.* **1982**, *23*, 3695−3698.
(15) Brouillard, R. *Phytochemistry* **1983**, *22*, 1311−1323.
(16) Brouillard, R.; Dangles, O. In *The Flavonoids, Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; Chapter 13, pp 565−588.

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